Structure and Function of "Metalloantibiotics"

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Abstract: Although most antibiotics do not need metal ions for their biological activities, there are a number of antibiotics that require metal ions to function properly, such as bleomycin (BLM), streptonigrin (SN), and bacitracin. The coordinated metal ions in these antibiotics play an important role in maintaining proper structure and/or function of these antibiotics. Removal of the metal ions from these antibiotics can cause changes in structure and/or function of these antibiotics. Similar to the case of ''metalloproteins,'' these antibiotics are dubbed ''metalloantibiotics'' which are the title subjects of this review. Metalloantibiotics can interact with several different kinds of biomolecules, including DNA, RNA, proteins, receptors, and lipids, rendering their unique and specific bioactivities. In addition to the microbial-originated metalloantibiotics, many metalloantibiotic derivatives and metal complexes of synthetic ligands also show antibacterial, antiviral, and anti-neoplastic activities which are also briefly discussed to provide a broad sense of the term ''metalloantibiotics.'' © 2003 Wiley Periodicals, Inc. Med Res Rev, 23, No. 6, 697–762, 2003

Key words: albomycin; aminoglycosides; anthacycline; antibiotics; aureolic acid; bacitracin; bleomycin; cisplatin; function; gramicidin; ionophore; metalloantibiotics; quinolones; siderophore; streptonigrin; structure; tetracycline

1. INTRODUCTION

Antibiotics can interact with a variety of biomolecules, which may result in inhibition of the biochemical or biophysical processes associated with the biomolecules. This can be illustrated in the interaction of the peptide antibiotic polymyxin with glycolipids which affects membrane function,[1] in the intercalation of the anthracyclines (ACs) into DNA base pairs which stops gene replication,[2] in the

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imbedding of the lipophilic antibiotic gramicidin[3] and the insertion of the amphiphilic antibiotic protein colicin A into cell membrane[4] which disturb normal ion transport and trans-membrane potential of cells, in the inhibition of transpeptidase by penicillin which affects cell wall synthesis,[5] and the inhibition of aminopeptidase by bestatin, amastatin, and puromycin which impairs many significant biochemical processes.[6] While most antibiotics do not need metal ions for their biological activities, there are several families of antibiotics that require metal ions to function properly. In some cases, metal ions are bound tightly and are integral parts of the structure and function of the antibiotics. Removal of the metal ions thus results in deactivation and/or change in structure of these antibiotics, such as bacitracin, bleomycin (BLM), streptonigrin (SN), and albomycin. In other cases, the binding of metal ions to the antibiotic molecules may engender profound chemical and biochemical consequence, which may not significantly affect the structure of the drugs, such as tetracyclines (TCs), ACs, aureolic acids, and quinolones. Similar to the case of ''metalloproteins,'' these families of antibiotics are thus dubbed ''metalloantibiotics'' in our studies and are the title subjects of this review.

The term ''antibiotic'' was originally coined by Selman A. Waksman and was used in the title of a book of his, *Microbial Antagonisms and Antibiotic Substances* published in 1945, and was defined as '' . . . produced by microorganisms and which possess the property of inhibiting the growth and even of destroying other microorganisms.''[7] However, many clinically useful ''antibiotic drugs'' nowadays are either synthetic or semi-synthetic, including many β-lactams, (fluoro)quinolones, and amino-glycosides. These (semi-)synthetic drugs and many synthetic metal complexes and organometallic compounds that exhibit ''antibiotic activities'' can be considered ''synthetic antibiotics'' as the counterparts of the originally defined ''microbial-originated antibiotics'' from a broad sense of the term. In this review, we focus on those nature-occurring metalloantibiotics and also briefly discuss a few synthetic metalloantibiotics to provide a broader view of the term ''metalloantibiotics.'' The structures and anti-microbial, anti-viral, and/or anti-cancer activities of these natural and synthetic metalloantibiotics will be discussed to provide further insight into their structure–function relationship.

Metal ions play a key role in the actions of synthetic and natural metalloantibiotics, and are involved in specific interactions of these antibiotics with proteins, membranes, nucleic acids, and other biomolecules. For example, the binding of Fe/Co–BLM, Fe/Cu–SN, Mg–quinolone, Mg–quinobenzoxazine, Mg–aureolic acid, and cisplatin with DNA impairs DNA function or results in DNA cleavage (Section 2); the involvement of Mg/Fe in the binding of TCs to the regulatory TetR protein turns on the mechanism for bacterial resistance to TCs (Section 3); the binding of metallobacitracin to undecaisoprenyl pyrophosphate prohibits the recycling of the pyrophosphate to phosphate which in turn inhibits cell wall synthesis (Section 4); and the binding of metal ions to ionophores or siderophores allows their transport through cell membrane which can cause disruption of the potential across the membrane, enables microorganisms to acquire essential iron from the environment, or delivers antibiotics to foreign microorganisms (Section 5). The structural and functional roles of metal ions in metalloantibiotics have been further advanced in recent years from extensive biological, biochemical, and physical studies,[8] which are discussed herein to provide an overview of this important and unique group of antibiotics.

2. DNA-BINDING METALLOANTIBIOTICS

DNA can bind many different biomolecules and synthetic compounds, including proteins, antibiotics, polyamines, and synthetic metal complexes and organometallic compounds.[9] In the case of the very specific protein–DNA interaction, transcription is regulated to turn on or off a specific biological process. DNA is also a target for therapeutic treatment of disorders and diseases, such as cancers, via direct ligand binding to it or binding to DNA-regulating biomolecules which in turn imparts DNA

function indirectly. Several clinically used anti-cancer antibiotics, such as BLM and the ACs, are DNA-binding (and cleaving) agents. A better understanding of the structure of these antibiotics and their DNA complexes, and a better understanding of the relationship of structure, function, and toxicity of these drugs can provide information for the design of more effective but less toxic drugs for therapeutic treatments. The investigation of the interaction between DNA and synthetic compounds or metal complexes can also further our understanding of DNA–ligand binding specificity which would provide clues for rational design of DNA-specific drug in the future.[10] The structure and function of a few natural and synthetic DNA-targeting metalloantibiotics are discussed in this section.

A. Bleomycin

Bleomycin (BLM, also known as Blenoxane) was first isolated as a Cu^{2+}-containing glycooligopeptide antibiotic from the culture medium of *Streptomyces verticullus*,[11] and was later found to be also an antiviral agent.[12] It was soon found to be an anticancer agent and has ever since become one of the most widely used anticancer drugs,[13] most commonly used in treatment of testis cancer, lymphomas, and head and neck cancer, as well as the AIDS-related Kaposi's sarcoma in combination with cisplatin and adriamycin. However, it can cause life-threatening side effect, including lung fibrosis. BLM contains a few uncommon amino acids, such as β-aminoalanine, β-hydroxyhistidine, and methylvalerate, two sugars (gulose and mannose), a few potential metal-binding functionalities such as imidazole, pyrimidine, amido, and amino groups, and a peptidyl bithiazole chain considered to be the DNA recognition site (Fig. 1). Similar to many other nature products, BLM is produced as a mixture of several analogues with BLM A_2 and B_2 being the most abundant.[11] BLM is the most extensively studied metalloantibiotic from several different view points, such as its metal binding property, structural studies with a variety of spectroscopic methods, mechanistic study of its oxidative DNA cleavage, investigation of its structure–function relationship, and its use as a non-heme model for investigation of dioxygen activation and DNA recognition/cleavage.[14] There are a few BLM-like antibiotics which exhibit similar physical, structural, and biochemical characteristics as BLM, which have been previously reviewed.[15]

1. DNA/RNA Binding and Cleavage

The antibiotic mechanism of BLM has been proposed on the basis of the results from the better studied Fe and Co derivatives (however, it is the Fe form that is considered the active form *in vivo* because of its higher abundance in the biological systems).[14] In the presence of reducing agents, the metal ion in Fe^{2+}- or Co^{2+}-BLM binds dioxygen and converts into an ''activated form'' HOO-M^{III}-BLM probably

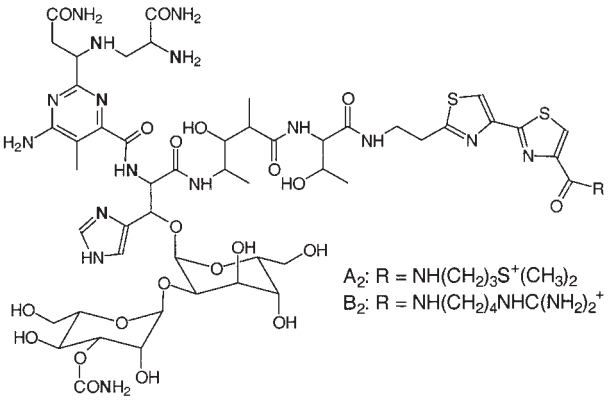

Figure 1. Schematic structure of bleomycin (BLM) A_2 and B_2. The proposed metal-binding ligands based on spectroscopic studies are in bold-phase.

via an superoxide-M^{III}-BLM intermediate. DNA cleavage by Fe–BLM is proposed to be carried out by the active $O=Fe^{V}$–BLM or $O=Fe^{IV}$–BLM species generated by O–O bond cleavage in the activated form[14,16] via oxidation at C4′ and C2′–H proton abstraction from the deoxyribose of DNA immediately following 5′GC and 5′GT sequences.[14f,17] The damaged deoxyribose then breaks down, and cleavage of DNA strand occurs. The mechanism for DNA cleavage by Co–BLM has been suggested to follow a similar mechanism via photo-activation.[18] Recent studies indicated that the sequence GTAC is a hot spot for double-stranded DNA cleavage by Fe–BLM at site T.[19] The sugar moiety of BLM is important in determining the specificity of the cleavage since Fe–BLM and deglycosylated Fe–BLM were reported to cleave $d(CGCTAGCG)_2$ at different sites.[20] In the presence of H_2O_2, Fe^{3+}–BLM generates hydroxyl · OH free radical in the vicinity of DNA which is expected to cause DNA cleavage *in vivo*.[21] More detailed discussion on the mechanism of DNA cleavage[14] and associated cytotoxicity[22] can be found in the cited review articles.

Several studies indicate that Fe^{2+}–BLM can also bind and cleave RNA molecules,[23] including tRNA and its precursors and rRNA.[24] The cleavage occurs mainly at the junctions between double-stranded and single-stranded regions in RNA molecules,[25] such as at C_{26} and A_{32} in *E. coli* $tRNA_1^{His}$.[25d] However, not all RNA molecules can be cleaved by Fe^{2+}–BLM, which include *E. coli* $tRNA^{Tyr}$ and $tRNA^{Cys}$.[25] These studies reveal that RNA cleavage by Fe^{2+}–BLM shows much higher selectivity as opposed to DNA cleavage that occurs at all 5′GC and 5′GT sites. Another significant difference is that the rate for RNA cleavage is significantly slowed in the presence of Mg^{2+} and abolished at 0.5 mM (but not in DNA cleavage at even 50 mM[25a]), which has been attributed to stabilization of RNA structure by this metal ion.[25d] It is interesting to note that a DNA molecule analogous to the T-stem loop of yeast $tRNA^{Phe}$ is cleaved by Fe^{2+}–BLM at 5′G**T** site as in the case of normal DNA cleavage, whereas the yeast $tRNA^{Phe}$ loop is cleaved at G_{53} after the corresponding GU sequence with a rate 16-times slower.[25a] Fe^{2+}–BLM can cleave DNA–RNA hybrids as well.[23b,26] However, the cleavage sites on the RNA strand are different from that of the RNA alone, and is inhibited at slightly higher Mg^{2+} concentrations equal or greater than 1 mM. In the meantime, the DNA strand in the hybrids is cleaved at all 5′GT sequences and 5′GC sequences to a less extent, similar to a regular double-stranded DNA.[26b]

2. Metal Binding and Coordination Chemistry

BLM was originally isolated as a Cu^{2+} complex which has since been extensively studied.[11] It has also been known to be an excellent ligand for binding with several different metal ions,[27] including Mn^{2+},[28] $Fe^{2+/3+}$,[29] $Co^{2+/3+}$,[30] $Ni^{2+/3+}$,[31,32] $Cu^{+/2+}$,[33,34] Zn^{2+},[33] Cd^{2+},[35] Ga^{3+},[36] and Ru^{2+}[37] ions as well as the radioactive ^{105}Rh for use in radiotherapy.[38] The d–d transitions of the Cu^{2+} complexes of BLM and analogues are detected at ~600 nm with a molar absorptivity ~110 M^{-1} cm^{-1}. The energy of the d–d absorption is higher than those of many "type 2" Cu^{2+} centers in the range of 650–750 nm, suggesting the presence of a strong ligand-field in a distorted 5- or 6-coordination sphere.[39] The metal coordination became clear after the structure of a Cu^{2+} complex of a biosynthetic intermediate of BLM was determined with crystallography.[40] This intermediate contains all the metal-binding moieties, but lacks the sugars and the peptidyl bithiazole moiety. In this complex, the Cu^{2+} is bound to the ligand via imidazole, pyrimidine, the amines of β-aminoalamine, and the amide nitrogen of β-hydroxyhistidine.

The identification of nitrogen-containing ligands in Cu^{2+}, Co^{2+}, and Fe^{3+}–BLM complexes has also been achieved by means of electron spin-echo envelope spectroscopy through the detection of ^{14}N hyperfine coupling.[41] This metal-binding mode has been considered to be conserved in Fe^{2+}–BLM. A previous observation of a perturbation on the ligand field and ligand-to-metal charge transfer transition bands in a few BLM congeners suggested the presence of an axial ligand which might be exchangeable.[42] A later nuclear magnetic resonance (NMR) study of the diamagnetic CO adduct of Fe^{2+}–BLM suggested a similar metal binding site as previously determined, except that the amide

group of α-D-mannose was considered to be involved in metal binding.[43] Despite the disagreement, a structure of the metal center with five coordinated ligands in a distorted octahedral geometry has emerged which leaves an open coordination site or an exchangeable site for oxygen binding.

3. Zn^{2+} and $Co^{2+/3+}$ Complexes and Their DNA Binding

The diamagnetic Zn^{2+}–BLM complex of BLM has been utilized as a structural model for the paramagnetic Fe^{2+}–BLM complex owing to the difficulty in high-resolution NMR studies of the paramagnetic species. Previous 2D-NMR studies of Zn–BLM strongly suggested that the metal is bound to BLM through the secondary amine of β-aminoalanine, the amido-*N* and imidazole of β-hydroxy histidine, pyrimidine, and the carbamoyl group of mannose.[44] This coordination chemistry of Zn–BLM has been suggested to be similar to that of the diamagnetic CO complex of Fe^{2+}–BLM based on NMR studies.[43] However, this metal coordination has recently been challenged by an NMR study of an analogous complex Zn–tallysomycin,[45] in which five N-containing donors are suggested, including the primary amines of β-amino-Ala, pyrimidine, and the peptidyl amide and imidazole of β-(OH)His with the pyrimidine at the apex and an SS chirality. This study also excludes the binding of the carbamoyl group. Instead, the disaccharide covers the sixth binding site. This disagreement in axial binding has also been raised in the study of HOO–Co^{3+} complexes of BLM and analogues discussed below.

BLM forms a complex with Co^{2+} under anaerobic conditions at pH 6.8, which exhibits a nearly axial electron paramagnetic resonance (EPR) spectrum with $g_{\perp} = 2.272$ and $g_{//} = 2.025$ and shows a large hyperfine coupling of $A_{//} = 92.5$ G attributed to the ^{59}Co nucleus of I = 7/2 and three superhyperfine-coupled lines of 13 G because of coupling with one ^{14}N (I = 1).[46] The sharp EPR features of this complex at 77 K with g ~2 and $g_{\perp} > g_{//}$ reflect the presence of a low-spin Co^{2+} center of S = 1/2 with the unpaired electron in the dz^2 orbital overlapping with one N-containing ligand, since a high-spin Co^{2+} center of S = 3/2 can only be observed at liquid He temperatures (and showing g ~2 and ~4 features) because of its fast electron relaxation rates.[47] The observation of a low-spin Co^{2+} center also concludes the presence of a strong ligand field as suggested based on the electronic spectrum of Cu^{2+}–BLM above. Upon oxygen binding at 77 K, the EPR spectrum is dramatically changed to give $g_{//} = 2.098$ and $g_{\perp} = 2.007$ and a very small hyperfine coupling with ^{59}Co of $A_{//} = 20.2$ G. The similar g values of ~2 and the small coupling with the ^{59}Co center strongly suggest that the unpaired electron density is not located at the Co^{2+} center, but very possibly on the bound oxygen, i.e., a ligand-centered EPR spectrum.[46a] The study of Co^{2+} binding of the less active deamido-BLM by means of EPR revealed that the fifth ligand is the amino group of β-amino-Ala. Upon the introduction of DNA, the spectrum of Co^{2+}–BLM is not changed whereas the spectrum of oxy-Co^{2+}–BLM is noticeably changed to give $g_{//} = 2.106$, $g_{\perp} = 2.004$, and $A_{//} = 18.9$ G.[46] This spectral change indicates that the binding of BLM to DNA via the bithiazole rings should affect the orientation of the bound O_2 molecule where the unpaired electron resides.

The Co^{2+} in Co^{2+}–BLM can form an activated ''green species'' HOO–Co^{3+}–BLM and an inactive ''brown species'' H_2O–Co^{3+}–BLM upon treatment with peroxide.[48] These low-spin diamagnetic Co^{3+} complexes of BLM and analogues have been extensively studied by means of 2D-NMR spectroscopy.[49,50] The coordination chemistry of BLM has further been established from these studies (Fig. 2A). The overall structure is similar to that revealed in the crystallographic study of the Cu^{2+} complex of the BLM bio-intermediate,[40] despite the lack of consensus regarding the axial ligands[49,50] (i.e., alanyl-NH_2 vs. mannose-CO–NH_2 binding).

