



# Iron(III)–Chelex resin complex as a prototypical heterogeneous catalyst for phosphodiester hydrolysis

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## Abstract

The  $\text{Fe}^{3+}$  complex (but not  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  complexes) of the cross-linked polystyrene Chelex resin is found to be an efficient and selective catalyst toward phosphodiester hydrolysis. The hydrolysis of bis-(*p*-nitrophenyl)phosphate (BNPP) by  $\text{Fe}^{3+}$ –Chelex complex exhibits a pre-equilibrium kinetics with a second-order catalytic proficiency (in terms of rate =  $k_2 [\text{Fe}–\text{Chelex}][\text{BNPP}]$ ) reaching one million at ambient temperature and pH 9.0.

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## 1. Introduction

In a metal-centered hydrolysis, the  $\text{p}K_{\text{a}}$  of a coordinated water molecule is dramatically lowered by the metal center of a high Lewis acidity and is a key factor for the activity. This coordinated water thus becomes a good nucleophile around neutral pH in the form of hydroxide for the attack at the substrate, as observed in metallohydrolases [1]. Many metal complexes, including  $\text{Co}^{3+}$ ,  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and lanthanide(III) complexes, have been synthesized as models of metallohydrolases and/or as catalysts for the hydrolysis of

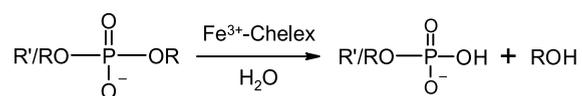
peptides, DNA, RNA, and nerve agent-like compounds [2–8]. Almost all these studies were conducted in homogeneous solutions to mimic the biological environment. While homogeneous catalysis is efficient and convenient, it suffers from difficulty in recovery of the catalyst and incorporation into a recycling or continuous flow process. These difficulties can be overcome by immobilizing catalytic centers on the surface of different matrices [9]. Despite extensive use in many types of reactions, heterogeneous catalysis has not been widely applied to hydrolytic processes. Nevertheless, some recent investigations demonstrated the efficacy of heterogeneous catalysis for hydrolytic reactions [10–12].

The majority of metallohydrolases are found to contain  $\text{Zn}^{2+}$  in the active center [1]. Despite the scarcity, iron centers have been found to catalyze

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hydration and hydrolysis in biological systems, such as the dinuclear Fe center in purple acid phosphatase [13,14] and the mononuclear center in nitrile hydratase [15]. Some Fe complexes have also been demonstrated to exhibit significant hydrolytic activities [16–18]. The high Lewis acidity of  $\text{Fe}^{3+}$  is consistent with the hydrolytic and hydration activities of these systems, which suggests that various Fe complexes should be further explored for hydrolytic catalysis. In this communication, we demonstrate that a very simple  $\text{Fe}^{3+}$ -resin system can perform efficient and selective heterogeneous catalysis toward the hydrolysis of phosphoester bonds.



## 2. Experimental

The commercially available cross-linked beaded polystyrene Chelex resin containing an iminodiacetate functional group  $-\text{N}(\text{CH}_2\text{COO}^-)_2$  can readily bind 2+ and 3+ transition metal ions to form neutral and positively charged complexes, respectively. The latter is expected to have high Lewis acidity suitable for hydrolytic catalysis. The  $\text{Fe}^{3+}$  complex of Chelex resin, which has been thoroughly washed and rinsed with de-ionized water of 18 M $\Omega$  and methanol to remove soluble matters, is prepared by adding slightly excess amount of freshly prepared 100 mM methanol solution of  $\text{FeCl}_3$  to Chelex resin suspended in methanol. The excess unbound  $\text{Fe}^{3+}$  can be removed by filtration and extensive washing with methanol, then washed with buffer for immediate use or air-dried for long-term storage. The  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  complexes of the resin are prepared similarly, using aqueous solutions of the corresponding metal salts. A direct metal titration determines the metal-binding capacity of the Chelex resin to be 1.97 meq/g, consistent with the specification provided by the manufacturer (2.0 meq/g).

