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Heterodinuclear $Fe^{III}Zn^{II}$-Bioinspired Complex Supported on 3-Aminopropyl Silica. Efficient Hydrolysis of Phosphate Diester Bonds

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Presented herein is the synthesis and characterization of a new $Fe^{III}Zn^{II}$ complex containing a Fe^{III}-bound phenolate with a carbonyl functional group, which was anchored to 3-aminopropyl-functionalized silica as the solid support. The catalytic efficiency of the immobilized catalyst in the hydrolysis of 2,4-bis(dinitrophenyl)phosphate is comparable to the homogeneous reaction, and the supported catalyst can be reused for subsequent diester hydrolysis reactions.

The development of rational approaches to the design of synthetic inorganic catalysts for the hydrolysis of phosphate esters is a subject continually attracting interest in bioinorganic chemistry.[1] Such interest arises mainly because the pK_a of a coordinated water molecule is dramatically lowered by the metal center of high Lewis acidity and thus the $M^{III/II}OH$ group generated becomes a good nucleophile at around neutral pH, as observed in metallohydrolases.[2] In this context, we have reported a series of heterodinuclear $Fe^{III}M^{II}$ complexes (M^{II} = Zn, Cu, Ni, Mn) containing the unsymmetrical ligand H_2L = 2-bis[{(2-pyridylmethyl)aminomethyl}-6-{(2-hydroxybenzyl)(2-pyridylmethyl)}aminomethyl]-4-methylphenol as structural and functional models for the active site of purple acid phosphatases (PAPs) over the past decade.[1,3] PAPs are metalloenzymes present in mammals and plants that belong to the family of binuclear metallohydrolases that catalyze the hydrolysis of a variety of phosphoester substrates within the pH range of 4−7.[1,2] In fact, they are the only metallohydrolases for which the need for a heterovalent active site ($Fe^{III}M^{II}$; M^{II} = Fe, Zn, Mn) for catalysis has been established.[1a] Particularly interesting are the structural features and the catalytic activity of the [Fe^{III}(μ-OH)Zn^{II}(L)] biomimetic in the hydrolysis of the diester substrate 2,4-bis(dinitrophenyl)phosphate (2,4-BDNPP), for which a mechanism similar to that proposed for red kidney bean PAP (rkbPAP)[2b,4] has been established.[3a] However, the limiting factor of these biomimetic homogeneous systems lies in the recovery and reusability of the catalyst. The heterogenization of the homogeneous catalysts, by anchoring the active complex onto the surface of a solid support, has been utilized as a convenient strategy to overcome this drawback.[5] Nevertheless, it is important to emphasize that only a very few examples of heterogeneous bioinspired catalysts as hydrolases have been reported to date.[6] Herein we report a synthetic route for the attainment of an appropriate modification of the ligand H_2L (H_2L^1 now containing a carbonyl group attached to the terminal phenol group; see the Supporting Information, SI), the X-ray structure of its $Fe^{III}Zn^{II}$ complex (Figure 1), the synthesis and characterization of the silica-bound $Fe^{III}Zn^{II}$ complex, and their homogeneous and heterogeneous hydrolase activities with the DNA-model diester substrate 2,4-BDNPP and DNA itself.

The molecular structure of **1** (Figure 1) shows that in the dinuclear [Fe^{III}(μ-OH)Zn^{II}(L^1)] unit the coordination/arrangement of L^1 and all metric parameters around the metal centers are similar to those observed in the isostructural complex containing ligand H_2L.[3a]

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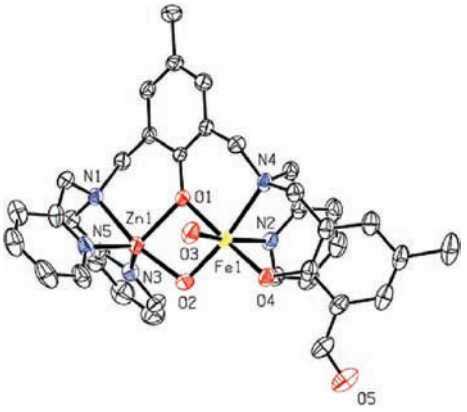

Figure 1. ORTEP plot of the cation complex in **1**.