These Co^{3+} complexes can bind double-stranded DNA to form DNA_2–Co^{3+}–BLM and DNA_2–(HOO)Co^{3+}–BLM ternary complexes. Several ternary complexes have been investigated by means of 2D-NMR techniques, and their structures determined.[51,52] A representative structure of the ternary complex $(CGTACG)_2$–Co^{3+}–(OOH)–deglycopepleomycin is shown in Figure 2. The coordination chemistry of the Co^{3+}–deglycopepleomycin complex in the ternary complex is very

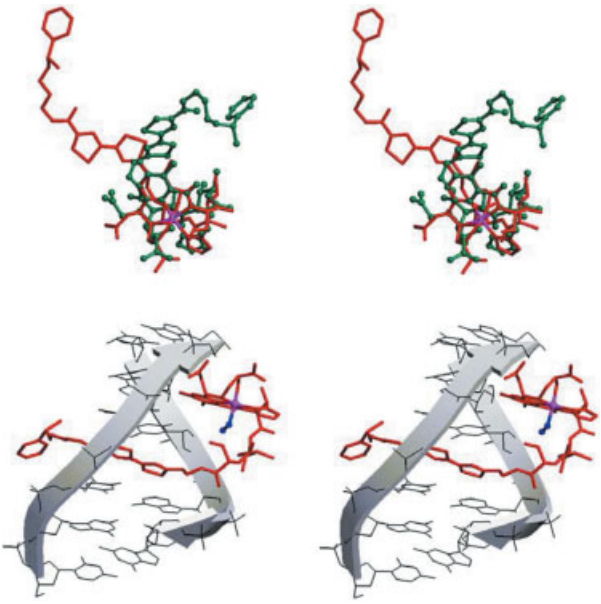

Figure 2. **Top**: The superimposed structures of the activated "green species" HOO-Co^{3+}-deglycopepleomycin (green ball-and-stick structure; Protein Data Bank ID 1A02) and the complex upon binding with $d(CGTACG)_2$ (red stick structure without showing the oligonucleotide) and (**bottom**) deglycopepleomycin-Co^{3+} (OOH)-$(CGTACG)_2$ derived from NMR studies and molecular dynamic calculations (Protein Data Bank ID 1A01.pdf). The Co^{3+}–deglycopepleomycin complex is shown in red color, DNA in gray, Co^{3+} in pink, and the metal-bound peroxide in blue.

similar to that of the DNA-free complex; however, the peptidyl bithiazole tail is pointed away from the metal center (Fig. 2A). The metal coordinated moiety is sitting in the minor groove of DNA duplex, and the bithiazole rings are found to interact with DNA double helix via intercalation which exposes the bound peroxide to the DNA. This intercalation binding mode was also observed in the binding of metal-free BLM to calf thymus DNA by means of low-frequency Raman spectroscopy.[53] The binding of these Co^{3+} complexes to DNA has brought the terminal oxygen of the Co^{3+}-bound peroxide close to the 4′-H of the scissile ribose (<3 Å), and has also resulted in several specific perturbations on the DNA structure. For example, in the case of $d(CCAG\mathit{T}ACTGG)_2$–$(HOO)Co^{3+}$–BLM,[51a] the bithiazole rings intercalate into the base pairs between T_5•A and A_6•T and the configuration of the T_5–A_6 riboses and the region C_2 through C_4 are found to deviate from B-form configuration.

The bithiazole was observed to span in the minor groove in the case of Zn^{2+}–BLM–DNA,[54] different from the intercalation binding mode in the Co^{3+}–pepleomycin–DNA complex.[52] This groove-binding mode was suggested to be probably the initial binding of the metal–BLM complex with DNA, prior to the more specific binding at 5′-GC or 5′-GT sequences (and intercalating into the base pairs next to the sequences).[52] However, intercalation has been suggested not necessarily a required interaction for DNA cleavage in a study wherein the bithiazole terminus of Fe–BLM is tethered to a porous glass bead, which shows similar efficacy in DNA cleavage as free Fe–BLM in solution.[55]

The importance of the lesion of one strand and the role of the bithiazole in the cleavage mechanism of double-stranded DNA have recently been very elegantly investigated. The binding of HOO-Co^{3+}–BLM to a double-stranded DNA with a lesion site was studied and structure determined with 2D-NMR.[56] This DNA has the sequence d(5′-$CCAAAG_6$_A_8CTGGG)•d(5′-CCCAG-T_{19}ACTTTGG), in which the underlined site has a 3′-phosphoglycolate lesion next to 5′-phosphato moiety and this ''cleaved strand'' is connected to the other strand of a complementary sequence (with

the corresponding lesion site occupied by an A) via two 5′-3′ hexaethylene glycol linkers. The metal-binding domain of Co^{3+}–BLM is located in the minor groove in close proximity of T_{19} and the bithiazole is most likely to partially intercalate between T_{19} and A_{20} according to nuclear Overhauser effect interactions (NOE, which is a function of the molecular rotational correlation time and inter-nuclear distance[57]). The structural model derived from this study suggests that the metal center interacts with G on the second strand at the 5′ end of the cleaved site on the first strand as well as a reorientation of the bithiazole rings upon cleavage of the first strand.

4. Paramagnetic $Fe^{2+/3+}$ Complexes

The binding of Fe^{3+} to BLM at slightly alkaline conditions forms a low-spin complex of S = 1/2 (g = 2.41, 2.18, and 1.89),[58] in which the sixth position is occupied by a hydroxide based on resonance Raman spectroscopy.[59] An oxy-form of Fe–BLM is formed by introducing dioxygen to Fe^{2+}–BLM in the absence of reducing agent and DNA. This oxy form has been determined to be a superoxide O_2^-–Fe^{3+}–BLM complex based on its ^{57}Fe Mössbauer spectrum.[60] In the presence of a reducing agent, the activated hydroperoxide HOO^-–Fe–BLM complex is formed which is the active species for DNA cleavage.[58b] A recent theoretical study suggested that both heterolytic and hemolytic cleavage of the O–O bond in the active peroxo complex are not favorable based on energetics and reaction specificity, which concludes a direct attack on DNA by the hydroperoxide of the active complex.[61]

The Fe^{2+}–BLM complex is paramagnetic (S = 2), which has been studied by means of NMR techniques.[62,63] In paramagnetic metal complexes, the NMR signals of nuclei near the metal center can be hyperfine-shifted outside the ''regular'' spectral range (i.e., ~13 ppm for ^{1}H-NMR and ~200 ppm for ^{13}C-NMR) by the unpaired electron(s) to afford a large spectral window that may reach more than 100 ppm for ^{1}H-NMR. In the meantime, the nuclear relaxation times are dramatically shortened which are proportional to the sixth power of metal-nucleus distances.[64] This paramagnetic complex exhibits many hyperfine-shifted ^{1}H-NMR signals in a spectral window of 230 ppm (Fig. 3), which have been assigned to the protons of the coordinated ligands or the protons near the metal by the use of 1D- and 2D-NMR techniques.[63] A structural model of this metal complex has been built by the use of the distance-dependent nuclear relaxation times as constraints. This structural model turns out to be similar to the structural models built on the basis of the Co^{3+}–BLM complexes and their ternary complex with the oligonucleotide duplex discussed above. The Fe^{2+} complex of a BLM congener peplomycin and its derivatives have been studied with X-ray absorption spectroscopy which reveals that an axial ligand may affect the Fe(II) $d\pi \rightarrow$ pyrimidine back-bonding as previously observed[42] which may stabilize the superoxide intermediate, consistent with the auto-oxidation rate of the metal center in the complexes.[65]

A recent ^{1}H-NMR study of the paramagnetic Co^{2+}–BLM complex at pH 6.5[66] corroborates the metal coordination chemistry obtained in the Fe^{2+}–BLM study.[63] A possible involvement of mannose-amido group as the sixth ligand was proposed. Co^{2+}–BLM at slightly higher pH of 6.8 was previously determined by means of EPR to have a low-spin Co^{2+} center,[46] which would exhibit only broad hyperfine-shifted ^{1}H-NMR features.[64] The observation of sharp ^{1}NMR features in Co^{2+}–BLM

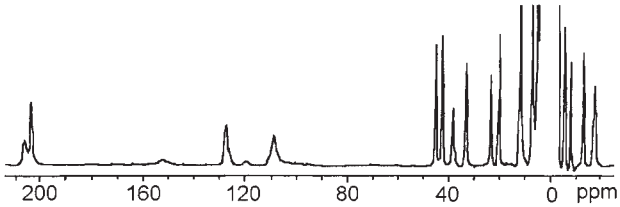

Figure 3. Hyperfine-shifted ^{1}H-NMR spectrum of high-spin Fe^{2+}–BLM in D_2O at pH meter reading of 6.5. The shifted signals outside the regular 0–10 ppm window are because of protons in close proximity of the paramagnetic Fe^{2+} ion.

reflects the presence of a fast-relaxing high-spin Co^{2+} center. The NMR and EPR studies suggest a possible presence of a high-spin to low-spin transition that is controlled by pH. However, this hypothesis cannot be verified because of the lack of cross investigations of the complex at lower pH with EPR at liquid He temperatures and the complex at higher pH with NMR in these studies.

5. Synthetic Analogues and Biosynthesis

A number of BLM-analogous compounds have been synthesized that contain the metal binding moieties of BLM.[67–69] These synthetic analogues form complexes with several metal ions, including Cu^{2+} and $Fe^{2+/3+}$, and are able to cleave DNA molecules similar to the cleavage pattern by BLM complexes. A recent revisit of the Fe–BLM mimicking complex Fe–PMAH[67c] (PMAH = 2-[*N*-(aminoethyl)amino)methyl]-4-[*N*-[2-(4-imidazolyl)ethyl]carbamoyl]-5-bromopyrimidine) showed that HOO–Fe^{3+}–PMA (with low-spin features of g = 2.22, 2.17, and 1.94 and a noticeable high-spin feature at g = 4.3) can be formed by reacting Fe^{3+}–PMA with H_2O_2, but not with iodosylbenzene and a base that was previously reported.[67c] This result is consistent with the observation in an early study of Fe^{3+}–BLM.[70]

Several lipophilic ligands analogous to the metal-binding moiety of BLM have been synthesized that comprise a 4-alkoxypyridine with methylhistamine or methylethylenediamine moiety and a long hydrocarbon chain in the alkoxy moiety for the lipophilicity.[71] These ligands bind Cu^{2+} and form micelles with critical micelle concentration in the range of $0.9–1.4 \times 10^{-4}$ M. The catalytic properties of these complexes were not tested in this study. Some pyridine-containing BLM analogues were synthesized, and investigated with spectroscopic and crystallographic techniques.[72] The λ_{max} of ~650 nm of Cu^{2+} complexes is significantly longer than that of Cu^{2+}–BLM[11] which indicates a weaker ligand field in the complexes of these analogues, whereas the g values of 2.21–2.22 and 2.04–2.05 of these complexes are close to those of Cu^{2+}–BLM and its analogues (~2.21–2.25 and ~2.06) which suggests the presence of a similar axially symmetric magnetic environment of the Cu^{2+} center in these complexes.[27]

BLM is a natural peptide–ketide hybrid (Fig. 1), like the cyanobacterial hepatotoxins such as cylindrospermopsin. The biosyntheses of many peptides and polyketides and their hybrid conjugates (including a number of antibiotics such as BLM, ACs, bacitracin, and some ionophore antibiotics) follow a nonribosomal pathway catalyzed by large clusters of peptide and ketide synthases/synthetases and peptide/ketide ''hybrid'' synthetases, respectively.[73,74] The genes of the synthases/synthetases of peptides and polyketides from microorganisms have recently been analyzed and cloned and the enzymes further studied,[73–75] including those of BLM and bacitracin (Section 4). BLM has been verified to be produced by synthetase clusters comprised of polyketide synthase and peptide synthetase modules.[76] These peptide and polyketide synthetases are comprised of a multi-domain modular structure for the catalysis of the initiation of the synthesis via ATP-activating formation of thioester linkage to the enzyme, elongation mediated by condensation of the thioester-linked amino acid and/or peptide on the peptide carrier domain following a mechanism not yet fully understood, and termination of the peptide or polyketide chain by a thioesterase domain via transfer of the final product to a serine in the thioesterase followed by hydrolysis.[77] The reactant amino acids or carboxylates are specifically recognized and covalently linked to the different domains before transferred to an intermediate peptide or polyketide chain. Changing of the stereochemistry is carried out by epimerization domains in the enzyme complex. The studies of several peptide and polyketide synthetases and their hybrids, including crystallographic studies of the adenylation domain and an NMR study of a peptidyl carrier domain,[78] have greatly enhanced our understanding of the structure and mechanism of this superfamily of ''mega enzymes.'' Since these synthetase complexes possess enzymatic activities toward the syntheses of secondary metabolites,[75] thus are potential targets for drug discovery in the production of potential bio-active peptides and polyketide as well as their hybrids.[79]

B. Aureolic Acids

The glyco-antibiotic aureolic acid family produced by *Streptomyces* species is comprised of several members with similar structures, including chromomycin A_3 ($ChrA_3$), mithramycin (Mit, produced by *S. plicatus* and also known as plicamycin), olivomycin, and variamycin, which exhibit activities toward Gram-positive bacteria, DNA viruses, and tumors.[80] However, high toxicity has limited their use as clinical antibiotics and anti-tumor agents. Mithramycin has been tested against several malignant diseases since its discovery,[81] and has a limited use for the treatment of the Paget's disease[82] and for the treatment of hypercalcemia[83] (however, controversies have also raised[84]). Both $ChrA_3$ and Mit have recently been found to be potent inhibitors of neuronal apoptosis induced by oxidative stress and DNA damage in cortical neurons.[85] Thus, these antibiotics may be effective agents for the treatment of apoptosis-associated neurological diseases, which suggests that sequence-selective DNA-binding drugs may serve as potential neurological therapeutics.

1. Structure of Aureolic Acids

These antibiotics contain a metal-binding β-ketophenol chromophore, a highly functionalized aliphatic side chain, and a disaccharide and a trisaccharide chains important for DNA binding and inhibition of DNA transcription (Fig. 4). The identity of the sugar chains and the sequence of the sugar linkage of this drug family were first established by partial hydrolysis of olivomycin A[86] and Mit.[87] The structure of $ChrA_3$ has later been further studied by means of ^{1}H- and ^{13}C-NMR spectroscopy.[88] The structure of Mit has also been studied by the use of synthetic and NMR techniques,[89] and has recently been fully determined by means of 2D-^{1}H and ^{13}C homonuclear and heteronuclear methods which is shown in Figure 4.[90] The structures of the DNA complexes of the drugs have also been investigated by the use of 2D-NMR techniques in recent years, which are discussed in a later section.

2. Role of Metal Ions in the Action of Aureolic Acids

A divalent metal ion, such as Mg^{2+}, Co^{2+}, Zn^{2+}, or Mn^{2+}, is required for aureolic acid to bind to a double helical DNA to form a $drug_2$–metal–$(DNA)_2$ ternary complex.[91–93] The metal–$drug_2$ complex in the ternary complex is bound to DNA in the minor groove with a high preference to GC sites and a length of approximately six base pairs based on ^{1}H-NMR,[94] DNA footprinting,[95] and biochemical[96] studies. Consequently, these antibiotics can inhibit transcription of the genes that have G-C-rich promoter sequences,[96] such as the *c-myc* proto-oncogene[97] (this binding might be non-specific[98]) that regulate cell proliferation and also controls the expression of β-galactosidase.[97,99] These studies led to further investigations of the interaction between this antibiotic family and double helical DNA of different sizes and sequences.[100] In addition to the above metal ions, Mit has been

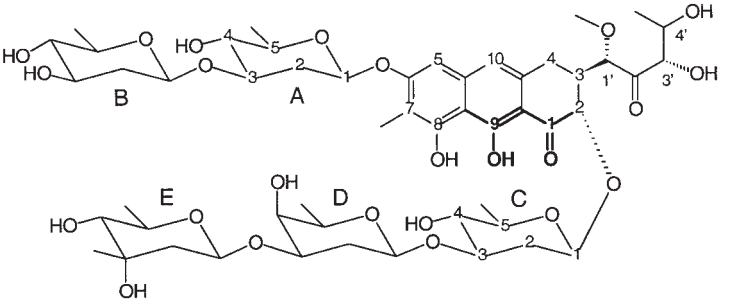

Figure 4. A schematic structure of mithramycin. The metal binding β-ketophenol moiety is shown with thick lines. All drugs in the aureolic acid family have the similar metal binding moiety, but vary in the sugar chains which have been suggested to cause different interactions with DNA.

determined to bind several other metal ions, including Ca^{2+}, Cd^{2+}, Tb^{3+}, Gd^{3+}, and alkali metals.[101] Metal:$drug_4$ complexes are suggested to form for Ca^{2+}, Tb^{3+}, and Gd^{3+}; however, which does not assist the binding of the drug molecules to DNA.

Some early studies of Mg^{2+} binding of Mit showed that two different complexes can be formed which exhibit different absorption and CD spectra, in which the better known 1:2 Mg^{2+}–Mit_2 complex formed at low Mg^{2+} concentrations and a 1:1 Mg^{2+}–Mit complex formed at higher Mg^{2+} concentrations.[93] Interactions of these two complexes with bulk DNA, polynucleotide, and an octanucleotide are observed to be different. For example, while the 1:1 complex interact with the B-DNA-representing poly(GC) · poly(CG) and the A-DNA-representing poly-G · poly-C in a similar fashion, the 1:2 complex shows distinct interaction patterns with the two DNA forms. Since cellular Mg^{2+} concentration varies significantly in neoplastic tissues,[102] these two complexes can be expected to form to certain extents and are considered important for *in vivo* action of the drug.[93e]

3. Role of Sugars

The importance of the sugar moiety in antibiotic activity of this family has been established in early study of the different congeners and derivatives of this antibiotic family.[103] Removal of the sugar moiety E (Fig. 4) from olivomycin A (affording olivomycin D) and $ChrA_3$ (affording $ChrA_4$) results in significant loss of antibiotic activity. Moreover, the derivatives with only one sugar and the aglycones (without any sugar) are inactive. The involvement of the sugar chains in stabilization of the 1:2 complexes M^{2+}–$(ChrA_3)_2$ (M = Mg and Ni) in methanol solution has been suggested.[104] On the contrary, the deglycosylated chromomycinone forms 1:1 complexes with these two metal ions. In addition, Ca^{2+} was determined to form only a 1:1 complex with $ChrA_3$ in methanol as opposed to a previous observation in aqueous solution.[101]

The metal complexes of $ChrA_3$ and Mit are found to interact differently with A- and B-representing DNA sequences. Since these two congeners differ in the sugar moieties (the sugars of $ChrA_3$ are acetylated), this observation indicates the significance of the sugar chains in the interaction of these antibiotics with DNA.[93] The difference between $ChrA_3$ and Mit has also been shown in their binding with the oligonucleotide $d(ACCGGGT)_2$, wherein $ChrA_3$ forms a $drug_2$–Mg^{2+}–$(DNA)_2$ ternary complex whereas Mit has been proposed to afford a ($drug_2$–Mg^{2+})$_2$–$(DNA)_2$ ternary complex based on their NMR spectra.[105] This difference has also been attributed to the difference in the sugar chains in these two congeners. The role of the sugar chains has further been investigated with a simple synthetic analogue, in which a simple triethylene glycol chain is attached to a β-ketophenolate aromatic ring structure as the aglycone of $ChrA_3$.[106] This simple model forms M^{2+}–$ligand_2$ complexes (M = Co and Mg) similar to $ChrA_3$. Preliminary study by these authors shows that this complex can bind DNA. The above studies further corroborate the significance of the sugar chains in metal binding and in the binding of this drug family to double-stranded DNA.

