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Metal–chelator polymers as organophosphate hydrolysis catalysts†

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A copper-containing polymer was prepared by free radical polymerization of a copper(II)–vinylbipyridyl complex with trimethylolpropane trimethacrylate. Hydrolysis of a phosphate monoester, 4-nitrophenyl phosphate, a diester, bis-4-nitrophenyl phosphate, and a triester, methyl parathion (*O*,*O*-dimethyl *O*-*p*-nitrophenyl phosphorothioate), in water–methanol (85 : 15) at pH 8.1 was investigated and compared to the hydrolytic ability of the analogous monomeric copper complex. Using the Michaelis–Menten formalism to characterize apparent catalytic constants, the rate of hydrolysis (k_{cat}) for each phosphate ester substrate was found to be about an order of magnitude faster and the K_m values up to two orders of magnitude lower for the polymeric catalysts compared to the homogeneous monomeric catalysts. The methyl parathion polymeric hydrolysis rate was 6.7×10^5 times faster than the uncatalyzed rate. Reasons for the enhanced catalytic activity, particularly at low substrate concentrations, are discussed.

Introduction

The role of metal ions in the enhanced hydrolysis of phosphate esters has long been known and the mechanism of these reactions extensively investigated.[1–3] Much of this interest has centered on synthesizing model complexes of metalloenzymes capable of hydrolyzing phosphate diesters, in particular, DNA and RNA.[4,5] To this end, an extensive range of complexes has been reported in which different metals were used,[6–10] polydentate ligands were varied,[8–11] and mono and dinuclear reactivities were also compared.[4,9,12]

While the investigation of phosphate triester hydrolysis is the subject of much current interest, less attention has been given to synthesizing complexes for this purpose. Hydrolysis reactions using Cu(II), Hg(II), Rh(III) and Ir(III) complexes,[13–16] as well as a recent report using an organometallic molybdenum complex,[17] have been studied. The limited literature on triester hydrolysis is surprising, as sulfur-containing triesters are among the most widely used insecticides in the United States. They are also relatively toxic, but this is greatly reduced after hydrolysis to the corresponding diesters.[18]

Given the environmental significance of triester hydrolysis, it is of interest to design easily recoverable heterogeneous catalysts that can rapidly hydrolyze these toxic substances. Such materials, other than soluble monomeric complexes, which have been reported include clay minerals,[19] clays treated with divalent metals,[20] and a variety of metal oxide surfaces.[21] Insoluble copper-containing polymeric compounds have also been shown to catalyze hydrolysis of model esters with a rate enhancement of two orders of magnitude compared to the reaction under similar conditions but in the absence of polymer.[22]

We have been exploring the use of heterogeneous catalysts in the hydrolysis of phosphate esters, including surface modification of silica particles,[23] as well as polymeric materials derived from the cross-linking monomer trimethylolpropane trimethacrylate (TRIM).[24] For the polymer, our results showed that while the soluble Cu(II)–diethylenetriamine complex gave no observable rate enhancement in the hydrolysis of *p*-nitrophenylphosphate, the same complex incorporated into a TRIM polymer resulted in a significant increase in reaction rate. Based on these results, it seemed possible that the incorporation of a catalytically active copper complex would give rise to even more rapid hydrolysis. Therefore, such an approach should lead to the development of heterogeneous, catalytic, and rapidly reacting materials suitable for hydrolysis of toxic pesticides and nerve agents.

In early research in this field, it was shown that 2,2′-bipyridyl (bpy) Cu(II) complexes are among the most efficient in hydrolyzing selected phosphorofluoridates, a class of compounds closely related to phosphate esters.[10] A more recent report detailed comprehensively the hydrolysis of other phosphate esters with Cu(II)(bpy).[14] The incorporation of such a complex into TRIM polymer would require the addition of a coupling linkage, such as a vinyl substituent, on the ligand. The significant amount of known bpy chemistry makes this complex readily amenable to such modifications. Here we report the synthesis of a Cu(II)-containing TRIM polymer and its hydrolytic reactivity with selected phosphate esters. In particular, the rates of hydrolysis of methyl parathion (MeP), a toxic phosphate triester, in the absence of Cu(II), with Cu(II) complex in solution, and with Cu(II) polymer are compared and differences discussed. Similar comparisons for the rates of hydrolysis of bis(*p*-nitrophenyl)phosphate (BNPP), a phosphate diester, and *p*-nitrophenylphosphate (NPP), a monoester are also made. For each substrate, the presence of Cu(II) increases the rate of reaction. Furthermore, in each case Cu(II) polymer was found to hydrolyze the substrate more rapidly than the analogous Cu(II) complex in solution.