Phosphoester hydrolysis is carried out by suspending a fixed amount of  $\text{Fe}^{3+}$ -Chelex in 4 ml of a substrate solution in 100 mM Tris buffer at pH 9.0

under constant stirring. The initial rate of the hydrolysis is determined spectrophotometrically based on the release of the chromophore *p*-nitrophenol ( $\epsilon_{405} = 1.91 \times 10^4 \text{ M}^{-1} \text{ cm}^{-1}$  at pH 9.0) from the substrate in the supernatant at different time intervals, and subtract the background hydrolysis using metal-free resin as the control. All hydrolytic reactions were performed four times and the average and the standard deviation determined. The initial rates for the hydrolysis of the substrates at different pHs are obtained similarly in appropriate buffer solutions of 100 mM. The “apparent concentration” of Fe-Chelex in the solution is expressed as the molar amount of Fe per unit volume of the solution, i.e., 49.3 mM for 100 mg of the complex in 4 ml of the reacting solution. The concentration of BNPP used for the determination of the rate law is in the range of 0.50–10.0 mM.

## 3. Results and discussion

The  $\text{Cu}^{2+}$ ,  $\text{Zn}^{2+}$ , and  $\text{Fe}^{3+}$  complexes of Chelex resin are completely insoluble in common solvents and in the buffers used for the kinetic analysis, thus representing a heterogeneous catalysis in nature. The model phosphodiester substrate bis(*p*-nitrophenyl)phosphate (BNPP), the phosphomonoester *p*-nitrophenyl phosphate, and *p*-nitrophenyl phenylphosphonate were used to evaluate the efficiency and specificity of the metal complexes of the resin towards hydrolysis of phosphoester bonds. The hydrolysis of BNPP by 100 mg  $\text{Fe}^{3+}$ -Chelex complex is readily observable at ambient temperature of 27 °C and pH 9.0 (Fig. 1), with a rate of  $(2.2 \pm 0.8) \times 10^{-4} \text{ mM min}^{-1}$  (i.e., a specific activity of  $2.2 \times 10^{-3} \mu\text{mol min}^{-1} \text{ mg}^{-1}$ ) for the hydrolysis of 10-mM BNPP. The complex remains active after at least three recycles simply by filtration and washing (○, Fig. 1). Moreover, the  $\text{Cu}^{2+}$  and  $\text{Zn}^{2+}$  complexes of Chelex resin were also found active, yet with much slower rates of  $1.3 \times 10^{-5}$  and  $1.1 \times 10^{-5} \text{ mM min}^{-1}$  toward the hydrolysis of 10-mM BNPP under the same conditions as the iron complex. To establish the rate law in terms of the amount of  $\text{Fe}^{3+}$ -resin complex for BNPP hydrolysis, the rate is measured in various amounts of the complex in a large range

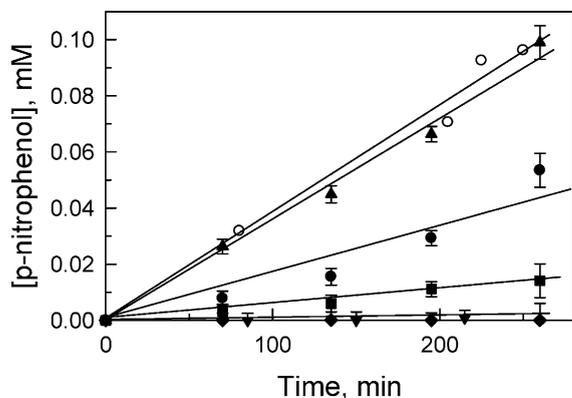


Fig. 1. The hydrolysis of BNPP (■, 1 mM; ●, 4 mM; and ▲, 10 mM) by 100-mg Fe<sup>3+</sup>-Chelex complex in 100 mM Tris buffer at pH 9.0 relative to the uncatalyzed reaction (◆, 1 mM and ▼, 10 mM) under the same conditions. The activity of a typical recycled Fe-Chelex complex is also shown (○).

equivalent to 3.0–49.3 mM. The rate of BNPP hydrolysis increases with increasing amount of Fe<sup>3+</sup>-Chelex complex (Fig. 2, inset), from which an observed first-order rate constant  $k_{\text{obs}}$  is obtained to be  $5.42 \times 10^{-8} \text{ s}^{-1}$  for the rate law  $\text{rate} = k_{\text{obs}}[\text{Fe-Chelex}]$ . The rate of BNPP hydrolysis by the same concentration of Fe<sup>3+</sup> in solution (which exists as rust) under the same conditions is negligible compared to hydrolytic rate by the