Total synthesis of aureolic acid has been attempted,[107] wherein stereoselective syntheses of aryl 2-deoxy-β-glycosides and the A-B disaccharide of olivomycin have been achieved. Since many antibiotics are found to be glycosylated, such as BLM, aureolic acid, aminoglycoside, and AC families discussed in this review, further exploration of glycosylated metal complexes and their DNA binding properties should be encouraged.

4. (Aureolic Acid)$_2$–Mg^{2+}–$(DNA)_2$ Ternary Complexes

The requirement of divalent metal ions for the binding of aureolic acid to DNA has been fully established by means of ^{1}H- and ^{31}P-NMR techniques, in which the spectral features of DNA are changed upon the binding of the drug in the presence of Mg^{2+}.[94] Several palindromic oligonucleotides have been used for NMR studies, wherein the addition of 1:2 Mg^{2+}–$(Mit)_2$ to DNA afford ternary complexes whose ^{1}H- and ^{31}P-NMR spectra are completely different from those of the parent DNA molecules. The interactions between the drug and DNA and between the two bound

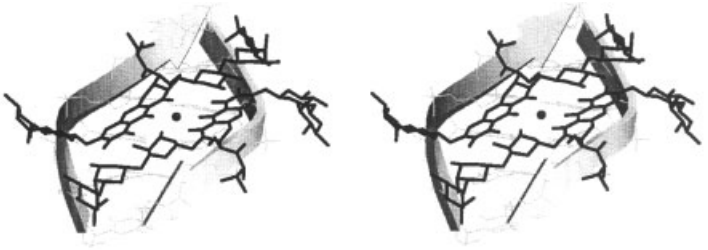

Figure 5. Stereo view of the structure of the ternary complex Mit_2–Mg^{2+}–$(TCGCGA)_2$ obtained with 2D-NMR techniques (Protein Data Bank ID 146D.pdb). The complex has a 2-fold symmetry with the two drug molecules residing in the minor groove of the DNA duplex.

drug molecules in the $drug_2$–Mg^{2+}–$(DNA)_2$ ternary complexes have been revealed with 2D-NMR techniques, from which the structures have been built as illustrated in Figure 5.

When a DNA sequence contains two GC sites separated by a few base pairs, such as the decanucleotide $(TAGCTAGCTA)_2$, binding of two equivalents of $(drug)_2$–Mg^{2+} complex to the DNA becomes possible.[108] The introduction of two Mit drug molecules and one Mg^{2+} to this decanucleotide forms a complex with half of the DNA molecule bound with the drug complex, i.e., the $(TAGCTA\ldots)_2$ moiety on one end of the helix is bound with the drug complex whereas the same moiety on the other end is not. This binding mode breaks the symmetry of the palindromic DNA sequence, which results in doubling the number of NMR signals. Upon the addition of another equivalent of Mit_2–Mg^{2+} recovers the palindromic symmetry of the complex. The structure of this unique ternary complex $(Mit_2$–$Mg^{2+})_2$–$(TAGCTAGCTA)_2$ has been determined by the use of 2D-NMR techniques and molecular dynamic calculations, which can be retrieved from the Protein Data Bank (PDB ID 207D.pdb). The above studies laid a good foundation for future studies of aureolic acid binding to DNA of different lengths and sequences.

5. Paramagnetic (Aureolic Acid)$_2$–Co^{2+}–(DNA)$_2$ Terminal Complexes

The diamagnetic Mg^{2+} can be replaced with a paramagnetic Co^{2+} for the binding of aureolic acid to DNA duplex to afford a ternary $drug_2$–Co^{2+}–$(DNA)_2$ complex.[91a] Because of the paramagnetism of Co^{2+}, protons near the metal center are hyperfine-shifted[64] to afford a ^{1}H-NMR spectrum with a wide spectral window of ~100 ppm as represented in Figure 6 for the complex Mit_2–Co^{2+}–$(ATGCAT)_2$.[91a] There are more than 50 signals well resolved in the large spectral window,

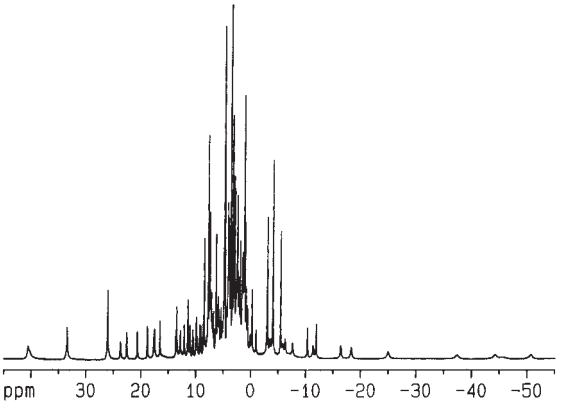

Figure 6. Hyperfine-shifted ^{1}H-NMR spectrum (360 MHz) of the ternary complex Mit_2–Co^{2+}–$(ATGCAT)_2$ at pD 8.0 obtained at 40°C.

representing a rare ''high resolution'' ^{1}H-NMR spectrum of a paramagnetic species. The good signal resolution allows further extensive study of this complex. The ternary complexes $(ChrA_3)_2$–Co^{2+}–$(TTGGCCAA)_2$ and other complexes of longer oligonucleotides[109] allow nuclear Overhauser effect (NOE)[57] to be clearly detected for better signal assignment. However, information about through-bond nuclear interaction cannot be obtained because of the large signal widths of the hyperfine-shifted signals attributable to large molecular size of the ternary complex. Nevertheless, combining the distance constrains derived from nuclear relaxation times and the geometry-related dipolar shift,[64] a structure of the ternary complex has been constructed (Protein Data Bank ID 1EKH.pdb and 1EKI.pdb)[109] which is similar to the structures derived from the previous 2D-NMR studies of the diamagnetic Mg^{2+} complexes of this antibiotic family discussed above.

C. Streptonigrin

Streptonigrin (SN, also known as rufochromomycin and bruneomycin) is a metal-binding quinone-containing antibiotic produced by *Streptomyces flocculus*[110] (Fig. 7). This antibiotic has been shown to inhibit several tumors and cancers (e.g., lymphoma, melanoma, and breast and cervix cancers) as well as viruses in some early *in vitro* and clinical observations.[111,112] While SN is active toward mammalian cells at the chromosome level, it is found to be much less effective against insect cell lines.[113] A recent study shows that SN also exhibits ionizing radiation-like damage toward *Ataxis telangiectasia* heterozygote cells.[114] Despite the potency of SN, high toxicity and serious side effects of this antibiotic have reduced its clinical value, and limit its use only as an experimental anti-tumor agent.[111,112] Nevertheless, because of its anti-tumor potency and unique structure, SN has served as a lead drug molecule for chemical modification and synthesis of new compounds to correlate the structure features with the biological activity and toxicity of this potent antibiotic.[115]

1. Action of Metallo-SN

SN is known to bind different transition metal ions to function properly.[116,117] The interaction of metal–SN complexes with DNA has been proposed on the basis of some optical studies.[118] A redox active metal ion such as Fe and Cu is required for this antibiotic to exhibit full antibiotic and anti-tumor activities.[119,120] The redox-active Fe and Cu complexes have been shown to accelerate SN-mediated DNA scission in the presence of NADH, thus enhance the anti-tumor activity of this antibiotic.[121–123] These results indicate that metal ions are possibly directly involved in the action of SN. However, the metal binding mode and structure of these metal complexes could not be definitely determined in these studies. Particularly, two different configurations of the drugs are possible for metal binding (Fig. 7) with the metal bound through either the quinolinequinone-amine functionalities based on the crystal structure[124] or the quinolinequinone-picolinate functionalities that requires a significant twist of the crystal structure.

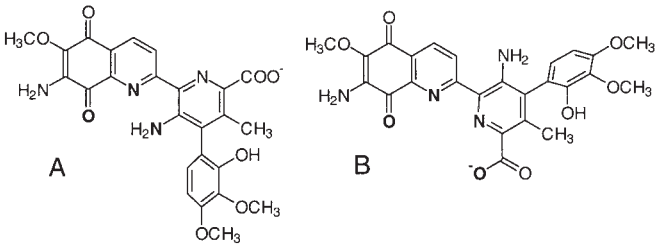

Figure 7. Schematic structures of streptonigrin (SN). The structure **A** is metal-free drug determined by means of crystallography, whereas the structure **B** represents the configuration upon metal binding as determined by means of NMR relaxation. The formation of structure **B** requires a dramatic twist of the C2–C2′ bond in structure **A**.

Since SN contains a quinone moiety, it may share some common mechanistic characteristics with other quinone-containing antibiotics[125] such as the ACs (discussed in Section 2.D "Anthracyclines") in terms of *in vitro* and *in vivo* DNA and RNA cleavage and inhibition of cancer growth via interference with cell respiration and disruption of cell replication and transcriptional control.[116,119,126] The metal–SN complexes can be reduced to their semiquinone forms by NADH, which then can induce cleavage of DNA. This process is inhibited by superoxide dismutase and catalase, indicating the involvement of superoxide and peroxide.[119,121] Reduction of this antibiotic in the presence of a bound metal ion is also confirmed by the detection of EPR signals attributable to the reduced semiquinone form.[127] Metal chelators and an antioxidant are found to prevent SN-induced DNA damage and cytotoxicity,[128] which supports the involvement of metal ions in the action of SN.

2. Metal Complexes of SN

Zn^{2+} binds SN to afford a few different complexes with different metal binding modes at various temperatures, in which a 1:1 metal–drug complex is the predominant complex.[129] A recent study of the crystal structure of a Zn^{2+} complex that mimics the metal-binding moiety of SN showed the binding of the metal to the quinolinequinone-picolinate functionalities,[130] corroborating the structures of several paramagnetic metal complexes of the drug determined by means of NMR techniques discussed below. The interaction of Zn^{2+}–SN with DNA and oligonucleotides has been investigated with ^{1}H- and ^{31}P-NMR spectroscopy. This study concluded the requirement of metal ion for SN binding to DNA[131] and revealed sequence preference in DNA binding of this antibiotic, in which the binding of Zn^{2+}–SN to $d(GCATGC)_2$ shows noticeable spectral changes whereas the complex does not affect the spectra of $d(ATGCAT)_2$.

SN can bind several different paramagnetic metal ions, including Co^{2+}, Fe^{2+}, and Yb^{3+} ions, with large formation constants to form 1:1 metal–SN complexes.[132] The paramagnetic Fe^{2+}, Co^{2+}, and Yb^{3+} complexes of SN have been studied with ^{1}H-NMR spectroscopy and relaxation, and their structures have been determined.[132] The study of Fe^{2+}–SN complex is particularly important since it is considered an active form of this drug that exhibits enhanced activity toward DNA destruction both *in vitro* and *in vivo*.[122] The hyperfine-shifted ^{1}H-NMR signals of these paramagnetic complexes have been fully assigned. The proton-metal distances derived from the relaxation times of the hyperfine-shifted signals in these complexes match those of the complex with the metal located at the quinolinequinone-picolinate site (structure **B**, Fig. 7), but not the quinolinequinone-amine site based on the crystal structure (structure **A**). This configuration requires a significant twist of the C2–C2′ bond by $\sim 180^{\circ}$ in the crystal structure[124] of the drug.

The introduction of poly[dA-dT] to reduced Cu^{+}–SN complex causes some small changes in chemical shift of the ^{1}H-NMR signals of the complex (0.22–0.31 ppm), which was suggested to be attributed to the binding of this complex to the DNA duplex.[123] The hyperfine-shifted ^{1}H-NMR signals of Co^{2+}–SN complex are found to be significantly changed upon addition of calf thymus DNA or poly[dA-dT] (the chemical shifts of two hyperfine-shifted signals are shifted by 20–40 ppm),[132] which are also indicative of direct binding of the complex with DNA. Along with the DNA binding study of Zn^{2+}–SN complex, these studies indicate the significance of metal ions in the action of this antibiotic.

D. Anthracyclines

Anthracycline (AC) antibiotics[133] are produced by *Streptomyces* species. Soon after their discovery, they were found to exhibit a wide spectrum of antineoplastic activity toward both solid and hematologic tumors and cancers.[134] In addition, an AC antibiotic has recently be found to exhibit antifungal activity.[135] Despite their severe cardiotoxicity[136] (e.g., cardiomyopathy) and other side effects,[137] these antibiotics have been widely used as dose-limited chemotherapeutic agents for the treatment of human cancers such as acute leukemia. The side effects have been attributed to the

toxicity of these drugs toward mitochondria,[138] leading to disturbance of bioenergetics, inhibitions of enzymes, oxidation of lipids, disorders of membrane, and oxidative stress. The less toxic adriamycin (doxorubicin) has currently been widely prescribed as a chemotherapeutic agent in association with other antineoplastic agents, such as BLM and cisplatin. In addition, new AC antibiotics and their chemical derivatives are still found or synthesized,[139,140] which may provide potential clinical use in the future.

The antineoplastic activity of AC antibiotics has been mainly attributed to their strong interactions with DNA in the target cells. The AC family members possess a quinone-containing chromophore and an aminoglycoside side chain.[133] The structures of the representing members of this family daunomycin (daunorubicin) and adriamycin are shown in Figure 8.[141] There are a few members of the AC family that contain more extensive sugar chains, such as β-rhodomycin contains a monosaccharide and a trisaccharide, cinerubins, marcellomycin, and rhodirubins have a trisaccharide, and musettamycin has a disaccharide chain.[133] The redox activity of the AC ring plays a key role in the action of these drugs. In addition, the metal ion bound to the 11,12-β-ketophenolate site is also thought to be involved in some actions of these antibiotics.

1. Action of AC and Metal–AC Complexes

The action of this drug family has been considered to be attributable to their redox activity and DNA-binding capability.[133,142,143] Two pathways have been proposed for these drugs to deform DNA structure and terminate biological function of DNA:[2] (a) intercalation of the drugs into the base pairs with the sugar chain sitting in the DNA minor grooves which involves hydrogen bonding, electrostatic, van der Waals, and hydrophobic interactions (a representing AC–DNA structure is shown in Fig. 9); and (b) a free radical damage of the ribose. The intercalation of AC drugs to DNA dramatically distort the DNA structure which thus prohibits transcription. A number of crystal[144] and NMR[145] structures of different AC–DNA complexes have been resolved. A representing AC–DNA structure nogalamycin$_2$-d(TGTACA)$_2$ is shown in Figure 9.[144h] These structural studies allow detailed comparison of sequence specificity of the drug binding and the different modes for the binding of different drugs with DNA.

These antibiotics can be reduced to their semiquinone forms by biological reducing agents, such as NADH and NADPH. Superoxide anion radical ($O_2^{\bullet -}$) and H_2O_2 can be produced from dioxygen upon receiving electrons from the semiquinone. Then, hydroxyl radicals can be generated, which can attack cell components, such as membrane and DNA, and impair cell functioning. In the presence of ascorbic acid and H_2O_2, hydroxyl radicals can also be generated by Cu^{2+} and Fe^{3+}–adriamycin.[146] The radicals generated during the redox cycle of ACs and their Fe complexes have been considered the cause of the cardiotoxicity.[147] However, a recent study showed that the capability of producing free radicals of ACs is not directly related to their cardiotoxicity. For example, although the 13-hydroxy derivatives of ACs are more cardiotoxic, they are less effective producers of oxygen-based free radicals.[148] The 13-hydroxy metabolites of ACs have been found to impair intracellular iron homeostasis, which provides new perspectives on the role of iron in cardiotoxicity of ACs.[149]

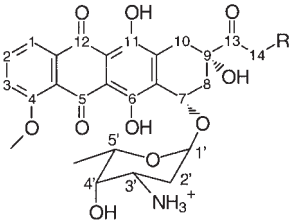

Figure 8. Schematic structures of daunomycin (R = H) and adriamycin (R = OH).

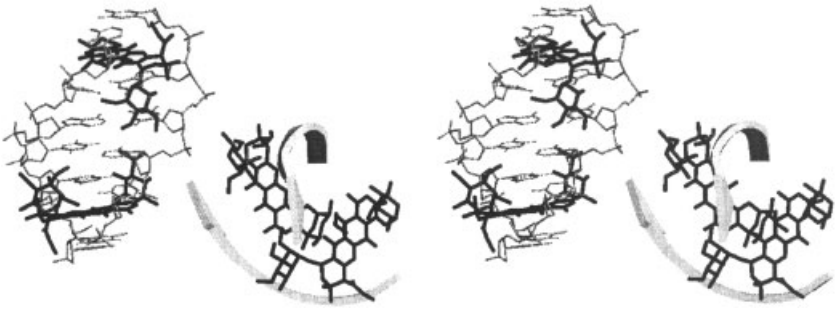

Figure 9. Crystal structure of nogalamycin$_2$–d(TGTACA)$_2$ complex in which the anthracycline (AC) rings are intercalated into DNA base pairs. The complex is packed in the crystal lattice as a dimer with the monomers nearly perpendicular to each other. The DNA duplex is shown in ribbon structure in one of the subunits.