Results and discussion

A Cu(II)-containing polymer consisting of the functionalized monomer, 4-vinyl-4′-methyl-2,2′-bipyridine : Cu(II) (Cu(II)(vbpy)), cross-linked to TRIM was synthesized. Copper levels in the polymer were determined to be 18.0 mg Cu per g of polymer. Given that the theoretical copper content is 16.8 mg Cu per g

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polymer, the Cu(II)(vbpy) incorporation was deemed quantitative.

Rates of spontaneous and Cu(II)(bpy)-catalyzed hydrolysis of NPP, BNPP, and their analogues have previously been reported.[9,14,25,26] Differing experimental conditions, however, make it difficult to compare directly with our present results. Our experiments were carried out at 20 to 22 °C in 85 : 15 water–methanol with 100 mM MOPS [3-(*N*-morpholino)propanesulfonic acid sodium salt] at pH 8.1. This concentration of methanol is not expected to affect copper hydrolysis of phosphate esters.[18]

TRIM polymers, obtained from the protocol described here, form a fine powder with a very high surface area of 406 $m^2\ g^{-1}$. The polymer matrix is also microporous, with an average pore diameter of approximately 2.5 nm While much of the powder is made up of particles greater than 10 μm that settle quickly, dynamic light scattering of the supernatant from sonicated samples shows a bi-modal size distribution of suspended particles with diameters centered about 5.3 and 0.15 μm. Given the large surface area and open pore structure, we make the approximation that all copper centers within the polymer are available for reaction with substrate molecules. With this assumption, our calculations for the apparent polymer rate constants will be of a lower rate limit. The TRIM polymer does not appear to be easily exhausted either, as we have taken the polymer to over 100 × turnover without catalytic poisoning.

The spontaneous rate constants for hydrolysis, *i.e.*, without Cu(II), as calculated from the slope of nitrophenol concentration against time, were consistent with zero order reaction, as expected, for each substrate. Hydrolysis rates using TRIM polymer with no Cu(II) present did not vary significantly from spontaneous hydrolysis rates. Double-reciprocal Lineweaver–Burk graphs [*e.g.*, Fig. 1(a)] were used to calculate the kinetic rate parameters, V_{max}, K_{cat}, and K_M, the apparent maximum reaction rate, apparent first-order kinetic rate constant, and the Michaelis constant, respectively, for reactions between the three substrates and monomeric Cu(II)(vbpy). NPP and MeP hydrolysis by Cu(II)(vbpy)-TRIM polymer were also analyzed using Lineweaver–Burk plots. The polymer catalysis of BNPP showed saturation kinetics for the substrate concentration range studied and, therefore, the kinetic constants were obtained directly from the graphs [Fig. 1(b)]. The rate constants for each catalyst–substrate system are shown in Table 1.

