Synthetic dephosphorylation reagents: rate enhancement of phosphate monoester hydrolysis by Cu(II)-metallated adenine nucleobase polymers

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Significant rate enhancement of *p*-nitrophenyl phosphate hydrolysis, catalyst turnover and recycling has been observed for metallated, 9-allyladenine-containing cross-linked polymers.

Nucleic acids can coordinate to metal ions through the participation of base keto oxygen atoms, heterocyclic ring nitrogen atoms, sugar hydroxy groups and phosphate oxygen atoms.[1] The importance of metal ion binding is not simply limited to phosphate charge neutralization, but is also essential for stabilization of the nucleic acid structure and for RNA catalysis.[2,3] The design of our dephosphorylation agents has drawn inspiration from nucleic acids[4a–c] and we have invoked metal-ion coordination ability of purine nucleosides and nucleotides,[5a–h] for the synthesis of metallated, cross-linked nucleobase polymers. These polymeric molecules have been utilized for the hydrolysis of a model phosphate monoester and the kinetic parameters have also been determined.

Phosphate ester hydrolysis plays an important role in energy metabolism and in a variety of cellular signal transduction pathways in biological systems.[6a,b] The design of synthetic models of this reaction has been an attractive area of research and consequently, a wealth of information regarding artificial phosphatases and nucleases is available in literature.[7a–d] Most of these models utilize ligand bound transition and inner-transition metal ions for the catalysis of phosphate ester hydrolysis.[8a–k] It is assumed that metal ions play an important role in hydrolysis through the formation of metal–aqua complexes and by providing electrostatic neutralization of the negative charge on the phosphate group, thus making it more susceptible to nucleophilic attack.[8c] Owing to polarization effects, water molecules coordinated to metal ions can be substantially more acidic compared to free water molecules.[9]

The synthesis of our metallated nucleobase polymers involve AIBN-initiated polymerization of 9-allyladenine[10] with a cross-linking agent such as 1,4-divinylbenzene (DVB) or ethylene glycol dimethacrylate (EGDMA), in the presence of added metal ions. Adenine derivatives have been shown to coordinate to metal ions such as Cu^{2+}, Mn^{2+}, Zn^{2+} and Co^{2+}, predominantly through purine ring nitrogens.[5a–h] Keeping this fact in mind, we have prepared a polymeric matrix containing multiple adenine rings for extensive metal ion coordination. The metallated polymers so obtained were insoluble in common solvents and thus the present study is an example of heterogeneous catalysis of phosphate monoester hydrolysis. Atomic absorption spectroscopy and inductively coupled plasma analysis were used to estimate the amount of Cu(II) incorporated within the polymeric matrix (Table 1). It was found that incorporation of Cu(II) in EGDMA cross-linked polymer was 2.5 times that when compared to the DVB polymer. A much higher loading could be explained due to the presence of oxygen atoms in EGDMA, which can provide additional sites for metal ion coordination. Preliminary EPR studies have also been performed with these polymers.[11]

We have employed *p*-nitrophenyl phosphate (pNPP), a routinely used model monophosphate ester substrate, to evaluate the phosphatase activity of our adenine polymers and time-dependent release of *p*-nitrophenolate anion ($\varepsilon_{400} = 1.65 \times 10^4$ M^{-1} cm^{-1}) was used to determine kinetic constants. Remarkable rate enhancement was observed for pNPP hydrolysis in the presence of metallated polymers. The pseudo-first order rate constants (k_{obs}) were determined and it was found that polymers **1** and **2** displayed *ca.* 2,700- and 11,000-fold rate enhancement for pNPP hydrolysis (Table 1), respectively, as compared to the uncatalyzed reaction (4.92×10^{-7} min^{-1}, pH 7 at 25 °C[8h]). These observations prompted us to perform a more thorough kinetic evaluation and therefore, the Michaelis–Menten kinetic parameters for metallated adenine nucleobase polymers **1** and **2** were determined. Lineweaver–Burk plots (1/V *vs.* 1/[S], Fig. 1) were used to calculate Michaelis constants (K_m) and maximal velocities (V_{max}). For polymer **1**, containing DVB cross-linker, the K_m and V_{max} values were found to be 1.01 mM and 2.55×10^{-5} mM min^{-1}, respectively. While for polymer **2**, containing EGDMA cross-linker, the corresponding K_m and V_{max} values were found to be 0.21 mM and 4.6×10^{-5} mM min^{-1}, respectively (Table 2, Fig. 1).

Table 1 Estimated copper-loading and pseudo-first-order rate constants for pNPP hydrolysis in the presence of polymers **1** and **2**[a]

Polymer[b]	9-Allyladenine: cross-linker: Cu(II)	Mg of Cu(II) (g polymer)$^{-1}$ [c]	k_{obs}/min^{-1}	k_{rel}
1	DVB, 1:3:1	63.60	1.32×10^{-3}	2.7×10^3
2	EGDMA, 1:4:1	160.00	5.47×10^{-3}	1.1×10^4

[a] All hydrolytic reactions were performed in duplicate in 3 mL of 0.01 M *N*-ethylmorpholine buffer in 50% aqueous methanol (pH 8.0, 30 °C). [b] Polymer weights were 1 mg in 3mL of buffer, corresponding to 0.33 and 0.84 mM of Cu^{2+}, if polymers **1** and **2** were completely soluble in buffer and substrate concentrations were 3.3 and 8.4 mM, respectively. [c] Determined by AAS (AAS-300 Analyst, Perkin Elmer) and ICP (Integra XL, GBC) measurements.