It is important to note that in addition to the terminal Fe-bound water molecule [Fe−O3 = 2.082(6) Å], which has been proposed as the nucleophile in the hydrolysis of diesters catalyzed by $[Fe^{III}(\mu\text{-OH})Zn^{II}(L)]$[3a] and rkbPAP,[4] the structure of **1** presents a carbonyl group located in the ortho position of the terminal Fe^{III}-bound phenolate ligand.

From the solid structure of **1**, it is possible to visualize that this functional group is ideally oriented for further chemical reactions and thus can be used in the development of new synthetic protocols to immobilize the catalytic center on a solid support, for example, 3-aminopropyl-modified silica (see the SI).

Potentiometric titration of **1** in water/CH_3CN (1:1, v/v) showed the neutralization of 3 mol of KOH/mol of complex in the pH range of 2−12. Fitting the data with the *BEST7*[7] program resulted in the following deprotonation constants: $pK_{a1} = 3.33$, $pK_{a2} = 5.07$, and $pK_{a3} = 8.21$ (Figure S2 in the SI). These values are comparable to those obtained for the isostructural $[Fe^{III}(\mu\text{-OH})Zn^{II}(L)]$ complex (pK_{a1} = 2.93, $pK_{a2} = 4.81$, and $pK_{a3} = 8.30$),[3a] and the slightly higher values in **1** are probably due to the presence of the electron-donating substituent methyl group in the para position of the terminal phenolate group in L^1.

Complex **1** was covalently linked to 3-aminopropyl-functionalized silica gel and electrostatically to nonfunctionalized SiO_2, and these materials were identified by IR, electron paramagnetic resonance, and UV−vis spectroscopies (see Figures S3 and S4 in the SI). Homogeneous and heterogeneous kinetic experiments on the hydrolysis of the activated substrate 2,4-BDNPP by **1**, **Si3AP-1**, and **SiO_2-1** were carried out by following spectrophotometrically the absorbance increase of the liberated 2,4-dinitrophenolate anion, under conditions of excess substrate.

The homogeneous catalytic activity of **1** in the hydrolysis of 2,4-BDNPP showed a bell-shaped profile (Figure S5 in the SI) with an optimum at around pH 7.0. Sigmoidal fits of the curve reveal pK_a values of 4.9 and 7.9, which are in good agreement with the values (pK_{a2} = 5.07 and pK_{a3} = 8.21) obtained from the potentiometric titration experiments. As proposed for the $[Fe^{III}(\mu\text{-OH})Zn^{II}(L)]$ complex,[3a] these results demonstrate that the $[(OH)Fe(\mu\text{-OH})Zn(OH_2)]$ form of the complex is the catalytically active species in cleaving 2,4-BDNPP. In this species, the phosphodiester substrate can replace the water molecule bound to the Zn^{II} ion and the adjacent iron(III) hydroxide promotes the nucleophilic attack on the phosphorus atom with concomitant release of 2,4-dinitrophenolate.[3a] The decrease in reactivity at pH > 7.0 most probably arises from the presence of the fully deprotonated form $[(OH)Fe^{III}(\mu\text{-OH})Zn^{II}(OH)]$ in which the leaving tendency of the hydroxide ion bound to the Zn^{II} ion becomes lower.[3]

Table 1. Kinetic Parameters for 2,4-BDNPP Hydrolysis Promoted by Complexes **1**, **Si3AP-1**, and **SiO_2-1**

catalyst	$V_{max} \times 10^9$ ($mol \cdot L^{-1} \cdot s^{-1}$)	$K_M \times 10^3$ ($mol \cdot L^{-1}$)	$k_{cat} \times 10^4$ (s^{-1})	k_{cat}/K_M ($mol^{-1} \cdot L \cdot s^{-1}$)
1	18.5	3.55	9.02	0.25
Si3AP-1	8.90	1.54	1.42	0.09
SiO_2-1	2.83	0.60	0.45	0.08