ACs are known to bind various metal ions, including transition metal, main group, lanthanides, and uranyl ions.[150,151] A number of articles reported that some metal ions, e.g., $Fe^{2+/3+}$, $Cu^{+/2+}$, Pd^{2+}, Pt^{2+}, and Tb^{3+}, play an important role in altering the biochemical properties of ACs.[152–155] These studies point a new direction in the pursuit of chemotherapeutic efficacy and lowering toxicity of these antibiotics. The binding of metal ions may cause a significant influence on the redox property of these drugs as shown in their Yb^{3+} complexes,[156] thus affecting their activities. The interactions of DNA and other cell components with metal–AC complexes, and their subsequent damage by the AC complexes of redox-active metal ions, including iron and copper,[157,158] have been previously studied by the use of various physical and biochemical methods. Adriamycin has been suggested not to undergo flavo-associated reduction upon intercalation.[159] However, a site-specific modification of DNA bases suggests a possible binding through intercalation,[157b] although specific electrostatic interactions cannot be completely ruled out. Pulse radiolysis studies indicate that adriamycin semiquinone can mediate a long-range electron transfer to as far as ~100 base pairs in DNA,[160] which may also serve as a mechanism toward DNA base modification.

2. Fe–AC Complexes

It has been shown that several different metal ions, including alkaline earth metals,[161] first-row[161] and heavy[154] transition metals, and rare earth metals,[155,161,162] can bind AC antibiotics very tightly in aqueous and methanol solutions, with the metal bound to one or both of the two β-ketophenolate moieties depending on the solution conditions. Iron is involved in the actions of several antibiotics, such as BLM discussed in Section 2.A ''Bleomycin'', SN in Section 2.C ''Streptonigrin'', and possibly ACs,[153] which serves as a redox center and can generate free radicals in the presence of dioxygen under reduction conditions which can damage cell components. The binding of Fe^{3+} with daunomycin has been studied by the use of ^{57}Fe Mössbauer, EPR, and X-ray absorption spectroscopies, in which several different complexes are seen at mM drug concentrations.[153a,163] Despite the similar structures of daunomycin and adriamycin, the Mössbauer spectra of their Fe^{3+} complexes are noticeably different which has been attributed to their slight difference in structure and reactivity.[164]

The binding of Fe^{3+} with several other ACs has recently been revisited.[165] The results suggest that Fe^{3+} binds these drugs to form 1:1 Fe–drug complexes with the metal bound at 11,12-β-ketophenolate site, and 2:1 Fe_2–drug complexes with the metal bound at both β-ketophenolate sites. The formation of mononuclear, dinuclear, and polynuclear metal–AC complexes are also suggested. The Fe^{3+} complexes of these drugs are very complicated systems since their spectra are dependent upon the preparation procedure, equilibrium time, metal-to-drug ratio, and drug concentration.[163–165]

Different complexes are also formed for lanthanide(III) binding with AC antibiotics observed in an early study, which is discussed in the next section.

A 1:2 Fe^{3+}–adriamycin complex was proposed to form a stable complex with calf-thymus DNA in solution. This drug–Fe–DNA tertiary complex is distinct from both the Fe^{3+}–adriamycin complex and the DNA-intercalated Fe^{3+}-free adriamycin on the basis of optical and chromatographic studies.[157b] In another study, Fe^{3+}–ACs have been suggested not to intercalate into DNA base pairs until the Fe^{3+} ion is released, despite the strong binding of Fe^{3+} with the drugs.[157c] A recent mutagenesis study indicated that Fe^{3+} is directly involved in the mutagenicity caused by doxorubicin through oxidative DNA damage, which further strengthens the role of Fe in AC action.[166] Formation of intracellular Fe–AC complexes have also been confirmed with different methods.[167] Further studies are still needed to clarify the mechanistic and structural roles of Fe in the action of this family of antibiotics.

3. Lanthanide–AC Complexes

Lanthanide(III) ions (Ln^{3+}) have been very widely utilized as substitutes and spectroscopic probes[168] for biological Ca^{2+} owing to their very similar ionic radii, binding properties, and coordination chemistry, yet with much higher affinity constants because of the higher charges of Ln^{3+} ions (thus is able to probe weak Ca^{2+} interactions).[169] Indeed, both Ln^{3+} and Ca^{2+} ions have been reported to bind ACs, in which Ln^{3+} ions show >3 orders higher in affinity constants.[156] Early NMR studies of the paramagnetic Yb^{3+}–daunomycin complex did not yield useful information for the description of the coordination chemistry of the complex because of the formation of a mixture and the lack of full assignment of the paramagnetically shifted ^{1}H-NMR features.[170] The binding of several Ln^{3+} ions, including Pr^{3+}, Eu^{3+}, Dy^{3+}, and Yb^{3+}, with ACs in both aqueous and methanol solutions under different conditions has recently been revisited by means of electronic spectroscopy, cyclic voltammetry, and NMR techniques.[156,171]

Like in the case of Fe^{3+}-bidning to AC drugs, different complexes are also formed for lanthanide binding with ACs. A 1:1 Yb^{3+}–daunomycin complex has been successfully prepared in solution, and its hyperfine-shifted ^{1}H-NMR spectrum fully assigned by means of 2D-NMR techniques (Fig. 10).[156,171] On the basis of the conclusive signal assignment, the configuration of the complex in solution has been determined to be similar to that of the metal-free drug in solution[172] and in the crystal structure,[173] and the metal binding site determined to be the 10,11-β-ketophenolate moiety. The AC drugs can bind Ln^{3+} to form complexes in solution with metal-to-drug ratios of 1:1, 1:2, 1:3, and 2:1 depending upon proton activity in the solution.[156] All of the complexes have been characterized by means of 2D-NMR techniques.[156] The complication in the earlier NMR studies has been attributed to the formation of the different complexes at different proton activities, which is likely to be the case for other metal complexes of the ACs.

4. Interactions of ACs and Their Metal Complexes With Other Biomolecules

In addition to their DNA intercalation and redox activity, AC antibiotics have been observed to interact with other biomolecules that may also influence cell functioning and may be the cause of the side effects of these drugs. For example, (a) adriamycin and its Fe^{3+}, Cu^{2+}, and Co^{2+} complexes can cause influence on effector cells of humoral and cell immune response.[174] (b) Fe–adriamycin complex was found to damage erythrocyte ghost membranes, which is attributable to the production of superoxide and hydrogen peroxide by the complex.[157a] (c) The Fe^{2+}, Cu^{2+}, and Co^{2+} complexes of adriamycin are potent inhibitors of propanolol-induced Ca^{2+}-dependent K^+ efflux, but not Pb^{2+}-dependent K^+ efflux, whereas Fe^{3+}–adriamycin can activate K^+ permeability of erythrocytes. However, the AC rings of adriamycin alone enhances Ca^{2+}-dependent K^+ efflux from erythrocytes. These influences are attributed to the influence on cellular Ca^{2+} transport rather than direct action on K^+ channels.[175] (d) AC drugs can cause poor wound healing[176] as a result of impaired biosynthesis of

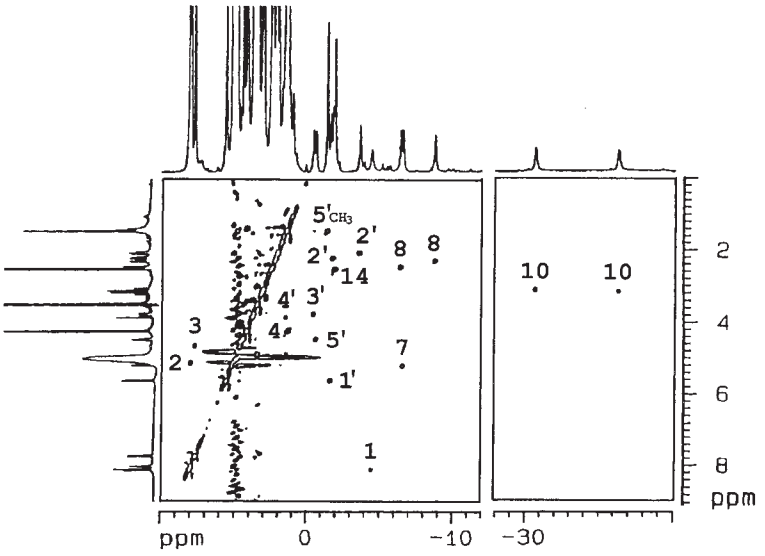

Figure 10. The ^{1}H-EXSY-NMR spectrum of 1:1 Yb^{3+}–daunomycin complex in methanol. The signals due to the metal complex (**top** trace) can correlate with those of the free drug (trace on the **left**) in this spectrum, shown as cross peaks in the "2D map" (labeled with numbers corresponding to the structure in Figure 8). The spectrum obtained in aqueous solution exhibits similar features as in methanol.

collagen.[177] The inhibition of AC drugs against the Mn^{2+}-containing prolidase has been observed to be parallel to the impairment of collagen synthesis.[178] The binding of the AC drugs to the Mn^{2+} in the active site of prolidase has been suggested to be the cause of the inhibition. Moreover, the higher Mn^{2+}-binding affinity of daunomycin than that of adriamycin has been considered to contribute to its greater potency in inhibition of collagen biosynthesis. (e) The Fe^{3+}–adriamycin complex is determined to be a potent inhibitor of protein kinase C,[179] and the Cu^{2+}–AC complexes are considered to serve as a vehicle to carry Cu^{2+} to protein kinase C which results in inhibition of the enzyme.[180] Direct binding of the complexes with the enzyme has been ruled out. These studies suggest that the interactions of AC drugs with different bio-targets must be taken into consideration for further drug design and future studies of the bioactivity and toxicity of these drug family.

E. Aminoglycosides

Aminoglycosides form a unique and structurally diverse family of antibiotics (Fig. 11), which include the famous Waksman's streptomycin and the widely used neomycin (an ingredient in "triple antibiotic" ointment along with bacitracin and polymyxin B). Despite their nephrotoxicity and ototoxicity, these antibiotics have remained their clinical values and also serve as lead drugs for rational design of next-generation antibiotics.[181]

1. RNA-Binding and Aminoglycoside Action

Aminoglycoside antibiotics are known to bind RNA which is considered the key mechanism in their antibiotic activities.[181–183] This binding decreases translational accuracy and interferes with translocation of the ribosome.[184] For example, neomycin-like aminoglycosides bind rRNA near the aminoacyl site, preventing chemical modification on the nucleotides in the aminoacyl site.[185] Neomycin B has been determined to bind to the transactivation-responsive element of HIV-1 RNA.[186] Neomycin has also been determined to inhibit the self-cleavage of the ribozyme from human hepatitis δ virus by direct replacement of the active divalent metal ions.[187] Moreover, aminoglycosides are known to bind and cleave hairpin ribozyme in the absence of Mg^{2+}, however, with much smaller rate

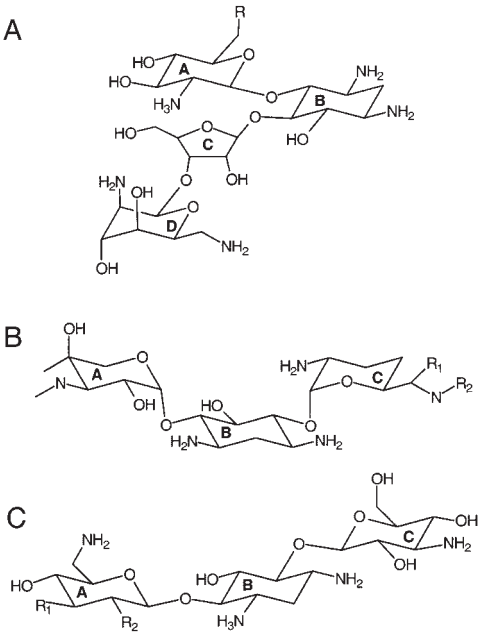

Figure 11. Schematic structures of aminoglycosides (**A**) neomycin B (R = NH_2) and paromomycin (R = OH), (**B**) gentamicin C_1 ($R_1 = R_2 = CH_3$), gentamicin C_2 ($R_1 = CH_3$; R_2 = H), and gentamicin C_{1A} ($R_1 = R_2$ = H), and (**C**) kanamycin A (R_1 = OH; R_2 = OH), kanamycin B (R_1 = OH; $R_2 = NH_2$), and tobramycin (R_1 = H; R_2 = OH).

constants k_{cat} in most cases except neomycin and apramycin of 18 and 13 times smaller, respectively, than that of Mg^{2+}-catalyzed cleavage.[188]

The interaction of aminoglycosides with RNA has been investigated by the use of small RNA nucleotides that contain the drug recognition site.[189] The solution structure of a 27-mer RNA molecule, and the structures of this RNA nucleotide bound with paromomycin and gentamicin have also been determined with NMR spectroscopy.[190] The structures of an *E. coli* decoding region A-site oligonucleotide with and without a bound paromomycin have also been resolved by means of homonuclear and heteronuclear NMR techniques, wherein the two structures are found similar except at the antibiotic binding region.[190,191] Crystal structures of ribosomal 30S RNA subunit[192] and its complexes with paromomycin, streptomycin, and spectinomycin have been resolved (Fig. 12, Top).[193] These structures have provided structural details about the conserved A1492 and A1493 region as well as detailed interactions of aminoglycoside antibiotics with RNA (Fig. 12, Bottom) which afford structural basis for the understanding of the action of aminoglycosides. Another crystal structure of RNA–aminoglycoside complex has also been recently determined, wherein two ribosomal decoding A-sites are bound with two paromomycin molecules.[194] In both solution and crystal structures, the rings **A** and **B** of the antibiotics (cf. Fig. 11) are found to be involved in specific interactions with RNA via H-bonding with G and A nucleotides, whereas rings **C** and **D** in paromomycin and neomycin contribute to the drug binding affinity to RNA. Consequently, methylation of G or A nucleotide can lead to different bacterial resistances to this family of drugs.[195] Nevertheless, different drugs are found to bind at different locations in 30S RNA, which could be metal-dependent (cf. Section 3 for TC binding to RNA).

2. Metal Binding and Bioactivities

Metal ions have been determined to be involved in some unique activities of aminoglycosides. The binding of iron to gentamicin (Fig. 11) has been postulated to induce free radical formation which

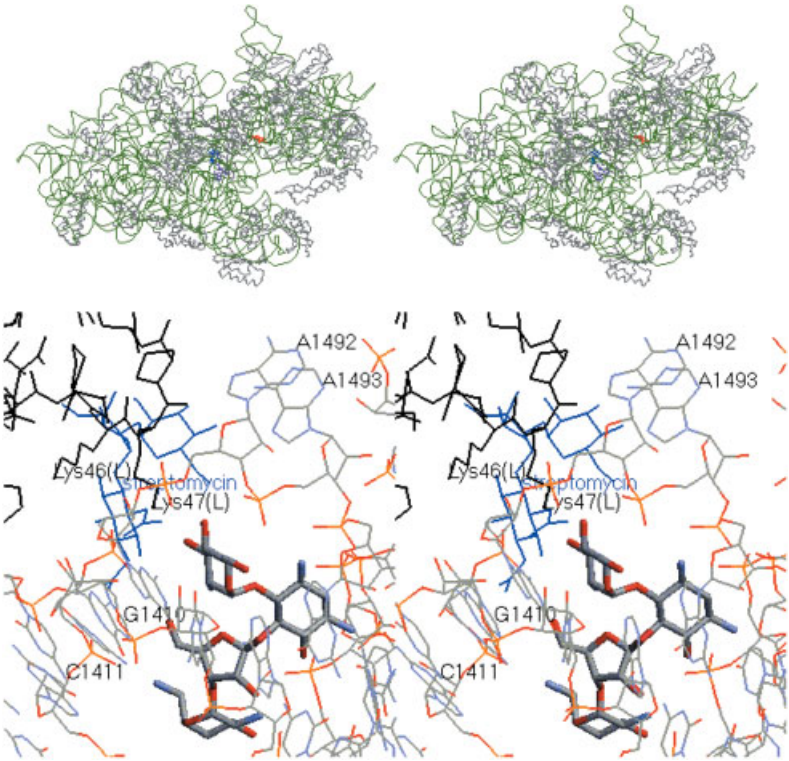

Figure 12. Crystal structure of antibiotic-bound 30S rRNA complex (**top** structure, Protein Data Bank ID 1FJG.pdb) and the details about the paromomycin-binding environment (**bottom**). The RNA molecule is shown in green, proteins in gray, and the aminoglycoside antibiotics paromomycin, streptomycin, and spectinomycin are shown in purple, blue, and red colors, respectively in the top structure. There are 96 Mg^{2+} ions and 2 Zn^{2+} ions (bound to the peptide chain) found in this structure (not shown in the figure); however, the metal ions are not involved in the binding of the antibiotics.

causes peroxidation of lipids.[196] The $Fe^{2+/3+}$ complexes of gentamicin have recently investigated with NMR, in which a low-spin 2:1 drug-to-Fe^{2+} complex as well as a 1:1 and a 2:1 drug-to-Fe^{3+} complexes have been proposed to form.[197] These redox-active iron complexes were implied for aminoglycoside toxicity.

The macrolide antibiotic erythromycin has a structure different from the streptomycin-like antibiotics, yet it contains two sugar moieties (one being a *t*-aminosugar), carbonyl, and hydroxyl groups which potentially can serve as metal binding ligands. An erythromycin–iron complex was observed to exhibit superoxide scavenging activity that was not seen for the antibiotic without the metal.[198] However, the physical and structural properties of the metal binding site and the structure of the complex were not determined in the study.