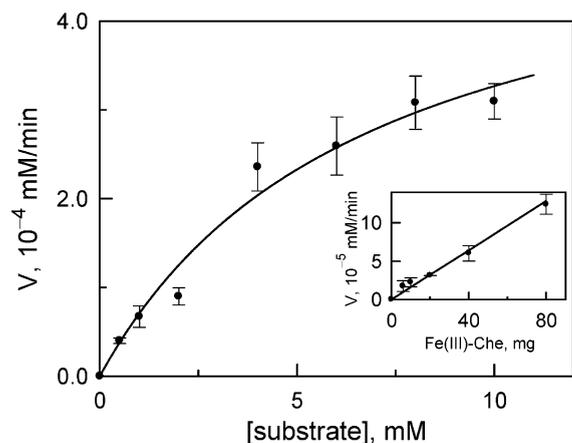
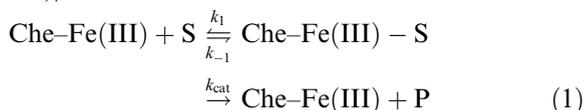


Fig. 2. Plot of the initial hydrolytic rate of BNPP at different concentrations by 100-mg Fe(III)-Chelex resin at pH 9.0 and 27 °C. The solid trace is the best fit to the pre-equilibrium kinetics (Eq. 1). The inset shows the initial rate for the hydrolysis of 10-mM BNPP by different amounts of Fe(III)-Chelex resin, from which an observed first-order rate constant  $k_{\text{obs}}$  can be obtained.

complex. Moreover, the clear reaction solution after Fe<sup>3+</sup>-Chelex is removed did not show noticeable activity, indicating a genuine heterogeneous catalysis. The significantly higher activity of the Fe<sup>3+</sup> complex suggests that Fe-centered hydrolysis should be further explored, despite the greater attractiveness of Cu<sup>2+</sup> and Zn<sup>2+</sup> complexes in hydrolytic chemistry [2–8,10–12]. The reasonably fast catalysis by the Fe<sup>3+</sup> complex allows an extensive kinetic investigation to be performed.

In order to establish the rate law for the heterogeneous hydrolysis of BNPP by Fe<sup>3+</sup>-Chelex complex, the rate is further determined at various [BNPP] values at ambient temperature of 27 °C and pH 9.0. The rate is found to be non-linear with respect to [BNPP] (Fig. 2), suggesting an enzyme-like pre-equilibrium kinetics. Such kinetics can be described as the binding of the substrate BNPP (S) to the catalytic center on the surface of the resin, followed by the conversion of the bound substrate into the products (Eq. (1)). The rate law for this reaction can be expressed as Eq. (2) by means of steady-state approximation, in which  $k' = (k_{-1} + k_{\text{cat}})/k_1$ .



$$\text{rate} = \frac{k_{\text{cat}}[\text{Che-Fe}][\text{S}]}{k' + [\text{S}]} \quad (2)$$

The data can be well fitted to the rate law (Eq. 2) to give a first-order rate constant  $k_{\text{cat}} = 1.8 \times 10^{-7} \text{ s}^{-1}$ ,  $k' = 6.7 \text{ mM}$ , and a second-order rate constant  $k_{\text{cat}}/k'$  of  $2.7 \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$  at  $[\text{S}] \ll k'$  to give the rate law  $\text{rate} = (k_{\text{cat}}/k')[\text{Che-Fe}][\text{BNPP}]$  (Fig. 2). This second-order rate constant is comparable to those of homogeneous hydrolysis of BNPP catalyzed by some simple metal complexes. For example, second-order rate constants are found to be in the range of  $(0.18\text{--}2.8) \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$  calculated from corresponding pseudo-first order rate constants at pH 8.36 and 55 °C [19] and  $(5.4\text{--}11.5) \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$  at pH 10.9–11.5 and 35 °C [20] for several mono- and dinuclear Zn<sup>2+</sup> complexes; and  $(6.0\text{--}80) \times 10^{-5} \text{ M}^{-1} \text{ s}^{-1}$  at pH 7.2 and 35 °C for Zn<sup>2+</sup> and Cu<sup>2+</sup> complexes of cyclic triamines [21,22]. The auto-hydrolytic rate

$k_0 = 2.06 \times 10^{-9} \text{ s}^{-1}$  of BNPP at 27 °C and pH 9.0 can be extrapolated from directly measured rates at higher temperatures and pH 7.0, assuming  $\text{OH}^-$  is the nucleophile [23]. The catalysis of BNPP hydrolysis by  $\text{Fe}^{3+}$ -Chelex exhibit a significant catalytic proficiency that is 87-fold higher than auto-hydrolysis in terms of the first-order rate constant expressed as  $k_{\text{cat}}/k_0$  [24], which represents a shortening of the half-life from  $\sim 20$  years to  $\sim 80$  days for rate =  $k_{\text{cat}}[\text{Che-Fe}]$  at high  $[\text{S}]$ , and  $9.8 \times 10^5$  times higher rate in terms of the second-order rate constant expressed as  $(k_{\text{cat}}/k')/(k_0/55.5)$  [25] for rate =  $(k_{\text{cat}}/k')[\text{Che-Fe}][\text{BNPP}]$  at low  $[\text{S}]$ .