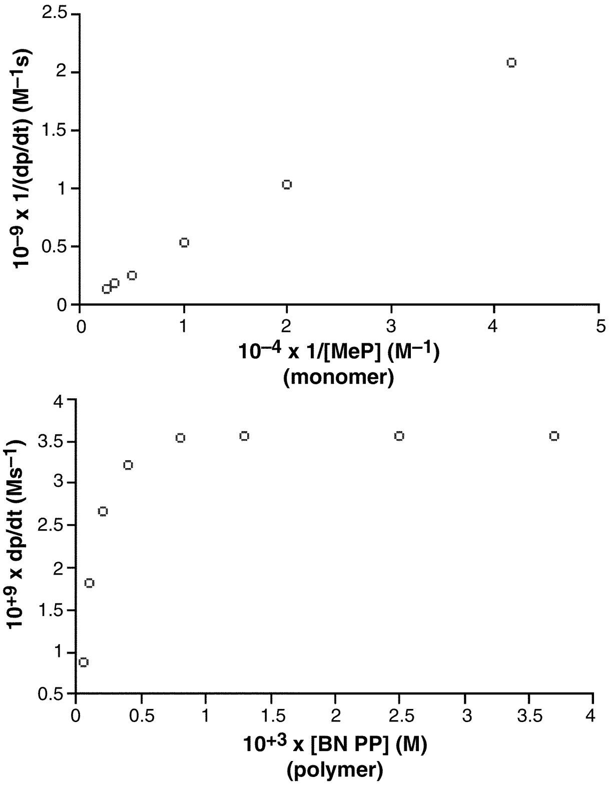


Fig. 1 (a) Dependence of the initial rate of hydrolysis of MeP by Cu(II)(vbpy) (1.16×10^{-5} M) on the concentration of MeP (20–22 °C, 85 : 15 H_2O–CH_3OH, 0.1 M MOPS, pH 8.1), inversely plotted as a Lineweaver–Burk graph. (b) Saturation kinetics for the hydrolysis of BNPP by Cu(II)(vbpy)-TRIM polymer [overall concentration of Cu(II)1.52×10^{-4} M] as a function of the concentration of BNPP (20–22 °C, 85 : 15 H_2O–CH_3OH, 0.1 M MOPS, pH 8.1).

Table 1 Summary of catalytic constants for the hydrolysis of MeP, BNPP and NPP

Substrate	Catalyst	K_{cat}/s^{-1}	Ratio k_{cat}/k_{uncat}
NPP	none	2.6×10^{-9}	—
NPP	Cu(vbpy) (solution)	1.4×10^{-7}	54
NPP	Cu(vbpy) (polymer)	1.5×10^{-6}	580
BNPP	none	9.3×10^{-10}	—
BNPP	Cu(vbpy) (solution)	9.9×10^{-7}	$1.0 \times 10^{+3}$
BNPP	Cu(vbpy) (polymer)	2.3×10^{-5}	$2.5 \times 10^{+4}$
MeP	none	3.0×10^{-8}	
MeP	Cu(vbpy) (solution)	7.6×10^{-3}	$2.5 \times 10^{+5}$
MeP	Cu(vbpy) (polymer)	2.0×10^{-2}	$6.7 \times 10^{+5}$

[a] NPP = nitrophenylphosphate; BNPP = bis(nitrophenyl)phosphate; MeP = methyl parathion.

Spontaneous hydrolysis rates, under our experimental conditions, follow the established trends of the tri- and monoester hydrolysis of MeP and NPP, respectively, and are more rapid than that for the diester, BNPP.[27] That is, the order of reactivity is MeP > NPP > BNPP.

As previously observed for the metal–chelator complex, Cu(II)(bpy),[14] significant rate enhancements over spontaneous hydrolysis are observed in the presence of our copper ion-functionalized monomer, Cu(II)(vbpy). The monomer solutions yielded rates that were 2 to 5 orders of magnitude faster, depending on substrate, than the corresponding spontaneous hydrolysis. The enhancement for BNPP, approximately 3 orders of magnitude, is similar to that previously reported for Cu(II)(bpy) under different reaction conditions.[14] With this large rate enhancement, the order of reactivity for functionalized monomers is changed from that of spontaneous hydrolysis to MeP > BNPP > NPP.

It is generally agreed that, during hydrolysis, the substrate first coordinates to Cu(II), replacing a Cu-bound water molecule. Hydrolysis then occurs by intramolecular attack by a second coordinated water or hydroxy group coordinated to the phosphorus.[2,3,14,26] The reactivity sequence can be understood by observing that, of the three phosphate esters, the phosphorus of the triester is most positive, allowing a more facile nucleophilic attack. Similarly, the monoester, with the least positive phosphorus, is hydrolyzed the slowest by Cu(II)(vbpy).[14]

Of more interest are the polymeric catalytic hydrolysis constants for these substrates. As expected, the polymeric catalysts' rates are faster than the spontaneous hydrolysis rates. More interesting, however, is that, for all three substrates, the polymer rates are also up to an order or magnitude higher than Cu(II)(vbpy) in solution. It was expected that the binding of the matrix forming TRIM would have adversely affected the geometry of the active site and decreased the ability of the substrates to optimally attach to the coordination complex. In addition, it was also likely that a portion of the Cu(II) sites would be buried within the polymer and be less accessible to substrate binding. We discuss below some possible bases for the apparent enhanced activity.