Several other aminoglycoside antibiotics have been determined to bind Cu^{2+}, including lincomycin,[199] kasugamycin,[200] kanamycin B,[201] tobramycin,[202] genticin,[203] and the semi-synthetic amikacin[204] (Fig. 11). In addition, a few simple amino sugars have also been reported to bind Cu^{2+}, which serve as simple model systems for metal-binding of aminoglycoside antibiotics.[205] In all the cases, the binding of Cu^{2+} to the aminoglycosides are highly pH-dependent, and afford multi-species around neutral pH based on the results from potentiometric and EPR studies. The Cu^{2+}–aminoglycoside complexes are observed to exhibit oxidative activity, which can catalyze oxidation of nucleotides in the presence of H_2O_2.[199–201,204] Hydrolytic cleavage of DNA[206] and RNA molecules[207] and the RNA of the HIV-1 viral Rev response element[207] under physiological conditions by Cu^{2+}–aminoglycoside complexes was also observed. The metal ion in these complexes has been

proposed to bind to the drugs through a chelating vicinal aminohydroxyl binding moiety of the drugs. The binding site of Cu^{2+} in kanamycin A has been determined to be the 3′–NH_2 and 4′–OH groups of ring **C** (Fig. 11C) by means of ^{13}C-NMR relaxation and potentiometric measurements.[201,206]

F. Quinolones

Quinolones are comprised of a large family of antibacterial agents such as nalidixic acid, pefloxacin, norfloxacin, ofloxacin, and ciprofloxacin (Fig. 13).[208,209] The first-generation nalidixic acid is active only against Gram-negative bacteria, whereas the later generations, such as the fluoroquinolones with a fluorine atom on the number 6 carbon (Fig. 13B), have been modified to become effective antibacterial agents which exhibit a broad spectrum of activity highly against Gram-negative bacteria and less active against Gram-positive bacteria and also show significant activity against anaerobic bacteria. Fluoroquinolones have been further modified to produce quinobenzoxazines (Fig. 13C), which are found to show anti-tumor activities (whereas the parent quinolones lack such activities) believed to be attributable to their interaction with topoisomerase II.[210] Ciprofloxacin (Cipro® of Bristol-Myers) is a prototypical fluoroquinolone which has been brought on the stage in recent antibioterrorism reactions. It has become ''the antibiotic of choice'' for fighting against anthrax caused by *Bacillus anthracis* prior to the release of significant amount of toxin by the bacterium, despite the fact that several other antibiotics are also effect against this bacterium.[211] Since this family of drugs have become widely used, one should also bear in mind the risk of serious side effects such as tendinopathy as a consequence of quinolone treatment.[212]

1. Metal Complexes of Quinolones

Quinolones can bind several divalent metal ions, including Mg^{2+}, Ca^{2+}, Mn^{2+}, $Fe^{2+/3+}$, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, and Al^{3+},[213,214] and may result in change in their activity. Mg^{2+} and Al^{3+} were found to decrease the activity of the drugs,[215] whereas Fe^{3+} and Zn^{2+} complexes were found to exhibit higher activities.[216] The crystal structures of the Ni^{2+} and Cu^{2+} complexes of cinoxacin and ciprofloxacin have been solved, in which the metals are found to bind to the α-carboxylketo moiety to form 1:2 metal-to-drug complexes.[214] The complexes have a pseudo-axial symmetry with the two drug ligands bound symmetrically at the equatorial positions. The axial symmetry is also seen in the EPR spectra of Cu^{2+}–$(drug)_2$ complexes.[213e,214c] The drug was also determined by means of crystallography to form a 1:3 Co^{2+}:$drug_3$ complex.[217] A few metal complexes (Fe^{3+}, Cu^{2+}, and Bi^{3+}) of quinolones were prepared in acidic solutions, from which crystals were obtained and structures solved.[218] However, the metal ions in these crystals do not bind directly to the drugs owing to protonation of the carboxylate group, which may not be relevant to the drug action under

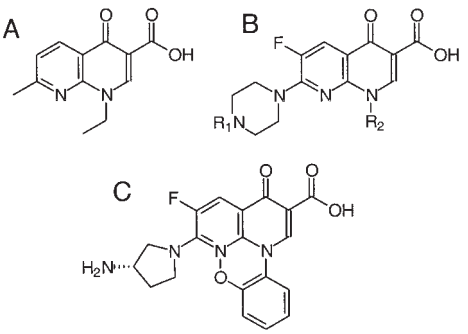

Figure 13. The structures of (**A**) nalidixic acid; (**B**) the prototypical fluoroquinolones (F substitution at position 6) ciprofloxacin (Cipro®); R_1 = H; R_2 = cyclopropyl, norflozacin; R_1 = H; R_2 = ethyl, and pefloxacin; R_1 = CH_3; R_2 = ethyl; and (**C**) a prototypical quinobenzoxazine, A-62176.

physiological conditions. The formation of M^{2+}(quinolone)(2,2′-dipyridine) ternary complexes (M = Co, Ni, and Cu) was observed by means of electrospray ionization and laser desorption mass spectroscopy.[219] A recent theoretical study suggested that metal binding to these drugs is associated with the action of these drugs, and fluorescence quenching measurements indicate the presence of a π–π stacking which has been suggested to be associated with the DNA intercalation capacities of the drugs and their Cu^{2+} complexes.[220]

2. Mechanism of Quinolone Action

The binding of quinolones to DNA-gyrase or topoisomerase IV has been considered the key step in the action of these drugs, which prohibits DNA religation activity and distorts DNA in the complex.[221] Recent studies on the mapping of the functional interaction domain of topoisomerase II revealed that the quinolone-action site on the enzyme overlaps with those sites for the DNA cleavage-enhancing drugs, including etoposide, amsacrine, and genistein.[222] DNA has been considered the target for quinolone drugs, and a cooperative quinolone–DNA binding model of DNA gyrase in the presence of ATP is proposed.[223] Norfloxacin exhibits a Mg^{2+}-dependent binding to plasmid DNA in the absence of the enzymes,[224] wherein metal–drug, metal–DNA, and drug–metal–DNA complexes are detected. The drug does not bind to DNA in the absence or in the presence of an access amount of Mg^{2+}. Intercalation of norfloxacin into DNA is proposed in the study, and Mg^{2+} is proposed to serve as a ''bridge'' for the carboxylate of the drug to interact with DNA. However, DNA unwinding efficiency of ~10° by this drug is only marginal for a weak intercalation.[224,225] Fluorine-19 NMR study of the binding of pefloxacin with double stranded DNA also revealed the participation of Mg^{2+} in the binding.[226] Moreover, the Mg^{2+}-dependent single-stranded DNA binding affinities of several 6-substituted quinolones are found to correlate with the gyrase poisoning activity of these drugs,[227] confirming the involvement of Mg^{2+} in such interaction and the significance of the substitution at position-6 and supporting the mechanism derived from quinolone–DNA interaction.

Quinobenzoxazines have been proposed to bind DNA duplex in the presence of Mg^{2+} to form a ternary complex in the form of $drug_2$–Mg_2^{2+}–DNA, in which one drug molecule is proposed to intercalate into the DNA base pairs while the other is ''externally bound.''[228] The two Mg^{2+} ions serve as salt bridges which interact with both molecules of the drug and the phosphoester backbone of DNA. These drugs have been determined to form a 1:1 or 2:2 complex with Mn^{2+} in methanol by means of Job plot[229] (in which absorption is measured against different metal-to-ligand ratios), which also implies a possible formation of ternary complexes between 2:2 metal–quinolone complexes and DNA. The metal–quinobenzoxazine complex interacts with DNA in a cooperative manner, i.e., a 4:4 metal–drug complex is proposed to interact with DNA as a unit, in which two drug molecules intercalate into DNA base pairs while the two ''external'' drugs have π–π interaction and are expected to interact with the enzyme topoisomerase II or gyrase. The 2:2 metal–drug complex is also suggested to be assembled in the presence of topoisomerase II based on the results from photocleavage assay, the use of mismatch sequences, and competition experiments.[230] The formation of the 2:2 metal–drug complexes suggests that different quinobenzoxazine or quinolone drug molecules should be utilized to form ''hybrids'' for the pursuit of optimal structure–activity relationship.

G. Cisplatin

The antibiotic activities of the platinum complexes *cis*-diamminedichloroplatinum (also commonly known as cisplatin, *cis*-$[Pt^{II}(NH_3)_2Cl_2]$; $R_1 = NH_3$ and $R_2 = Cl$ in Fig. 14) and *cis*-$Pt^{IV}Cl_6(NH_4)_2$ were found serendipitously by Barnett Rosenberg to cause dramatic elongation of *E. coli* during a study of the influence of electric fields on the growth of the bacterium by the use of a platinum electrode in a buffer solution containing NH_4Cl.[231] The abnormal growth of this bacterium was later identified to be caused by the oxidation of the Pt electrode to form the Pt(IV) salt, which was confirmed via chemical synthesis of the compound. In the meantime, the Pt(II) compound *cis*-$[Pt(NH_3)_2Cl_2]$ was

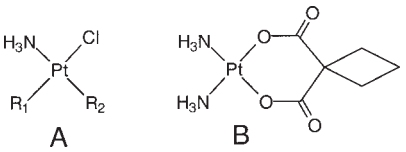

Figure 14. Schematic structures of (**A**) cisplatin ($R_1 = NH_3$ and $R_2 = Cl$) and "transplatin" ($R_1 = Cl$ and $R_2 = NH_3$) and (**B**) the less toxic analogue carboplatin.

also identified to be a potent antibiotic agent, causing the same effect as the Pt(IV) salt. Thus, these synthetic metal complexes can be considered metalloantibiotics from a broad sense of the term as they can inhibit the growth of microorganisms. After its discovery, cisplatin was soon found to be a potent anti-cancer agent and is nowadays one of the most prescribed anti-cancer drugs which has been used for the treatment of several different cancers and tumors, including head and neck tumor and testicular, lung, breast, and ovarian cancers.[232] DNA is considered the main biological target of cisplatin. The coordination chemistry and reactivity of cisplatin and the interaction of cisplatin with DNA have been extensively studied by means of ^{1}H-, ^{31}P-, and ^{195}Pt-NMR spectroscopy and X-ray crystallography, and has previously been reviewed in a number of publications.[233]

1. Cisplatin–DNA Complexes

The chemistry of *cis*-$[Pt(NH_3)_2Cl_2]$ has been thoroughly investigated in the late 1890s by Alfred Werner.[234] The two bound chloride ions in cisplatin are relatively labile, which can undergo exchange with nucleophiles such as amine bases. Upon introduction of DNA, cisplatin binds to the N_7 nitrogen of two adjacent guanidine bases or guanidine–adenine bases in the major groove, or two proximal guanidine bases on different strands in the minor groove which distorts the DNA structures by bending the helix by 40–60° and a helical twist of 25–32°. This binding pattern and structural perturbation on DNA have recently been revealed by means of crystallography[235] and NMR spectroscopy[236] (Fig. 15).

The DNA binding mode of cisplatin cannot be achieved by its stereoisomer "transplatin" ($R_1 = Cl; R_2 = NH_3$ in Fig. 14),[237] in which the two *trans* chloride cannot bind to adjacent guanidine bases as in the case of cisplatin. The lability of the Pt–Cl bond still allows nucleophilic substitution to occur in transplatin which can result in DNA binding. However, the DNA binding of transplatin is significantly different from that of cisplatin, wherein cross-strand linkage becomes predominant.[233,238] The observation of significant cytotoxicity of the *trans* analogues with pyridine in place of the ammonia[239] and high anti-tumor activity of *trans*-imino analogues[240] suggest that "transplatin" analogues are worth further exploration for design of new platinum antineoplastic agents.[237]

2. Cisplatin Conjugates

Cisplatin has been linked to bioactive molecules to form conjugates which exhibit unique properties in terms of DNA binding and anti-tumor activity. For example, adriamycin (Section 2.D "Anthracyclines") can form complexes with $PtCl_4^{2-}$ to afford cisplatin-like complexes,[154a] such as *cis*-dichloro-*t*-butylamine-adriamycino-platinum which has been determined to be active against murine carcinoma and leukemia.[154b] This complex has been suggested to interact with DNA by intercalation of the AC rings rather than covalent binding to the Pt center. A hormone-anchored cisplatin complex has been prepared in which testosterone is bound to cisplatin in place of the diammine groups via a thiosemicarbazone linkage.[241] This conjugate exhibits a higher activity than cisplatin against human breast cancer cell line MCF-7. The binding of cisplatin with proteins, including serum albumin[242] and transferrin,[243] has also been reported which is considered to play important role in the metabolism and bioactivity of this drug. The interaction of proteins with cisplatin may possibly mediate cell response to the drug, which has been recently reviewed.[244]

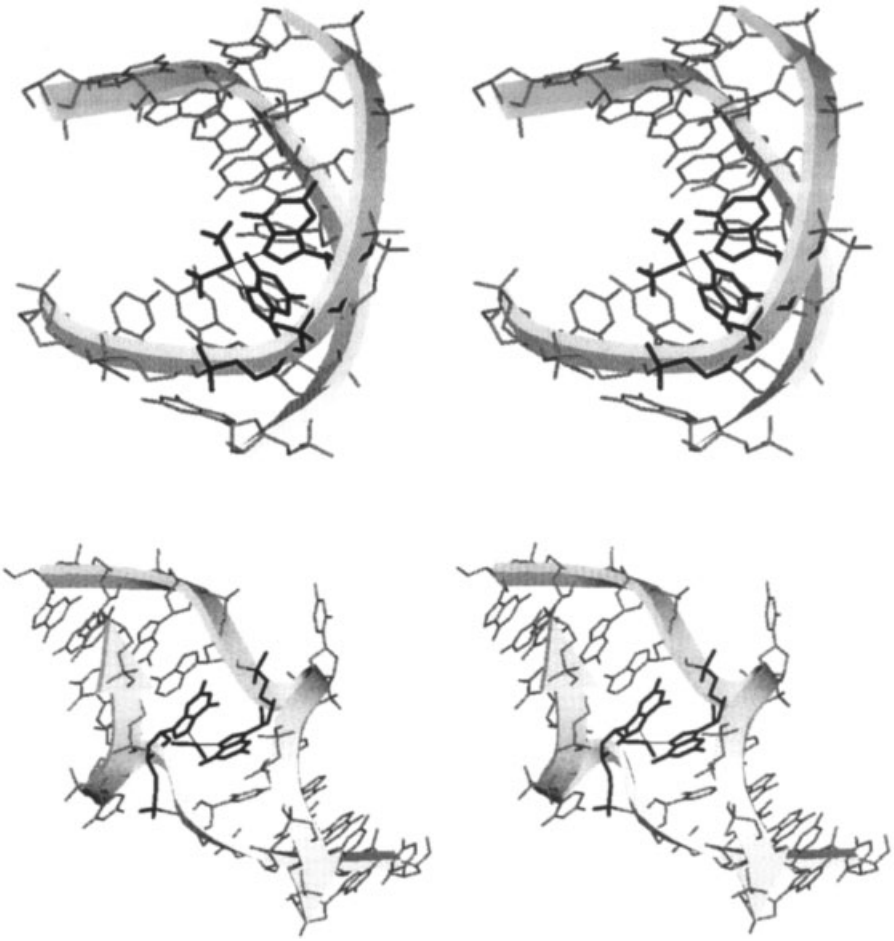

Figure 15. Stereo view of the structures of d(CCTGGTCC)•d(GGACCAGG) obtained with NMR spectroscopy (**top** structure; Protein Data Bank ID 1AU5.pdb), in which cisplatin is bound at the center GG nucleotides of one strand, and d(CCTCGCTCTC)•d(GGAGCGAGAG) obtained with X-ray crystallography (**bottom** structure; Protein Data Bank ID 1A2E.pdb), in which cisplastin is bound to the center G from each strand. The binding of cisplatin to DNA significantly distorts DNA structure, particularly in the case of the cross-strand binding.

3. Cisplatin Analogues

A large number of cisplatin-like compounds have been synthesized, their molecular properties thoroughly characterized, and their anti-tumor activities evaluated.[233,245] Of these new analogues, the compound carboplatin (*cis*-diammine-cyclobutane-1,1-dicarboxylatoplatinum, Fig. 14B) exhibits lower toxicity than cisplatin and has currently been used clinically for cancer treatment. The other compounds such as nedaplatin (*cis*-diammine-1-hydroxoacetatoplatinum) and oxaliplatin (1,2-diaminocyclohexane-oxalatoplatinum) also exhibit potential antineoplastic activities, which have gained approval for clinical use in some countries and are under extensive evaluation.[233,245,246]

Several cisplatin analogues with two Pt centers have recently been prepared, possessing a general formula of $[(trans\text{-}PtCl(NH_3)_2)_2\text{-}\mu\text{-}L]^{2+}$ in which L is a diamine linker.[247] Because of the presence of two DNA-binding motifs in each molecule, binding of these dinuclear platinum complexes to DNA duplex affords intrastrand and/or interstrand cross-link, wherein the bending of DNA at the binding site is much less than that caused by cisplatin.[247,248] These dinuclear platinum compounds exhibit anti-tumor activities differently from cisplatin and may also be different from each other, and are

potential new anti-tumor agents. Analogous compounds with multi-platinum centers have also been prepared and show significant anti-tumor activities and a cellular response different from cisplatin, and have been under clinical trial.[249] The DNA-binding pattern of these new compounds has also been investigated which shows a similar bifunctional manner as dinuclear platinum compounds.[250]

Platinum(IV) complexes have been known to exhibit anticancer activities.[231] Several Pt(IV) complexes have entered clinical trials;[251] however, they have not been widely used because of lower activities than cisplatin or high toxicity and viability of drug uptake, including *cis*,*trans*,*cis*-$[PtCl_2(OH)_2(isopropylamine)_2]$ (iproplatin, CHIP, or JM9),[252] $[PtCl_4$(D,L-cyclohexane-1,2-diamine)] (tetraplatin or ormaplatin),[253] and *cis*,*trans*-$[PtCl_2(OAc)_2(NH_3)(NH_2C_6H_5)]$ (JM216 or satraplatin[254]). The bioinorganic chemistry of Pt(IV) complexes has recently been extensively reviewed.[255]

H. Organometallics

Organometallic compounds are a large family of unique synthetic metal-containing organic compounds, which are characterized by the presence of direct metal–carbon bond(s). Several organometallic compounds have been found to exhibit antineoplastic activities.[256] Of these, the ''metallocene'' compounds $M(IV)Cp_2Cl_2$ (Cp = cyclopentadienyl; M = Ti, V, Nb, and Mo) show significant activities toward several experimental animal tumors and human tumors on nude mice, whereas the Zr and Hf analogues do not show anti-tumor activity.[257] The Ti compound has entered clinical trials.[258] In addition to the metallocenes, there are a number of non-platinum metal complexes which have been extensively studied and tested for their anti-tumor activities and are covered in recent reviews.[233e,256]

Although metallocenes were originally considered to bind DNA similar to cisplatin, recent studies indicated that they do not bind tightly to DNA at neutral pH.[259,260] Nevertheless, DNA is still a binding target of these compounds under certain conditions,[256] as suggested by NMR studies.[261] $TiCp_2Cl_2$ has been suggested to exhibit an anti-tumor mechanism different from cisplatin,[262] showing inhibitory activity toward protein kinase C and DNA topoisomerase II.[259] The hydrolysis of these compounds into $M(IV)Cp_2(H_2O)_2^{2+}$ has been proposed to render their anti-tumor activities.[259] The different pK_a values of the bound water molecules in $MCp_2(H_2O)_2^{2+}$ result in different charges on the compounds, which relates to their capability of entering cells. The high acidity of $TiCp_2(H_2O)_2^{2+}$ with pK_a values of 3.51 and 4.35, which afford a neutral species at pH 7.0, and its reasonable stability with $t_{1/2} = 57$ hr for Cp dissociation[263] may account for its high anti-tumor activity.

