Modeling of Prebiotic Catalysis with Adenylated Polymeric Templates: Crystal Structure Studies and Kinetic Characterization of Template-Assisted Phosphate Ester Hydrolysis

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This paper is dedicated to Prof. Dr. Fritz Eckstein on the occasion of his 70th birthday

Abstract: We have synthesized and characterized novel, copper-metalated, polymeric templates that contain adenine nucleobases. These promote hydrolysis of non-natural and natural phosphate ester substrates in a highly efficient and catalytic fashion. The crystal structure of the copper-containing adenylated monomer reveals the formation of a polymeric array, through coordination to both N1 and N7 atoms. Possible implications of these studies for prebiotic catalysis, involving synergism between adenine and copper ions, are also discussed.

Keywords: adenine · copper · heterogeneous catalysis · prebiotic · polymerization

Introduction

A hypothetical scenario of a prebiotic RNA world, in which RNA served as a genetic template and possessed catalytic features necessary for self-replication, has long been postulated.[1] However, due to the lack of a credible mechanism for prebiotic RNA synthesis and RNA's inherent instability, it seems likely that life processes originated with a nucleic-acid-like polymer possessing the desired templating and catalytic features.[2] Although artificial selection has discovered nucleic acid enzymes with predetermined catalytic activities,[3] a plausible mechanism for de novo nucleic-acid template synthesis still remains elusive. In this context, numerous chemical and biochemical studies under simulated prebiotic conditions have appeared, thanks to pioneering efforts by Orgel, Miller, Ferris, and others.[4]

Studies pertaining to prebiotic catalysis and the chemical origin of life have attracted considerable attention over the years.[5] One theme of these investigations relates to the preponderance of homogenous versus heterogeneous catalysis in the primordial era. In this context, Bernal pointed towards the possible assistance of mineral surfaces for biological polymerization reactions.[6] Towards this end, adsorption of adenine and other nucleotides on graphite, zeolites, feldspars, and montmorillonite, and a favorable assistance from Cu^{II} for optimal binding to the mineral surfaces have already been documented.[7]

In general, mimicry of artificial nucleases employs ligands of synthetic or natural origin, in conjunction with an appropriate metal ion, to accomplish phosphate ester hydrolysis.[8, 9] Reaction catalysis is mostly homogenous; however, some reports have also documented the use of these reagents in a heterogeneous environment.[10] Most of the reported examples use small, monomeric ligands that are often not suited to mimic the intricate three-dimensional architectures of biomolecules, such as protein enzymes. We have recently described the activity of nucleobase-containing polymeric frameworks, which conform to classical Michaelis–Menten kinetics, for the hydrolysis of activated phosphate esters.[11] Interestingly, one such template also displayed a remarkable enzyme-like activity for oxygen insertion in an aromatic C–H bond,[12] a reaction catalyzed by hydroxylase/oxidase protein enzymes and required for the biosynthesis of the essential amino acid tyrosine and catecholamine neurotransmitters.[13] As a bio-inspired approach, we report the construction of two new adenylated polymeric templates and their catalytic assistance of 2′,3′-cyclic adenosine monophosphate (2′,3′-cAMP) cleavage. Thorough kinetic analyses have been performed to confirm the true catalytic nature of metalated polymeric adenylated templates, and preliminary attempts have been made to understand the underlying mechanism, by employing activated phosphate esters.

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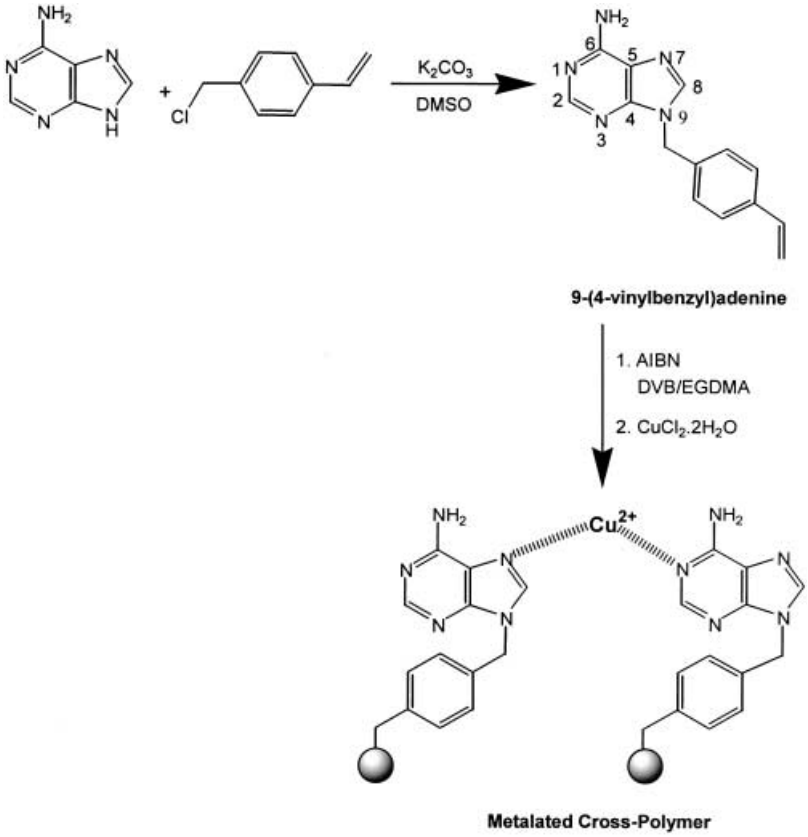
Results and Discussion