The determination of the initial rates in a homogeneous medium at pH 7.0 as a function of the substrate concentration reveals saturation kinetics with Michaelis−Menten-like behavior (Figure S6 in the SI). The nonlinear Michaelis−Menten regression was used, and the calculated kinetic parameters are listed in Table 1. Under these experimental conditions, **1** shows a 5040-fold acceleration rate compared to uncatalyzed hydrolysis ($k = 1.89 \times 10^{-7}\ s^{-1}$). This value is comparable to, although slightly higher than, that found for the $[Fe^{III}Zn^{II}(\mu\text{-OH})(L)]$ complex (4800),[3a] with this being evidence that the Lewis acidity of the Fe^{III} center is directly affected as the nature of the para substituent at the terminally Fe^{III}-bound phenolate is changed from −H to $-CH_3$.

The heterogeneous reactions using **Si3AP-1** and **SiO_2-1** as the catalysts were performed under experimental conditions identical with those employed in homogeneous media (see the SI) using a reference cell without addition of the catalyst.[8] In both cases, as with the reaction in homogeneous media, the dependence of the initial rates on the concentration of 2,4-BDNPP at optimum pH (7.0) shows Michaelis−Menten behavior (Figures S7 and S8 in the SI), and nonlinear regression fits result in the kinetic parameters given in Table 1. In fact, these kinetic parameters reveal that $K_{ass} \cong 1/K_M$ is about 2 and 6 times higher for **Si3AP-1** and **SiO_2-1**,[9] respectively, when compared to the reaction in homogeneous media, while the catalytic turnover constant k_{cat} is approximately 6 and 20 times lower, respectively, for the heterogeneous reactions. Nevertheless, considering the second-order rate constant for **1**, **Si3AP-1**, and **SiO_2-1**, it can be observed that these systems have distinct but still comparable catalytic efficiencies, with k_{cat}/K_M being in the range of 0.08−0.25 $mol^{-1} \cdot L \cdot s^{-1}$, thus indicating that the heterogeneous data are in good agreement with the solution data. The higher affinity of 2,4-BDNPP for the anchored systems **Si3AP-1** and **SiO_2-1**, presumably originates from the solid support itself. Particularly, for **Si3AP-1** we propose stabilization of the substrate via hydrogen bonds with the residual amino group of 3-aminopropyl-functionalized silica (Figure 2), while the decrease in the catalytic activity is most probably due to partial occupation of the labile Fe^{III} and Zn^{II} coordination sites by the silanol groups of the solid support. It is important to note that the catalyst concentration anchored on the solid support refers to the concentration of complex **1**,

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(8) The rate of the background reaction in the presence of modified Si3AP or SiO_2 is similar to that of autohydrolysis of 2,4-BDNPP at pH 7.0.

(9) Better saturation kinetics were obtained for the heterogeneous reactions most probably because of the higher stabilization of the substrate−catalyst intermediate (see $K_{ass} = 1/K_M$ in Table 1).

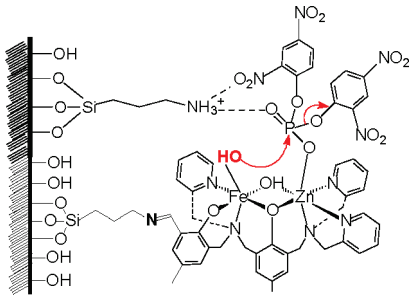

Figure 2. Proposed mechanism for hydrolysis of 2,4-BDNPP promoted by **Si3AP-1**.

enabling a direct comparison between the catalytic activity of this unit in homogeneous and heterogeneous media.