The hydrolysis rates observed maintain the same MeP > BNPP > NPP order of the monomeric solution complexes, Cu(II)(vbpy), except the rate enhancements are now between 3 to almost 6 orders of magnitude higher than the spontaneous rates. In comparison, a previously reported heterogeneous copper-containing polymer reported a 400-fold enhancement in hydrolysis of a phosphate triester over the background rate, while another group reported rate enhancements of about 600 and 300-fold for NPP and BNPP, respectively, using another cross-linked polymer system.[22] In contrast, we observe a maximum 6×10^5 increase here. The k_{cat}/K_M ratios also compare favorably with those of a previously reported *homogeneous* copper-containing polymer.[25] It is to be noted that our k_{cat} for MeP hydrolysis is 2.0×10^{-2} s^{-1}, a value that is only around 3 orders of magnitude slower than for naturally occurring phosphotriesterases.[28]

It is of further interest to compare K_m values for Cu(II)(vbpy) in solution with that of the polymer (Table 2). K_m can be defined operationally as the concentration of the substrate at which the catalysis rate is half of the maximum reaction velocity. We see that K_m, for each substrate, is lower for the polymers than for the solution complexes. While the difference is not significant for NPP, K_m for the BNPP polymer is more than one order of magnitude and that for MeP about two orders of magnitude lower than solution values. This means that, not only does the TRIM polymer possess a higher maximum rate of hydrolysis of phosphate esters than Cu(II)(vbpy) in solution, but that the polymer hydrolysis approaches the maximal rates at much lower concentrations of substrate than the corresponding monomeric complexes. For example, at a Cu(II) concentration of 1.16×10^{-5} M and an MeP concentration of 2×10^{-4} M, hydrolysis by the polymer occurs 50 times faster than by the Cu(II)(vbpy) monomer.

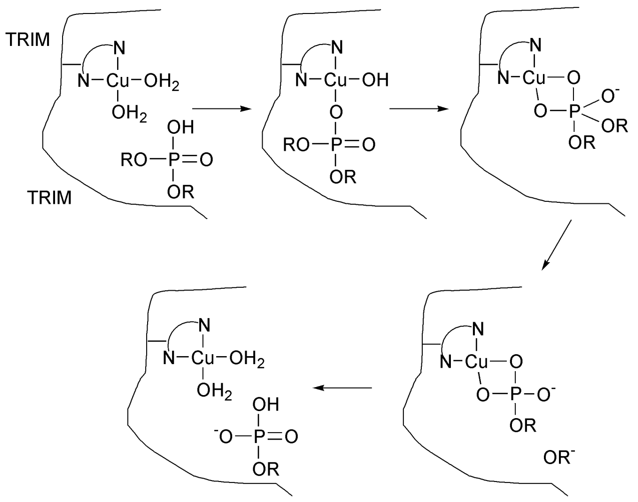
Given the reactivities observed, factors leading to the rapid rates of hydrolysis require some discussion. During the course of kinetic experiments, it was noted that, in the initial stages of experiments, the disappearance of substrate from solution occurred faster than product formation. Furthermore, the production of 4-nitrophenolate continued well beyond the time when substrate no longer remained in solution. Experiments carried out to measure the substrate concentrations immediately before and after contact with the Cu(II)(vbpy)-TRIM polymer showed significant amounts of substrate were immediately adsorbed from solution by the polymer. This effect was most noticeable for MeP, but still significant for BNPP and NPP. In fact, TRIM polymer, without Cu(II)(vbpy) also adsorbed substrate, although less efficiently for BNPP, and even less so for NPP.

The adsorption of substrate can be cited as the reason for the observed low K_m for the polymer. At low substrate concentration, adsorption by the polymer raises the local concentrations of phosphate esters on the polymer surface, where Cu(II) is also present in high concentrations. With all other factors being equal, this enhanced localized substrate concentration will lead to an increase in observed rates of reaction above that expected based on bulk substrate concentrations. Thus, reactions at low substrate concentrations are much more rapid for these polymers compared to their solution complexes, resulting in lower K_m.

Adsorption does not, however, satisfactorily explain the increased maximum rates. Inspection of the Lineweaver–Burk equation shows that an increase in localized substrate concentration will only affect the slope but not the intercept of the graph, which corresponds to V_{max}. In addition, factors such as heterogeneity, Cu(II) accessibility, and the adsorption of some 4-nitrophenolate by the polymer, should all retard the observed rates of reaction. Thus, the increase in rate must arise from differences in the microenvironment between polymer and solution systems. One possible mechanism for the enhancement was previously proposed by Morrow and Trogler (Scheme 1).[14] In this context, once the phosphate is bound, the reaction proceeds through a five coordinate phosphorane with the subsequent cleavage of the 4-nitrophenolate leaving group. The intermediate phosphorane is stabilized in the Cu(II)(vbpy)-TRIM polymer compared to in Cu(II)(vbpy) solution, as the alkyl groups of the ester are in the hydrophobic environment of the polymer surface rather than in aqueous solution as for the monomeric Cu(II)(vbpy). Other factors, such as differences arising from distortions of coordination geometry, could also affect rate enhancements. Subtle changes in coordination geometry around Cu(II) have been shown to lead to changes in hydrolytic reactivity.[11]