MCp_2Cl_2 can form conjugates with adriamycin (Section 2.D ''Anthracyclines'') to give 1:2 metal-to-drug complexes (M = V and Zr) and 1:1 and 1:2 complexes (M = Ti).[264] While the Zr conjugate does not show activity toward P-388 leukemia, the Ti complexes exhibit activity comparable to the free drugs. The structures of these metal conjugates were not determined in the previous study. The metal ions are suggested to assist the binding of the drug to DNA and red blood cell membrane. However, these metal complexes do not catalyze electron transfer from NADH to dioxygen as does adriamycin (Section 2.D ''Anthracyclines''), which possibly may decrease the cardiotoxicity of adriamycin. Thus, these conjugates seem to serve as bifunctional anti-tumor compounds, i.e., to release adriamycin and the $M–Cp_2$ complex.

3. METAL–TC COMPLEXES AND BACTERIAL RESISTANCE

The tetracyclines (TCs) have once been widely used as both external and internal medicines for an extended period of time because of their broad-spectrum activity toward both Gram-positive and -negative bacteria, and also their activity toward rickettsiae, chlamydiae, and protozoans, such as the prototypical TC aureomycin (Fig. 16) produced by *Streptomyces aureofaciens*.[265] The antibiotic activity of TCs is attributed to their binding to the ribosome which inhibits protein synthesis.[266] Their usage has been limited in recent years because of side effects, including staining of teeth and increase

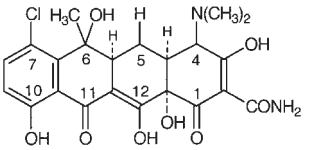

Figure 16. Schematic structure of aureomycin (7-chlortetracycline). Substitute OH for 5-H and H for 7-Cl afford terramycin (5-oxytetracycline).

in bacterial resistance. However, recent studies of the mechanism for bacterial resistance of this drug has afforded new insight into rational design of analogues and searching for new analogues of this broad-spectrum antibiotic family, such as the novel 9-glycylamido derivatives the ''glycylcyclines,'' for defending bacterial infections.[267,268] One of the glycylcyclines 9-*t*-butylglycylamido-minocycline (GAR-936, tigilcycline) is currently under phase II clinical trials.[269]

A. Metal Binding

The metal-binding capability of TCs has been well documented,[150] including the binding with alkaline earth and transition metal ions (VO^{2+}, Cr^{3+}, Mn^{2+}, $Fe^{2+/3+}$, Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}) and Al^{3+}.[270–274] TCs have been determined to be present mainly as Ca^{2+}-bound form (and Mg^{2+}-bound form to a lesser extent) in the plasma when they are not bound to proteins such as serum albumin. Thus, the bio-availability of TCs should be dependent upon the physical and biochemical properties of their metal complexes instead of their metal-free forms. Metal binding to different TCs are found to be slightly different which has been suggested to be correlated to their pharmacodynamic effect.[271b]

The acidic oxy-groups at positions 1, 3, 10, 11, and 12 of TC are the potential metal binding/chelating sites (Fig. 16). The acidity of these groups has been determined to follow the order of 3–OH > 12–OH > 4-ammonium > 10–OH.[150] The 11,12-β-ketoenol moiety has been considered to be the primary metal binding site,[275] which has also been determined to be the Mg^{2+} binding site in the repressor TetR–Mg^{2+}–TC ternary complex (see later). A recent study indicated that TC forms 2:1 TC:metal complexes with 3d transition metal ions in non-aqueous solutions, in which the metal is bound at the 2-amido 3-enol chelating site.[270] Moreover, the formation of metal–TC complexes with different stoichiometries, including 2:1, 1:1, and 1:2 metal:TC ratios, has also been suggested in the previous studies.

B. RNA Binding

The antibiotic activity of TCs is attributed to their binding to the ribosome.[266,276] TCs have been reported to bind different forms of RNA, including the ribosome, bulk RNA, rRNA, and ribozymes. The studies of TC binding and interaction with RNAs have recently been reviewed.[277] The binding of TCs to bulk RNA is not specific, and may not be significant for their antibiotic activity.[278] On the other hand, this family of antibiotics bind to the ribosome at the 30S subunit with K_d of 1–20 mM (in addition to many other low affinity sites). This binding induces a conformational change that prevents tRNA from binding to the ribosome and results in interference of protein synthesis.[279] The interaction of TCs with 16S rRNA has recently been extensively studied with photo-modification, activity assay, mutation, and other methods, from which the TC-binding sites have been identified.[277,280]

The crystal structures of TC-bound small ribosomal subunit have recently been resolved,[281] further confirming the significance of such binding in the action of this antibiotic family. Two TC molecules are found to bind to the RNA (Fig. 17).[281a] One of the TC molecules may involve a Mg^{2+} ion (at the 11,12-β-ketophenolate site that is found in metal binding studies of TC discussed above) in

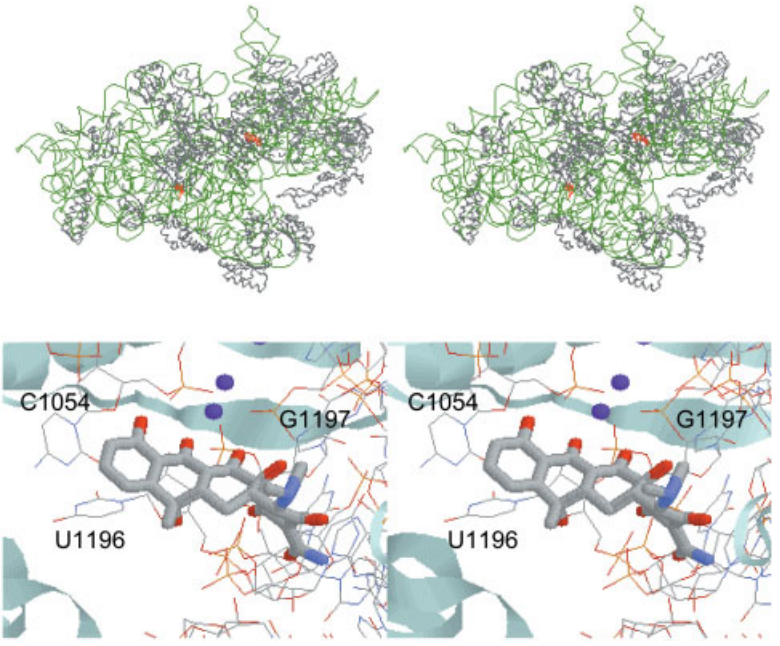

Figure 17. **Top**: The crystal structure of 30S rRNA with two tetracycline (TC) molecules bound (red). There are 96 Mg^{2+} ions found in the structure. The one located near 11,12-β-ketoenol moiety may be involved in the binding of the drug to RNA (**bottom** enlarged binding site of the TC on the **right**), as in the case of the binding of the drug to TetR receptor discussed below.

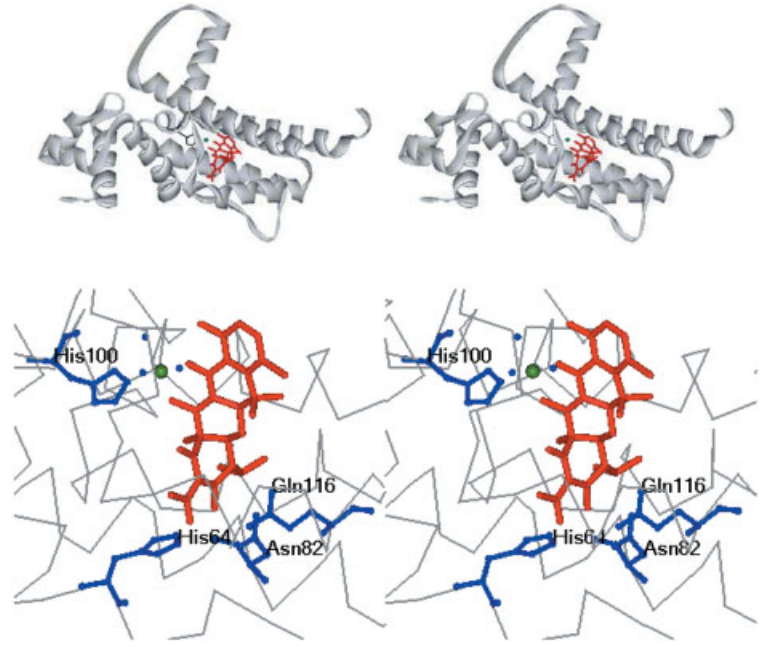

Figure 18. **Top**: Stereo view of one subunit of the ternary complex formed between class D TetR repressor and Mg^{2+}–aureomycin (Protein Data Band ID 2TCT.pdb). The Mg^{2+}–aureomycin complex is shown in red. The DNA-binding domain is located at the N-terminus on the **left**. The 2-fold symmetry of the TC–TetR dimeric complex allows the binding of the complex to the 15-base pair tet-operator. **Bottom**: Structure of the TC biding site in the TetR–Mg^{2+}–TC ternary complex. The drug complex is bound to the protein through His100 via Mg^{2+} (green), and is also H-bonded with the protein through three amino acid side chains.

binding to RNA through the phosphates of C1054, G1197, and G1198 (Fig. 17). The TC molecules occupy the sites that are distinct from those for the binding of aminoglycosides discussed in Section 2.E ''Aminoglycosides'' (cf. Fig. 12).

Inhibition of ribozymes by TCs has been studied, including groups I and II introns, hammerhead ribozyme, a ribozyme from hepatitis delta virus, and *Neurospora crassa* Varkud satellite RNA.[187,282] The concentration for 50% inhibition ($IC_{50\%}$) of ribozymes has been determined to be ~10–500 mM for several different TCs, with hydrophobic TCs showing higher inhibitions. The large $IC_{50\%}$ values indicate that these drugs are weak inhibitors for ribozymes, or may even serve as non-specific inhibitors.[277] The binding sites, the binding nature, the pattern for the inhibition, and the role of metal ions (particularly the RNA-significant Mg^{2+}) in the binding with ribozymes were not revealed in the previous studies.

C. Metal-Dependent Bacterial Resistance

Despite the high potency as broad-spectrum antibiotics, TCs are of little use nowadays because of their bacterial resistance.[265b,283] The predominant TC-resistance mechanism in Gram-negative bacteria is active efflux of the drugs mediated by the antiporter membrane protein TetA which pumps out TC as a Mg^{2+} complex coupled with proton uptake.[284] The expression of TetA is controlled by the repressor protein TetR, whose binding to operator prevents transcription of both *tet*R and *tet*A genes. A conformational change of the TetR repressor is supposed to occur upon binding of TC in the presence of divalent metal ions.[285] The conformational change results in the release of the repressor from the operator and initiates the expression of TetA for active TC efflux. The crystal structures of the repressor TetR and the ternary complex TetR–Mg^{2+}–TC have been resolved which confirm the induction of the conformational change of the repressor upon the binding of the Mg^{2+}–TC complex.[286,287]

A structure of Mg^{2+}–TC-bound TetR is shown in Figure 18(Top). TetR is a dimeric protein with 10 α-helical structures, of which the first three helical bundles from the N-terminus of each subunit serve as the DNA binding site. The *tet*-operator is composed of 15 base pairs shown below, which has a 2-fold symmetry (boxed sequences) with respect to the center T-A base pair.

T	C	T	A	T	C	A	T	T	G	A	T	A	G	G
A	G	A	T	A	G	T	A	A	C	T	A	T	C	C

Upon Mg^{2+}-TC binding, significant conformational changes of TetR are observed, including changes in the drug binding site and the DNA binding site.[286] Significant changes are also observed at helix-9, suggesting that the opening at the C-terminus of helix-9 serves as the entrance for the drug as this opening is significantly narrowed after TC binding.[286,287] Mg^{2+} in the ternary TetR–Mg^{2+}–TC complex is found to bind to the drug at the 11,12-β-ketoenol moiety (as suggested in early metal-binding studies, see above) and to TetR via His100, in addition to three water molecules (Fig. 18).

Fe^{2+} can form a ternary complex with TC and TetR in place of Mg^{2+}.[285,288] An *in vitro* induction assay shows that Fe^{2+}–TC is a stronger inducer of Tet repressor than Mg^{2+}–TC by more than 1,000 times, suggesting that Fe^{2+} may play a role in TC resistance *in vivo*.[288b] Specific sites of cleavage of TetR by the bound Fe^{2+} is achieved in these studies via Fenton chemistry, and have been identified by means of electrospray ionization mass spectroscopy. The cleavages are found to occur at residues 104 and 105, 56 and 136, and 144 and 147 in order of preference. This cleavage pattern is consistent with the geometric locations of the respective residues to the metal center found in the crystal structures. The determination of the roles of metal ion in the binding of TC to TetR and in the structure of the TC–M^{2+}–TetR complex is expected to lead to rational design of TC analogues that exhibit

broad-spectrum antibiotic activities yet devoid of bacterial resistance, such as the glycylcycline family.[267–269]

4. BACITRACIN AND CELL WALL BIOSYNTHESIS

Bacitracin is a metal-dependent dodecapeptide antibiotic excreted by *Bacillus* species, including *B. subtilis* and *B. licheniformis*. It is a narrow spectrum antibiotic directed primarily against Gram-positive bacteria, such as *Staphylococcus* and *Streptococcus*, via inhibition of cell wall synthesis.[289] Currently, this antibiotic is commercially produced in large quantities as an animal feed additive for livestock[290] and in human medicinal "triple antibiotics" ointments (along with polymyxin and neomycin).[291] The historical perspectives, the structure of metallobacitracin, and the structure–function relationship of this antibiotic have recently been reviewed.[292]

A. Congeners and Biosynthesis

This antibiotic is produced as a mixture of many closely related peptides, in which bacitracins A_1 and B_1 are the major components with the most potent activity (Fig. 19).[293] Several congeners of this antibiotic have previously been isolated and characterized by means of amino acid sequence and mass spectroscopy.[293] Bacitracin contains four D-amino acids, including D-Glu4, D-Orn7, D-Phe9, and D-Asp11, and a thiazoline ring formed by condensation of the carboxylate of Ile1 with the $-NH_2$ and the –SH groups of Cys2 (Fig. 19). A cyclic heptapeptide structure is formed via an amide bond linkage between the side-chain ε-NH_2 of Lys6 and the C-terminus of Asn12. These unusual structural features may protect this peptide from degradation by proteases.

Like those structurally diverse peptides and polyketides and their hybrids such as BLM (Section 2.A "Bleomycin"), bacitracin congeners are also nonribosomal products of a large peptide synthetase complex.[294] The structure and mechanism of bacitracin synthetase resemble those of other peptide and polyketide synthetases, which are comprised of a multi-domain modular structure for the catalysis of the initiation of the thioester linkage to the enzyme, elongation of the thioester-linked amino acid, and termination of the peptide or polyketide chain by a thioesterase domain.[77]

Bacitracin synthetase has been known since its early studies to comprise of a complex modular structure as in the case of other peptide/polyketide synthetases. This enzyme catalyzes an ATP-dependent synthesis of bacitracin, starting from the N-terminus based on the observation of a few N-terminal peptidyl intermediates such as Ile-Cys, Ile-Cys-Leu, Ile-Cys-Leu-Glu, and several other N-Ile-containing peptides.[295] The role of ATP has been suggested to be involved in the formation of the labile aminoacyl adenosine intermediates. As in the case of other nonribosomal peptide/polyketide biosyntheses, the synthesis of bacitracin has been suggested to involve thioester-linkages

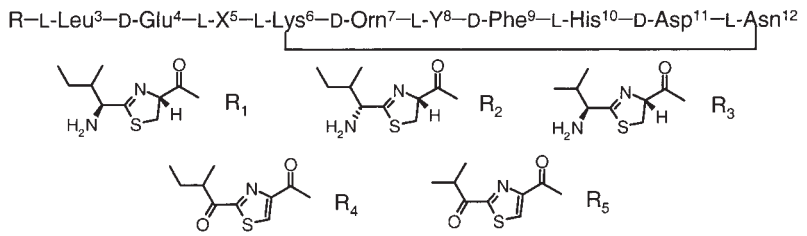

Figure 19. Amino acid sequences and N-terminal structures of a few congeners of bacitracin. Bacitracin A_1 contains Ile^5, Ile^8, and R_1; A_2, Ile^5, Ile^8, and R_2; B_1, Ile^5, Ile^8, and R_3; B_2, Val^5, Ile^8, and R_1; B_3, Ile^5, Val^8, and R_1; F, Ile^5, Ile^8, and R_4; E, Val^5, Val^8, and R_3; H_1, Ile^5, Ile^8, and R_5; H_2, Val^5, Ile^8, and R_4; H_3, Ile^5, Val^8, and R_4; I_1, Val^5, Ile^8, and R_5; I_2, Ile^5, Val^8, R_5; I_3, Val^5, Val^8, and R_4.

based on the observation of covalent peptide–enzyme complexes.[77] The thiazoline ring in bacitracin has been suggested to be synthesized at the stage of Ile-Cys formation on the basis of the detection of the oxidized thiazole product of the Ile-Cys intermediate.[296] The thiazoline ring and analogous thiazole ring are found in a number of peptide antibiotics and siderophores that are synthesized with a similar mechanism.[76,297] An early study revealed that the activity of bacitracin synthetase is affected by Mg^{2+}, Mn^{2+}, Fe^{2+}, and Co^{2+} (Zn^{2+} was not checked) as well as bacitracin,[298] suggesting a feedback control of the synthetase by bacitracin and metal ions.