The pH dependence of the catalytic activity of **Si3AP-1** reveals sigmoidal behavior with a pK_a of 5.70 (Figure S9 in the SI), which is in reasonably good agreement with the pK_{a2} obtained from the potentiometric titration of **1**, and again the [(OH)Fe(μ-OH)Zn(OH_2)] form of the complex seems to be the catalytically active species in cleaving 2,4-BDNPP.[3a]

Furthermore, the measured kinetic isotope effect of $k_H/k_D = 0.95$ for **1** and for **Si3AP-1** strongly supports an intramolecular nucleophilic attack by an Fe^{III}-bound hydroxide for both reactions. On the other hand, as was already reported for the biomimetic [Fe^{III}(μ-OH)Zn^{II}(L)],[3a] the absorption maximum of the iron(III) phenolate charge-transfer band is only slightly affected when the diester 2,4-BDNPP is added to the catalyst **1** or **Si3AP-1** (Figures S10 and S11 in the SI), suggesting monodentate binding of the substrate to Zn^{II} in the homogeneous as well as heterogeneous reactions. However, on the basis of the experimental results presented in this study, at present we do not have a reasonable explanation for the distinct pH−rate profiles; for the **Si3AP-1**-catalyzed reaction, it is sigmoidal, whereas for the free complex in solution, a bell-shaped pH−rate profile is observed. Finally, reactions between complex **1** or **Si3AP-1** and the monoester 2,4-dinitrophenyl phosphate (DNPP) were monitored under an excess of substrate, and after ca. 3 h of reaction, no changes in the UV−vis spectra were observed (Figure S11 in the SI). Addition of the diester 2,4-BDNPP to this solution after this time showed that the absorption band at 400 nm immediately started to increase, indicating the recovery of the catalyst and specific diesterase activity of the catalyst in the homogeneous[3a] as well as heterogeneous reactions. The detection of 10 turnovers in 24 h for **1** and **Si3AP-1** represents further evidence that during the catalytic cycle the intermediate containing the bridging monoester phosphate dissociates to regenerate the catalytically active species [(OH)Fe^{III}(μ-OH)Zn^{II}(OH_2)] when the diester substrate is present in excess. The advantage of using solid supported catalysts is that they are easily removed from the final product and have the potential to be recycled. The **Si3AP-1** catalyst was reused in the subsequent hydrolysis reaction of 2,4-BDNPP (see the SI), with the rate of reaction decreasing by around 30% compared to the previous hydrolysis reaction (Figure S12 in the SI). The reduction in activity may be attributed to some loss of the supported catalyst due to imine hydrolysis and subsequent washings.

To verify the ability of **1** (free and immobilized) to cleave DNA phosphodiester bonds, the complex activity at different concentrations toward DNA was tested (see the SI). Both **1** and **Si3AP-1** (Figure S13 and S14 in the SI) were able to cleave plasmid DNA in a concentration-dependent manner. An increase in circular (FII) plasmid DNA, with a proportional decrease in supercoiled DNA (FI), was observed with increasing complex concentration. Complex **1** may be considered to be slightly more active than **Si3AP-1** in DNA cleavage because the observed cleavage at a 10 μM complex concentration, as measured by the appearance (in percent) of FII DNA, corresponds to 47% and 33%, respectively, and these data are in agreement with the distinct catalytic efficiency observed in the hydrolysis of the model diester substrate 2,4-BDNPP. In the presence and absence of distamycin, **1** showed similar cleavage activity, indicating that this complex is not inhibited by this typical minor groove binder and therefore interacts with the DNA molecule in the major groove (Figure S15 in the SI).

In summary, we have shown the straightforward synthetic route for the preparation of an artificial bioinspired $Fe^{III}Zn^{II}$ metallohydrolase and a new synthetic protocol to immobilize this catalytic center on solid supports containing primary amine functional groups. In addition, it was also demonstrated that the hydrolase activity of the immobilized catalytic center is comparable to that of the homogeneous process ($Fe^{III}OH^-$ is the nucleophile) and that the supported catalyst can be reused for subsequent diester hydrolysis reactions. Finally, the immobilized hydrolase was also shown to be efficient in DNA cleavage, which indicates its potential as an artificial nuclease.

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Supporting Information Available: Crystallographic data of **1**, synthesis, characterization, and kinetics for **1** and **Si3AP-1**, and Figures S1−S13. This material is available free of charge via the Internet at http://pubs.acs.org. The atomic coordinates for this structure have been deposited with the Cambridge Crystallographic Data Centre as CCDC 757797. The coordinates can be obtained, upon request, from the Director, Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge, CB2 1EZ, U.K.