We have also evaluated polymer **2** under turnover conditions[12e] by increasing the substrate concentration, while keeping the amount of polymer constant. Pseudo-first-order rates were determined and it was found that polymer **2** displayed efficient catalysis even in the presence of a 10-fold excess concentration of pNPP (Table 3). A unique feature of our

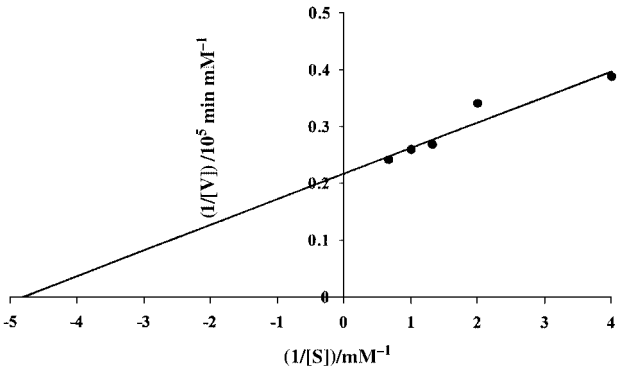

Fig. 1 Lineweaver–Burk plot of Cu(II)-metallated EGDMA cross-linked adenine nucleobase polymer **2**.

Table 2 Michaelis constants and maximal velocities of Cu(II)-containing adenine polymers for pNPP hydrolysis[a]

Polymer[b]	K_m/mM	V_{max}/mM min^{-1}
1	1.01	2.55×10^{-5}
2	0.21	4.6×10^{-5}

[a] All hydrolytic reactions were performed in duplicate in 5 mL of 0.01 M *N*-ethylmorpholine buffer in 50% aqueous methanol (pH 8.0, 30 °C), pNPP concentrations, [S]: 0.5–2.0 mM and 0.25–1.5 mM (for polymers **1** and **2**, respectively). [b] Polymer weights were 1 mg in 5 mL of buffer, corresponding to 0.2 and 0.5 mM of Cu^{2+}, for polymers **1** and **2** respectively, if the polymers were completely soluble in buffer.

Table 3 Turnover experiments[a]

Molar ratio of Cu(II) present in EGDMA adenine polymer[b]: pNPP	Pseudo-first-order rate constant k_{obs}/ min^{-1}
1:1	1.69×10^{-3}
1:3	2.39×10^{-3}
1:10	5.47×10^{-3}

[a] All hydrolytic reactions were performed in duplicate in 3 mL of 0.01 M *N*-ethylmorpholine buffer in 50% aqueous methanol (pH 8.0, 30 °C); [b] Polymer weights were 1 mg in 3 mL of buffer, corresponding to 0.84 mM of Cu^{2+}, if polymer **2** was completely soluble in buffer.

polymers is that they could be easily recycled. In a typical procedure, after pNPP hydrolysis, the reaction mixtures were centrifuged; polymers were filtered off and washed with copious volumes of 50% aqueous methanol. Washed and dried polymers were then reused for the catalysis of a subsequent hydrolytic reaction. Both of the polymers were reused thrice and the initial velocities, depending on the release of *p*-nitrophenolate anion, were found to be similar to the values obtained by using fresh polymeric catalysts (data not shown).

It is tempting to attribute a differential rate enhancement between the two polymers to the high loading of Cu(II) in polymer **2**. Unmetallated, cross-linked adenine polymers failed to catalyze pNPP hydrolysis over an extended period of time (data not shown), thereby indicating a crucial role of coordinated metal ion in accelerating the hydrolytic reaction. There are some literature reports that describe polymer-based, non-enzymatic hydrolysis of activated phosphate esters and RNA.[4c,12a–f] We have also introduced a novel matrix of cross-linked nucleobase polymers and have exploited metal coordination capability of adenine nucleobase for phosphate monoester hydrolysis. Turnover and recycling experiments indicate that these molecules are robust, possess high catalytic efficiency, and are amenable to recycling. Importantly, the heterogeneous nature of our catalyst can be utilized for its convenient removal at the completion of reaction.

The precise mechanism of monophosphate ester hydrolysis by metallated nucleobase polymers is unclear at the present time. Experiments are underway to elucidate the mechanism and to evaluate the catalytic assistance of these polymers for the hydrolysis of amides, esters, dinucleotides and nucleic acids. Moreover, we are also in the process of developing soluble, nucleobase containing polymeric matrices for effecting homogeneous catalysis of the above mentioned reactions.

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10 9-Allyladenine was prepared by reacting adenine (1 equiv.) with allyl bromide (0.9 equiv.) in the presence of sodium hydride (1.2 equiv.), using anhydrous DMF as the solvent at 30 °C for 12 h. Silica gel column chromatography (R_f 0.31, ethyl acetate) afforded the pure compound, which was characterized by ^{1}H NMR and MS [EI] *m*/*z* 175 (M^+, base peak).
11 Preliminary EPR measurements, at liquid nitrogen temperature (77 K), suggest the presence of rhombic symmetry for the EGDMA cross-linked polymer **2**, while isotropic symmetry and indication of interacting Cu(II) centers were observed for DVB cross-linked polymer **1**. Detailed EPR-based structural investigations and determination of magnetic properties of these polymers are currently in progress.
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