The bacitracin synthetase operon contains the gene *bacA*, *bacB*, and *bacC* which have been recently cloned and determined to encode three products BA1 of 598 kDa, BA2 of 297 kDa, and BA3 of 723 kDa.[294] BA1 contains five modules to incorporate the first five amino acids, an epimerization domain attached to the forth module for the inclusion of D-Asp4, and a cyclization domain for the formation of the thiazoline ring between Ile1 and Cys2; BA2 is comprised of two modules and an epimerization domain for D-Orn6 incorporation; and BA2 contains five modules for the addition of Ile8-Asn12 with two epimerization domains and the thioesterase domain, consistent with previous studies.[294] A disruption of the *bacB* gene results in a bacitracin-deficient mutant, confirming the involvement of this gene in bacitracin synthesis. Moreover, the expression of a foreign bacitracin synthetase in a host *B. subtilis* results in the production of bacitracin at high level, confirming the functional role of bacitracin synthetase and its association with bacitracin self-resistance genes.[299] The available genes of bacitracin synthetase and other peptide/polyketide synthases/synthetases afford us the tools for possible preparation of different congeners of peptide antibiotics with higher activities for combating bacterial infections.[79]

B. Metal Complexes and Antibiotic Mechanism

Bacitracin requires a divalent metal ion such as Zn^{2+} for its antibiotic activity,[300] and can form a 1:1 complex with several divalent transition metal ions, including Co^{2+}, Ni^{2+}, Cu^{2+}, and Zn^{2+}.[301] The Co^{2+}–bacitracin complex binds tightly to C_{55}-isoprenyl (undecaisoprenyl or bactoprenyl) pyrophosphate with a formation constant of 1.05×10^6 M^{-1}.[302] This binding capability of metallobacitracin presumably prevents the long-chain pyrophosphate from dephosphorylation by a membrane-bound pyrophosphatase, which subsequently inhibits cell wall synthesis because the hydrolytic product undecaisoprenyl phosphate is required to covalently bind UDP-sugars for transport of the sugars during cell wall synthesis.[303] Thus, the binding of metal–bacitracin complexes to undecaisoprenyl pyrophosphate is the key step in the inhibition of cell wall synthesis by this antibiotic since the sugars become unavailable as building blocks during cell wall synthesis. Although the formation of the Co^{2+}–bacitracin–undecaisoprenyl pyrophosphate ternary complex was suggested in previous studies,[302] the structure of different metal–bacitracin complexes and the structure–activity relationship of this antibiotic were not conclusively defined.

C. Coordination Chemistry of Metal Complexes

An early NMR study of Zn^{2+}–bacitracin suggested that His-10 and the thiazoline ring sulfur atom rather than the nitrogen atom were coordinated to the metal,[301] but did not implicate other moieties as metal binding ligands. A later EPR study on Cu^{2+}–bacitracin revealed a slightly rhombic EPR spectrum with $g_x = 2.058$, $g_y = 2.047$, and $g_z = 2.261$ and a large copper hyperfine coupling constants of $A_z = 534$ MHz, typical of a tetragonally distorted Cu^{2+} center.[301b] The detection of clear superhyperfine coupling is indicative of the presence of N ligands. The coordination environment of this complex was suggested to be comprised of ligands from thiazoline ring nitrogen, the His10 imidazole, the D-Glu4 carboxylate, and the Asp11 carboxylate. The results from a recent X-ray absorption spectroscopic study via the examination of the extended X-ray absorption fine structure (EXAFS) of Zn^{2+}–bacitracin in solid form indicated the involvement of three nitrogens and one oxygen in the first coordination sphere with a tetrahedral-like geometry.[304] The ligands are suggested

to be thiazoline nitrogen, His10 imidazole, D-Glu4, and possibly the N-terminal amino group. The binding of metal through thiazoline sulfur is excluded in this study. This study has provided further insight into the metal binding environment of Zn^{2+}–bacitracin and corroborates some previous observations.

The structure of the metal coordination has emerged from the spectroscopic studies discussed above and a recent NMR study of the paramagnetic Co^{2+} complex.[305] The hyperfine-shifted ^{1}H-NMR signals of Co^{2+}–bacitracin complex have been successfully assigned by the use of both 1D- and 2D-NMR techniques as shown in Figure 20. The metal-binding ligands have been conclusively identified in this NMR study, which are assigned to be the N_ε of His10, the carboxylate of D-Glu4, and the thiazoline nitrogen. The N-terminal amino group is not bound to the metal. The identification of several signals attributed to protons near the metal allows a model to be built using relaxation times as distance constrains (Fig. 21). It is interesting to note in the model that a hydrophobic pocket is formed by the side chains of Ile5 and D-Phe9, which presumably can serve as the binding site for the hydrophobic hydrocarbon chain of the sugar-carrying undecaisoprenyl pyrophosphate.

D. Structure–Function Relationship

Further investigation of the Co^{2+} complexes of several other congeners, including the active bacitracins B_1 and B_2 and the inactive stereoisomer A_2 and the oxidized form F (which have been characterized with mass spectrometry and 1D- and 2D-NMR techniques[306]), revealed that a proper metal binding is essential for bacitracin to exhibit antibiotic activity. That is, all the active congeners have similar metal binding properties and coordination chemistry as bacitracin A_1, whereas the metal binding patterns of the inactive bacitracins A_2 and F are different from that of the active forms, in which Glu10 in bacitracin A_2 and both Glu10 and the oxidized thiazole ring in F are not involved in metal binding.[305] This study thus reveals a relationship between the structure, metal binding, and antibiotic activity of this antibiotic.

The structure of metal-free bacitracin has been determined by means of 2D-NMR spectroscopy, which revealed that the side chains of D-Phe9 and Ile8 are in close proximity of Leu3.[307] However, the result from the study of the Co^{2+} complex indicates that D-Phe9 and Ile5 are close to each other.[305] This difference is possibly induced by the metal binding. Bacitracin is known to bind to serine proteases, and the crystal structures of bacitracin–protease complexes have recently been determined.[308] The protease-bound bacitracin has an extended structure, which prevents metal from binding to the antibiotic. This structure of bacitracin is different from both the metal-free and metal-bound forms in solution determined by means of NMR. Bacitracin has also been known to inhibit metalloproteases, presumably because of its metal-binding capability.[309] In addition to protease inhibition, bacitracin can also inhibit a membrane-bound protein disulfide isomerase,[310] and may serve as a selective inhibitor of β1 and β7 integrin following a not yet known mechanism.[311] Thus, the

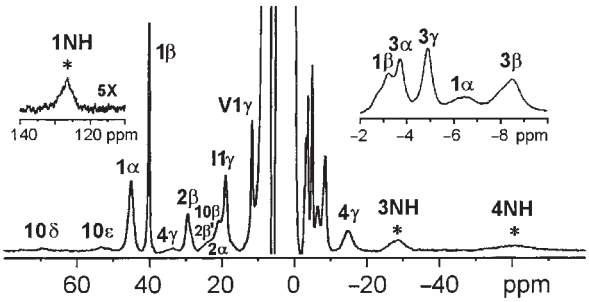

Figure 20. Proton NMR spectrum of the Co^{2+} complex of bacitracin in H_2O at pH 5.5. The signals have been assigned as indicated by the use of 1D- and 2D-NMR techniques.

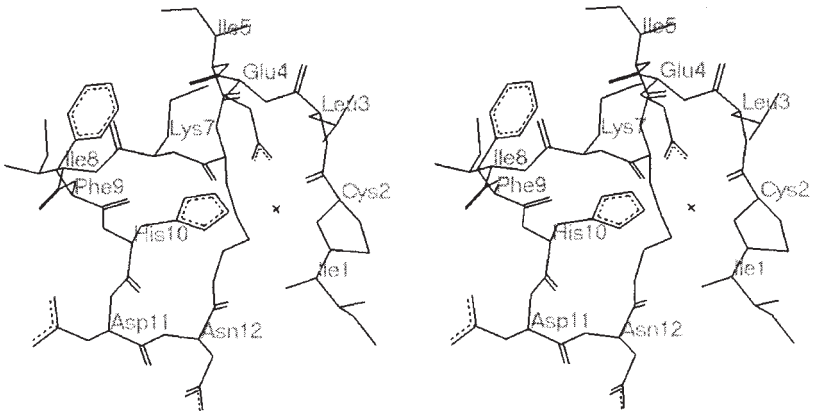

Figure 21. Stereo view of Co^{2+}–bacitracin A_1 produced by means of molecular modeling using nuclear magnetic relaxation rates as distance constraints. The metal ion is coordinated to the drug through the nitrogen of thiazoline ring, the carboxylate of Glu^4, and the ring N_ε of His^{10}. The N-terminal amine is not bound to the metal, but may be hydrogen-bonded to Asn^{12}. The side chains of Phe^9 and Ile^5 are in close proximity and may serve as a flexible hydrophobic binding site for lipid pyrophosphates. This structure is expected to be similar to the structures of the Co^{2+} complexes of bacitracins B_1 and B_2 with high antibiotic activities.

inhibitory property toward proteins may serve as a unique ''alternative activity'' of this antibiotic, in addition to its better documented inhibition activity toward bacterial cell wall synthesis.

5. IONOPHORES AND SIDEROPHORES

Ionophores[3,312–316] and siderophores[317] are relatively small molecules excreted by microorganisms which can selectively bind and transport alkali or alkaline earth metal ions and Fe^{3+}, respectively, across cell membranes and artificial lipid bilayers. These molecules can serve as antibiotics by (a) disturbing the ionic balance across membrane via ion transport (particularly, the transport of alkali and alkaline earth metal ions), such as nactins, lasalocid, and valinomycin, (b) creating pores on membranes which results in leaking of cations through the pores, such as gramicidins, and (c) competing for essential iron in the environment, such as ferrichromes. The antibiotic activity of ionophores have also entitled them to be practically used as growth promoters and for increasing agricultural products.[318]

A. Structure, Cation Binding, and Transport of Ionophores

Cation transport across the membrane by ionophores requires the participation of specific membrane proteins and is strictly regulated. The transport of cations results in disturbance of the ionic balance across the membrane upon release of the bound metal ions. This disturbance may slow down normal cell growth or even cause cell death. This family of cation-binding microbial products can thus be considered antibiotics. As opposed to the metalloantibiotics discussed in previous sections in which the metal ions serve as an integral part of the molecules to exhibit antibiotic activities, the metal ions themselves in metalloionophores serve as the ''magic bullets.'' The release of metal ions from the metalloionophores in the cells can cause imbalance of potential across cell membrane which engenders antibiotic activities of ionophores. In the case of ion-channeling antibiotics, the ''magic bullets'' are transported directly into the cells to result in antibiotic effect. The mechanism of this type of antibiotic activity has been adopted in a recent design of channel-forming antibacterial agents.[319]

1. Structure and Metal Binding

Metal binding to ionophores and siderophores is the key step that allows specific receptors on the cell surface to recognize the metalloforms of the molecules, which results in transport of metal ions across the membrane into the cell. Upon binding of metal ions, conformational changes of ionophores and siderophores may occur.[312] The structure of the metalloforms may vary dramatically, depending on the bound metal ions. In the case of enniatin (which has a cyclic[L-*N*-methyl-valine-D-hydroxyisovalerate]$_3$ structure), the parent ionophore has a structure very similar to its K^+ complex, whereas the Rb_2–enniatin complex has a structure quite distinct from that of the metal-free form.[320] This difference is attributed to the different ionic radii and binding affinities of alkali metal ions with enneatin. In many other cases, the binding of metal ions results in significant changes of the structures of ionophores from more extended conformations to more folded forms on the basis of the crystal structures of nactins (e.g., nonactin, tetranactin, and dinactin) and valinomycin and their metalloforms.[321,322]

A common structural feature of this family of antibiotics is the presence of an O-rich metal binding environment, including ether and ketide linkages, the carbonyl group of esters and amides, and carboxylates. Schematic structures of a few ionophores are shown in Figure 22. Such polyketide structure is synthesized by polyketide synthases via C–C bond (type I and II polyketide synthases) and C–O bond (type III polyketide synthase) formation, analogous to the peptide synthetase for bacitracin synthesis (Section 4) and peptide/ketide synthetase for BLM synthesis (Section 2.A "Bleomycin"). Type III polyketide synthase has been demonstrated to be involved in the synthesis of the C–O formation in the antibiotic nonactin (Fig. 22A).[75m]

Since these O-rich moieties are preferred ligands for alkali and alkaline earth metal ions, the preference in metal binding is thus not because of the ligands but controlled by different mechanisms

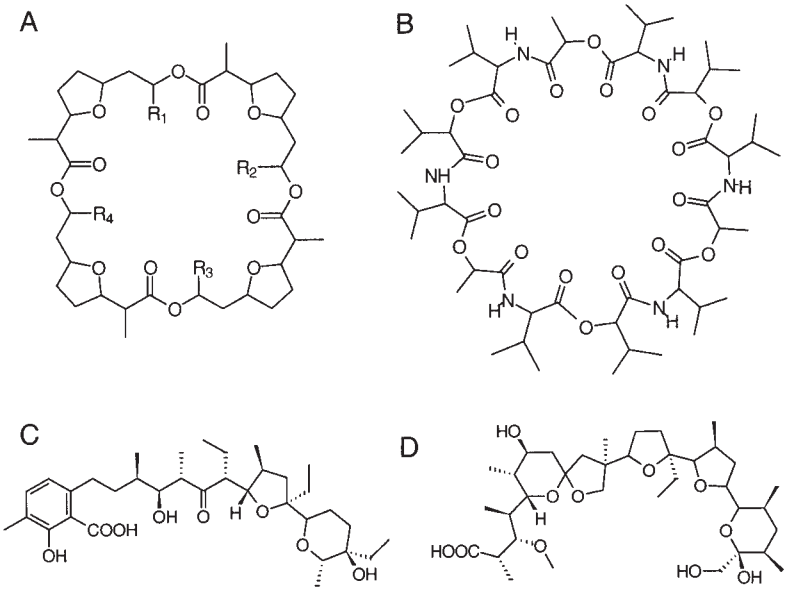

Figure 22. Schematic structures of nactins (**A**): Nonactin (R_1, R_2, R_3, and R_4 = CH_3), monactin (R_1 = C_2H_5; R_2, R_3, and R_4 = CH_3), trinactin (R_1 and R_3 = C_2H_5; R_2 and R_4 = CH_3), and tetranactin (R_1, R_2, R_3, and R_4 = C_2H_5); valinomycin, which has a cyclic structure with three repeating units of (L-Val–L-Lactate–D-Val–D-hydroxylisovalerate) (**B**); lasalocid (**C**), and monensin (**D**).

such as the different molecular structures, the different sizes of the metal-binding site, the different ionic radii of metal ions (i.e., 0.66, 0.95, 1.33, 1.48, and 1.69 Å for Li^+, Na^+, K^+, Rb^+, and Cs^+, respectively), the use of different moieties for metal binding, and/or the different degrees of hydration energy of cations. For instance: (i) while lasalocid with a linear structure has a larger apparent affinity for its Ca^{2+} binding than its K^+ binding in the presence of vesicle membrane,[323] the cyclic nactins exhibit higher preference toward monovalent cation binding and best in the binding with NH_4^+.[314] (ii) The larger binding cavity in nonactin than in tetranactin (Fig. 22A) results in a binding affinity of $Cs^+ > Na^+$ for nonactin, but $Na^+ > Cs^+$ for tetranactin.[324] (iii) The large ''metal binding cavity'' in valinomycin (Fig. 22B) allows its binding with one (e.g., K^+) or two (e.g., Ba^{2+}) metal ions.[312] (iv) X-ray crystal structures show that K^+ is bound to valinomycin at the ''internal binding site,'' whereas Na^+ at an ''external binding site.''[322] (v) While the apparent formation constants for K^+ and Na^+ binding to nonactin in dry acetone are in the same order, that for Na^{2+} is significantly decreased in the presence of water which indicates the importance of hydration in cation binding to nactins.[325]

2. Carboxylic Ionophores

This family of ionophores are comprised of a linear polycycloether backbone and a carboxylate group, as represented by lasalocid and monensin (structures **C** and **D**, Fig. 22). Most of these ionophores bind monovalent cations with a one-to-one ratio and bind divalent cations with a metal:ligand ratio of 1:2, in which the cyclic ether-O atoms serve as metal-binding ligand.[326] Recent FT-IR and ^{7}Li- and ^{23}Na-NMR studies suggest that lasalocid forms a fluxional 1:1 complex with Li^+ and 2:2 complexes with K^+, Rb^+, and Cs^+ ions in solid and in chloroform,[327] and 1:1 and 2:2 complexes with Na^+ in chloroform.[328] The neutral and acid complexes of lasalocid with alkali metals, Tl^+, Ag^+, and alkaline earth metal ions have been studied with ^{1}H- and ^{13}C-NMR, and have been suggested to possess similar structures.[329] Lanthanide(III) (Ln^{3+}) complexes of lasalocid with different metal-to-drug ratios have also been reported.[330] Extraction of water-soluble Ln^{3+}–acetylacetonato (acac) complexes into organic solvent by lasalocid was observed, in which ternary lasalocid–Ln^{3+}–acac complexes are proposed to form with high preference toward smaller Ln^{3+} ions.[331] Metal complexation of lasalocid has recently been reviewed.[332]

3. Gramicidin Family

Gramicidins are a family of peptide ionophores[3,333] produced by *Bacillus brevis* as a mixture of a few congeners with different amino acid compositions, of which Gramicidin A is the major component whose primary structure is shown below.[3]

Formyl-L-Val^1–Gly^2–L-Ala^3–D-Leu^4–L-Ala^5–D-Val^6–L-Val^7–D-Val^8–L-Trp^9–D-Leu^{10}–L-Trp^{11}–D-Leu^{12}–L-Trp^{13}–D-Leu^{14}–L-Trp^{15}–ethanolamine.