Table 2 Summary of apparent Michaelis constants, K_m

Substrate	Catalyst	K_m/M
NPP	Cu(vbpy) (solution)	1.2×10^{-3}
NPP	Cu(vbpy) (polymer)	8.3×10^{-4}
BNPP	Cu(vbpy) (solution)	2.3×10^{-3}
BNPP	Cu(vbpy) (polymer)	1.0×10^{-4}
MeP	Cu(vbpy) (solution)	4.4×10^{-3}
MeP	Cu(vbpy) (polymer)	5.1×10^{-5}



Scheme 1

In conclusion, a Cu(II)-containing polymer has been synthesized that shows enhanced catalytic activities over analogous homogeneous systems, with rates as high as five orders of magnitude above background hydrolysis. The polymer is, therefore, an efficient, heterogeneous, general catalyst for the hydrolysis of phosphate esters. In particular, these properties make this a promising material in the treatment of toxic phosphate triesters. Investigations continue into improving this reactivity further by using other complexes and polymer matrices. We are also continuing to assess the adsorption properties of the polymers, with and without metal catalysts incorporated, with a variety of substrates.

Experimental

Chemicals and reagents

4-Vinyl-4′-methyl-2,2′-bipyridine was prepared as previously described.[29] All other reagents and solvents were purchased from commercial sources and used as received.

Cu(II)(vbpy)-TRIM polymer

$Cu(NO_3)_2 \cdot 2.5\ H_2O$ (0.11 mmol) and 4-vinyl-4′-methyl-2,2′-bipyridine (vbpy) (0.11 mmol) were dissolved in ethanol (10 ml) and stirred for 5 min. TRIM (1.1 mmol) dissolved in ethanol (10 ml) was added and argon was bubbled through the solution with stirring at room temperature for 30 min. The solution was heated to 70 °C and 2,2′-azobisisobutyronitrile (0.01 mmol) added. The polymer began precipitating out of the reaction mixture after approximately 30 min. The reaction was cooled to room temperature after 90 min, filtered and washed thoroughly with ethanol to give the polymer as a pale blue solid. (171 mg).

Copper content of the polymer

This was determined by stirring 50 mg samples of the polymer in aqueous Na_2EDTA (3 ml, 0.1 M) overnight, measuring the resulting concentration of Cu(II)–EDTA complex using the visible absorbance at 734 nm and calculating the copper content of the polymer using a standardized curve.

Surface area measurements

Surface area and pore diameter measurements were performed on an ASAP 2010 chemisorption system (Micromeritics Instruments, Norcross, GA). Standard $N_{2(g)}$ adsorption analyses were carried out using the BET model for area and single-point pore volume calculations.

Particle sizing

A Coulter instrument (N4MD, Hialeah, FL) was used to measure the particle sizes of the polymer particles using dynamic light scattering. Data were analyzed in the histogram mode of the instrument. Samples were sonicated at room temperature in buffer for 5 min and the supernatant measured.

Kinetic measurements

Reactions were monitored spectrophotometrically at 402 nm, at which wavelength the concentration of 4-nitrophenolate, a product of hydrolysis for each substrate, could be measured. These measurements were made using a Hewlett Packard 8453 spectrophotometer. Reactions were carried out [20 °C, water–methanol (85 : 15), 0.100 M MOPS, pH 8.1] in duplicate and were not monitored past 5% completion. Data presented are the average of these experiments. For BNPP and NPP, samples for measurement were removed periodically from the reaction mixtures. For MeP, reactions were rapid and carried out in a cuvette. In this case no significant change in solution temperature occurred during the course of kinetic measurements. Corrections were made in each case for any light scattering due to polymer suspended in solution.

The copper concentrations for kinetic experiments using polymer were calculated using the copper content of the polymer and treating the reaction mixture as being homogenous. In order to compare polymer with solution catalysis, for each substrate, experiments were carried out with the same Cu(II) concentration whether using polymer or Cu(vbpy) monomer in solution. Because of lack of solubility and the different rates at which the substrates react, the same Cu(II) concentrations could not be used for the three substrates. The Cu(II) concentrations used were: for MeP, 1.16×10^{-5} M; for BNPP, 1.52×10^{-4} M; and for NPP, 7.86×10^{-4} M.

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