$\text{Fe}^{3+}$ -Chelex can also catalyze the hydrolysis of a phosphonate ester, *p*-nitrophenyl phenylphosphonate. A rate of  $1.3 \times 10^{-4} \text{ mM min}^{-1}$  was determined at pH 9.0 and ambient temperature with 100 mg of the complex, representing a small rate enhancement of  $\sim 9$  fold against the uncatalyzed rate of  $1.4 \times 10^{-5} \text{ mM min}^{-1}$ . Moreover, the  $\text{Fe}^{3+}$ -Chelex complex discriminates against the hydrolysis of the phosphomonoester *p*-nitrophenyl phosphate, showing negligible rate enhancement under the same experimental conditions. The low activity towards phosphomonoester hydrolysis by this mononuclear iron center might be attributed to the decrease in the Lewis acidity and/or the positive charge of the metal center due to the negatively charged acetate groups on the ligand which results in less effective neutralization of the negative charge on the monoester for the subsequent nucleophilic attack by the coordinated  $\text{OH}^-$ .

In order to determine the catalytic mechanism, the effect of pH on the hydrolysis of BNPP by the  $\text{Fe}^{3+}$ -Chelex complex has also been investigated. A sigmoidal rate-pH profile is revealed (Fig. 3), which can be fitted to a single-proton ionization process by considering that the deprotonated form is the active species. A  $\text{p}K_{\text{a}}$  value of 8.8 is obtained from the fitting. That the activity is attributable to a single ionization process is consistent with a hydrolytic mechanism mediated by a metal-bound nucleophilic  $\text{OH}^-$ . The relatively high  $\text{p}K_{\text{a}}$  value than those of many other metal complexes [2–8] reflects a relatively low Lewis acidity of the metal center in  $\text{Fe}^{3+}$ -Chelex, which might be due to the negative charge on the ligand. It is also noteworthy

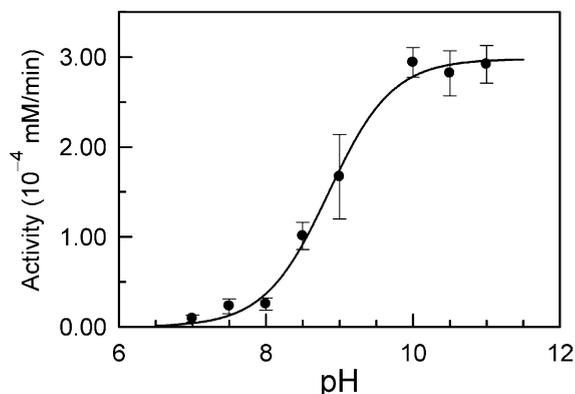


Fig. 3. Activity-pH profile for the hydrolysis of 10 mM BNPP in the presence of 100 mg  $\text{Fe}^{3+}$ -Chelex complex. The data are fitted to a single-ionization process according to the equation rate =  $V_{\text{max}}/(1 + [\text{H}^+]/K_{\text{a}})$ , in which the ionized form is considered the only active form,  $K_{\text{a}}$  the ionization constant, and  $V_{\text{max}}$  the rate of the fully ionized form.

that  $\text{Fe}^{3+}$  is not pulled out from the resin at neutral and higher pHs (Fig. 3), a condition that favors the formation of iron rust due to the low  $\text{p}K_{\text{a}}$  of  $\text{Fe}(\text{OH})_6^{3+}$  which has been determined to show negligible hydrolytic activity toward BNPP compared to  $\text{Fe}^{3+}$ -Chelex complex under the experimental conditions.

We have demonstrated that a simple iron-centered heterogeneous system can effectively promote hydrolysis of phosphodiester and phosphonate esters. Another very attractive feature of this system, and in heterogeneous catalysis in general, is its reusability without any loss of activity toward BNPP hydrolysis. The ease of handling, effectiveness, and selectivity toward phosphodiester demonstrated by  $\text{Fe}^{3+}$ -Chelex complex suggests that heterogeneous hydrolysis, and  $\text{Fe}^{3+}$ -centered hydrolysis as well, should be further investigated and its applications further explored.

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