L-Trp^{11} in gramicidin A is replaced by L-Phe in gramicidin B, and by L-Tyr in gramicidin C. This family of ion channeling antibiotics exhibit a different mechanism for their action from the cation-carrying ionophores described in the above section. Gramicidins can insert into the lipid bilayer as a dimer and span across the membrane, forming a unique β-double-stranded helix which creates a channel of ~4 Å wide for cations to permeate (Fig. 23).[334] This channel exhibits an cation selectivity of $H^+ > NH_4^+ > Cs^+ > Rb^+ > K^+ > Na^+ > Li^+ > N(CH_3)_4^+$ in 0.1 M salt,[335] yet does not show permeability to divalent metal ions Mg^{2+}, Ca^{2+}, Ba^{2+}, and Zn^{2+}. The divalent metal ions can bind to the β-double-stranded helix structure at the entrance of the channel which prevents the transport of monovalent cations.[336] The trend in the alkali metal binding affinity follows the same order as the relative ionic mobility, ionic radius, and hydration energy of the ions.[3]

The structures of several gramicidin congeners and their metal-bound forms have been determined with X-ray crystallography[337] and NMR spectroscopy in both micelles[338] and solid states[333,339,340] (Fig. 23). Similar configurations are observed for the peptide backbone in both solution and solid states.[337–341] However, deviations are observed among side chains, particularly

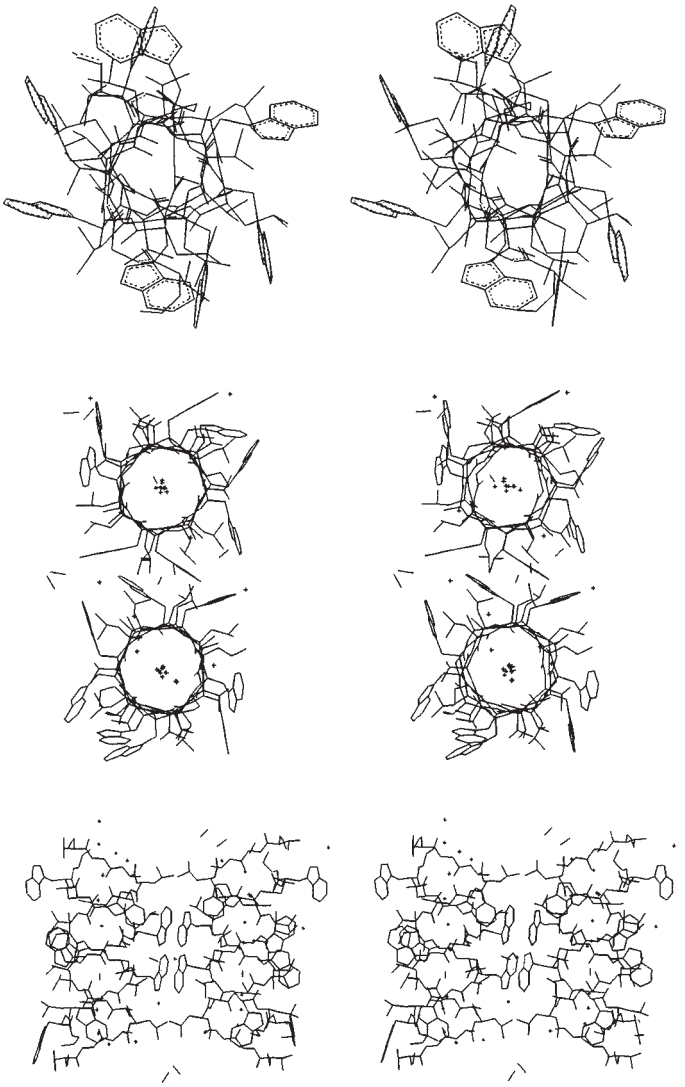

Figure 23. Structures of gramicidin A determined with NMR spectroscopy (**top**, Protein Data Bank ID: 1MIC.pdb) and X-ray crystallography (**middle**, top view; **bottom**, side view, Protein Data Bank ID: 1AV2). Three Cs^{2+} ions located in the channel are found in the crystal structure.

Trp-9. The structures in solution determined with NMR spectroscopy exhibit a higher degree of irregularity than the structures in solid state determined with crystallography, such as the shape of the channel (Fig. 23, top). The binding of monovalent metal ions does not seem to cause significant structural change of gramicidins. The structure similarity of this antibiotic with and without bound cations is quite reasonable since the insertion of gramicidins into membranes does not rely on the binding of metal ions as in the case of other ionophores described above. In addition, the similarity does not create further energy barrier for metal binding and transportation. According to these structures, the hydrophobic amino acid side chains are located outside the channel. This molecular arrangement allows gramicidins to exhibit extensive hydrophobic interaction with membrane upon insertion into the membrane, whereas the carbonyl groups are positioned inside the channel for interaction with and transportation of cations.

The structures of gramicidins A, B, and C in micelles obtained by means of 2D-NMR techniques are very similar, with background atom RMSD $\lesssim 0.5$ Å ascribed to similar structures.[338] The side chains in these three congeners also have similar configurations. Despite their very similar structures and monovalent cation specificity, gramicidins A, B, and C exhibit different cation binding and transporting properties, such as the conductance and activation energy for ion transport. The minor variations in the structures of the three congeners cannot explain these significantly different properties. Change in dipole moment of the side chains (i.e., dipole moments of 2.08, 0, and 1.54 D for Trp, Phe, and Tyr, respectively) was suggested to cause the difference. The decrease in the single channel conductance of Na^+ for several natural and synthetic gramicidin congeners was found to correlate with the replacement of a Trp by Phe, which decreases the dipole moment.[338a]

B. Iron Sequestering and Antibiotic Activities

The extremely small solubility product K_{sp} of $\sim 10^{-38}$ for $Fe(OH)_3$ makes soluble Fe^{3+} in aqueous solutions very scarce. To overcome this low availability of iron under aerobic conditions, microorganisms excrete Fe^{3+}-specific siderophores (Fig. 24) which bind Fe^{3+} with extraordinarily high affinity constants in the range of $\sim 10^{30}$–10^{52} M^{-1} and transport Fe^{3+} into cells via specific receptors.[317,342] Once the Fe^{3+} complexes of siderophores are transported into the cells, the iron can be released upon reduction. In addition to the iron transport activity of siderophores, the Cu^{2+}, Co^{3+}, and Ni^{2+} complexes of the siderophore desferal were found to exhibit an interesting ''alternative bioactivity'' toward the cleavage of plasmid DNA and oligonucleotides[343] which points a new direction for design of new ''chemical nucleases.''

1. Mechanism and Structure of Siderophores

The biosynthesis of siderophores is regulated by cellular concentration of Fe^{2+}.[344] When the concentration is low, Fe^{2+} is dissociated from an ferric uptake regulatory (Fur) protein, resulting in the binding of Fur to the operon that initiates the synthesis and excretion of siderophores for Fe^{3+} sequestering.[342] The Fe^{3+}–siderophore complexes can be recognized by species-specific receptors and transported into the cells. For example, although ferrichrome A serves as an iron carrier for fungi, it does not serve that purpose in bacteria; whereas ferrichrome does.[345] Thus, a rational approach to chemotherapy becomes possible when it is based on iron transport mechanism in microorganisms[317]

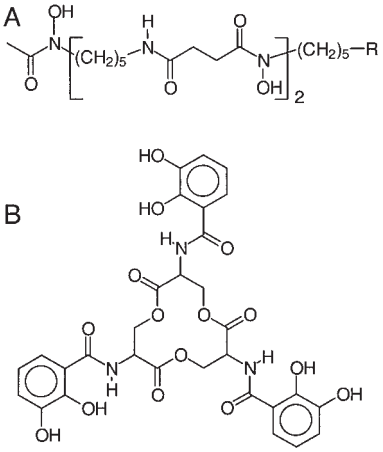

Figure 24. Two prototypical families of Fe-free siderophores: (**A**) hydroxamate-based ferrioxamines B ($R = NH_3^+$), D_1 ($R = NHCOCH_3$), and E ($R = NHCOCH_2CH_2$ that closes the chain to give a 3-fold symmetric ring structure) and (**B**) catechol-based enterobactin.

by (a) controlling iron transport via iron chelating, (b) transport of an antibiotic substance via conjugating with siderophores (e.g., albomycin discussed below), and/or (c) inactivation of siderophore receptors via inhibitor binding.

The crystal structures of several hydroxamate-containing Fe^{3+}-bound siderophores have been resolved which allow a detailed comparison of their structures and correlation of their structures with function. While the crystal structures show that the iron complexes of ferrioxamines B,[346] D_1,[347] and E[348] and desferrioxamine E[349] are mixture of Λ- and Δ-*cis* isomers, ferrichrome complexes[350] are shown to be exclusively the Λ-*cis* isomers. This *cis* configuration seems to be important for the recognition of these siderophores by membrane receptors (see next Section), wherein the ''carbonyl face'' is considered to play an important role since the ''oxime face'' is relatively more shielded than the carbonyl face in the structures of these siderophores.

2. Albomycin Structure and Receptor Binding

Albomycin is a prototypical siderophore antibiotic produced by *Streptomyces*. It is a broad-spectrum antibiotic active against several Gram-positive and -negative bacteria with low minimum inhibition concentrations, and is even active toward those bacteria resistant to penicillin, streptomycin, TC, and erythromycin.[317,351] This antibiotic is a natural conjugate comprised of an iron-binding tri-δ-*N*-hydroxy-δ-*N*-acetyl-L-ornithine site analogous to that of ferrichromes and an antibiotic moiety of thioribosyl pyrimidine (Fig. 25). Albomycin is recognized by the ferrichrome receptor in the outer cell membrane of *E. coli*,[352] and is activated after cleavage by peptidase N to release the antibiotic moiety. Thus, albomycin-resistant bacteria are found to lack either the receptor[353] or the peptidase.[354]

Iron-depleted siderophores are expected to have quite flexible and extended structures. Upon Fe^{3+} binding, the metal binding ligands are held together by the metal and exhibits a compact metal-binding configuration which is recognized by the ferrichrome receptors (cf. Fig. 25). A few structures of the membrane transporter protein FhuA (ferric hydroxamate uptake A protein) with and without a bound Fe^{3+}–sederophore have been determined (Fig. 25).[355] The Fe^{3+} binding moiety of the Fe^{3+} complexes of phenylferricrocin, albomycin, ferrichrome, and ferricrocin have quite similar coordination chemistry upon their binding to FhuA, and are bound to the receptor in a similar orientation and interacts with the same amino acid side chains, including Tyr and Arg side chains. The *cis* configurations of the Fe^{3+}–siderophore complexes bound to the uptake proteins are found to be the same as those of Fe^{3+}–siderophores without bound to the protein.[346–350] Similar interactions and configuration are also observed in the crystal structure of gallichrome–FhuD complex.[356] The binding of Fe^{3+}–ferichrome to FhuA induces a significant conformational change through the N-terminal domain, including an unwinding of helix 2 near the binding site and a ∼11-Å translation of the loop next to it, as well as a 17-Å moving of Trp22 on the oppose side from the binding site. How these changes result in the uptake of the Fe^{3+}–siderophore complexes and transport through cell membrane could not be revealed in the crystallographic studies.

Recently, a semi-synthetic rifamycin analogue CGP4832 was found to bind to and actively transported by FhuA protein, despite its distinctly different structure from those of albomycin and ferrichrome.[357] In addition to FhuA, the membrane transporters FepA (ferric enterobactin transport protein)[358] and FecA (ferric citrate uptake protein which recognizes and transports the dinuclear $Fe(III)_2$–$citrate_2$ complex)[359] are also characterized to contain the 22-stranded β-barrel structure as found in FhuA,[360] with a cross section of ∼35–45 Å. The above studies have provided the molecular basis for rational design of antibiotic siderophores that target bacteria through specific siderophore receptors of the bacteria.

C. Perspectives of Metal Ions in Medicine

In addition to the metalloantibiotics discussed in this review, a number of drugs and potential pharmaceutical agents also contain metal-binding or metal-recognition sites, which can bind or

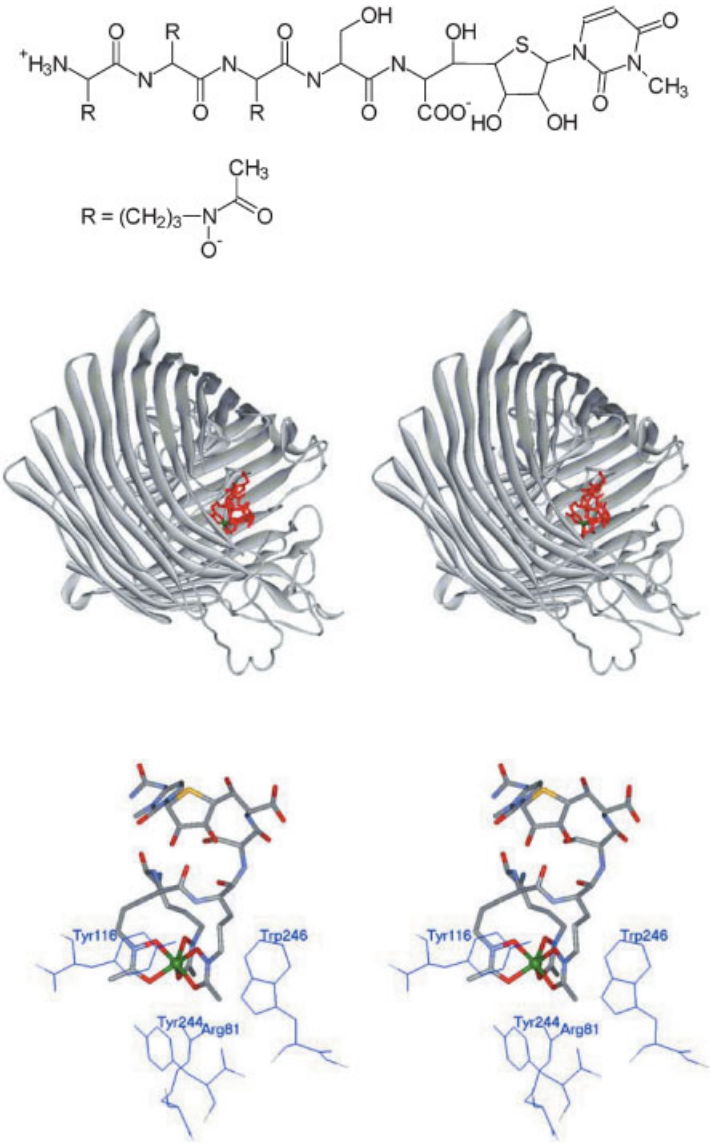

Figure 25. **Top**: Schematic structure of albomycin. **Middle**: The crystal structure of the Fe^{3+}–albomycin–FhuA complex (Protein Data Bank ID 1QKC). The Fe^{3+}–drug complex is shown in red color (Fe^{3+} in green), and the protein as gray ribbons. The binding site of the drug complex is located inside the FhuA "pocket." **Bottom**: The structure of Fe^{3+}–albomycin seen in the crystal structures of the Fe^{3+}–albomycin–FhuA complex, in which the residues involved in H-bonding with the $Fe3^{3+}$–albomycin are shown in blue color.

interact with metal ions and potentially influence their bioactivities and might also cause damages on their target biomolecules. Numerous examples these ''metallodrugs'' and ''metallopharmaceuticals'' and their actions can be found in the literature, for instance: (a) several anti-inflammatory drugs, such as aspirin and its metabolite salicylglycine,[361] ibuprofen,[362] the indole derivative indomethacin,[363] bioflavonoid rutin,[364] diclofenac,[365] suprofen,[366] and others[367] are known to bind metal ions and affect their antioxidant and anti-inflammatory activities; (b) the potent histamine-H2-receptor antagonist cimetidine[368] can form complexes with Cu^{2+} and Fe^{3+}, and the histidine H2 blocker

antiulcer drug famotidine can also form stable complex with Cu^{2+};[369] (c) the anthelmintic and fungistatic agent thiabendazole, which is used for the treatment of several parasitic diseases, forms a Co^{2+} complex with metal:drug ratio of 1:2;[370] (d) the Ru^{2+} complex of the anti-malaria agent chloroquine exhibits an activity two to five times higher than the parent drug against drug-resistant strains of *Plasmodium faciparum*;[371] (e) a number of $Ru^{2+/3+}$ and $Rh^{2+/3+}$ complexes are found to bind DNA and exhibit anti-tumor activities;[256,372] (f) the antiviral trifluoperazine forms complexes with VO^{2+}, Ni^{2+}, Cu^{2+}, Pd^{2+}, and Sn^{4+} which exhibit higher inhibition activities than the metal-free drug when tested on Moloney murine leukemia virus reverse transcriptase;[373] (g) the clinically useful β-lactamase inhibitor sulbactam can form complexes with Ni^{2+}, Cu^{2+}, and Fe^{3+};[374] (h) a few hormone-anchored metallodrugs have been prepared which show enhanced receptor binding and higher activities against cancer cells;[241,375] (i) the thiosemicarbazone-conjugated isatin (which shows a broad-spectrum bioactivity[376]) can bind late first-row transition metal ions and exhibit activity toward human leukemia cell lines, however, without inducing cell apoptosis;[377] and (j) metal complexes (including Be^{2+}, Mg^{2+}, Mn^{2+}, Co^{2+}, Ni^{2+}, Cu^{2+}, Zn^{2+}, Cd^{2+}, Pb^{2+}, Fe^{3+}, Al^{3+}, and La^{3+}) of several carbonic anhydrase inhibiting sulfonamides[378] have been investigated for their topical intraocular pressure lowering properties and as potential agents against gastric acid imbalances.

There are also a number of metallodrugs and metallopharmaceuticals which have been utilized for the treatment of diseases and disorders or as diagnostic agents,[233e,379,380] such as gold antiarthritic drugs, bismuth antiulcer drugs, gadolinium MRI contrast agents, technetium radiopharmaceuticals, metal-based X-ray contrast agents, and photo- and radio-sensitizers, vanadium as insulin mimics, and lithium psychiatric drugs. The metal ion Li^{+} can be considered the smallest effective metallodrug whose carbonate and citrate salts exhibit significant therapeutic benefit in the treatment of manic depression (bipolar mood disorder).[381] Some recent studies by means of 3D-MRI techniques indicate that the volume of the brain gray matter is increased in bipolar disorder patients treated with Li^{+}.[382] The status of Li^{+} in cells have been extensively studied and recently reviewed.[381,383] It is also interesting to point out that the metal ion Sb^{3+} may be regarded as the simplest ''metalloantibiotic'' from a broader viewpoint of the term, whose salts (including *N*-methylglucamine antimonite and Na-stibogluconate) have been utilized for the treatment of leishmaniasis against the protozoan parasite *Leishmania*.[384] The antiprotozoal mechanism of Sb^{3+} is thought to be attributed to its binding to trypanothione that is essential for the growth of the parasite.

This review has summarized the structure, function, and activity of several different families of metalloantibiotics, and has also pointed out the design and potential utilization of metal complexes for battling pathogenic microorganisms. Because of the increase in bacterial resistance toward many currently used antibiotics in recent years, further development of new antibiotics has become an urgent mission. Better understanding of the structure, function, and mechanism of existing metalloantibiotics and the mechanism of antibiotic resistance will lead to better design of metal complexes for this mission. As the chemical properties of metal ions can vary significantly and can also be further fine-tuned by proper design of drug ligands for targeting different biomolecules and biocomponents, new generations of various ''metalloantibiotics'' isolated from natural resources or obtained via chemical syntheses and/or modifications that exhibit more effective antiparasitic, antibacterial, antiviral, and anti-tumor activities can be foreseen.

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