Syntheses and characterization: 9-(4-Vinylbenzyl)adenine (9-VBA) was synthesized by treatment of 4-vinylbenzyl chloride with adenine, in the presence of potassium carbonate (Scheme 1). The vinyl group was introduced in order to achieve more effective polymerization than obtained with the allylated monomer used by us in previous studies,[11] which resulted in a better product yield. 9-VBA was co-polymerized either with 1,4-divinylbenzene or with ethyleneglycol dimethacrylate in the presence of azobis(isobutyronitrile) (AIBN), followed by metalation with $CuCl_2$ in methanol. The extent of nucleobase incorporation in templates **1** (DVB) and **2** (EGDMA) was determined by elemental analysis, while the amount of copper in the metalated templates was estimated by atomic absorption spectroscopy (AAS). The coordination around copper in the polymeric matrix was partially characterized by EPR spectroscopy. Templates **1** and **2** were found to display isotropic symmetry with $g_{iso} = 2.112$ and 2.120, respectively (Figure 1). Both the templates **1** and **2** were hydrophobic and completely insoluble in common solvents. To make the polymer compatible with hydrolysis in aqueous buffer, it was necessary to pre-wet it with the minimum possible volume of methanol. Thus, catalysis of phosphate ester cleavage was heterogeneous in nature.



Scheme 1.

X-ray structural studies: Metal–nucleobase coordination complexes have received considerable attention over the past several decades. Several strategies, pioneered by Lippert and others, have used such complexes to generate novel coordination architectures.[14] We have determined the X-ray crystal structure of the 9VBA-Cu complex in order to ascertain the nature of coordination around the copper atom. Green, plate-like 9VBA-Cu crystals were grown, with *N*,*N*-dimethyl formamide (DMF) as a solvent and hexane as a precipitant. The ORTEP diagram (Figure 2) of the unit cell shows two pentacoordinate copper atoms. Coordination occurs through the ring nitrogen atoms of modified adenine, two chlorine atoms, and an oxygen atom of DMF. The geometry around both copper atoms is distorted trigonal bipyramidal, with nitrogens N19 and N21 occupying the axial positions around Cu11, and N11 and N29 around Cu12. The equatorial positions are taken up by Cl11, Cl12, and O11, and by Cl21, Cl22, and O21, respectively. The axial angles N19-Cu11-N21 and N11-Cu12-N29 are close to linear, with angles of 170.3(3)° and 168.6(3)°, respectively. Distortions of the two copper centers from perfect trigonal bipryamidal geometry are evident from the equatorial angles Cl1-Cu11-O (95.2(17)° and 155.8(18)°), Cl2-Cu12-O (94.9(18)° and 158.0(18)°), and Cl-Cu-Cl (108.9(8)° and 107.1(8)°). The rest of the angles subtended at copper centers are close to right angles, as expected for trigonal bipyramidal geometry. The distortion may be due to the steric bulk of the vinylbenzyl substituent. Important bond lengths and bond angles are listed in Table 1. Simultaneous coordination to N1 and N7 has been observed before in Pt^{II}-metalated modified purine crystal structures.[15] In addition, coordination to other nitrogen atoms has also been observed for various copper-metalated adenine crystal structures.[16]

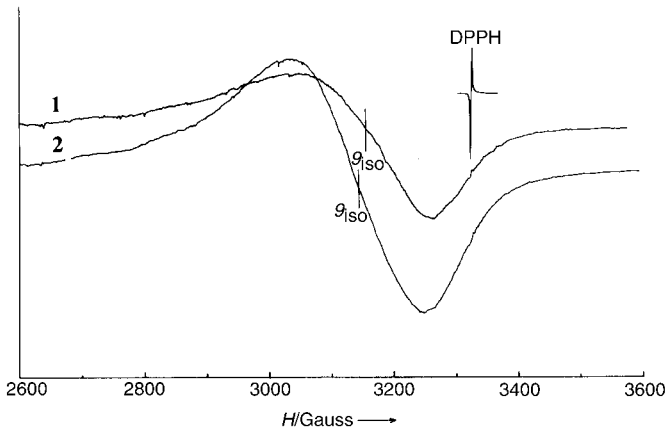


Figure 1. Solid-state EPR spectra for templates **1** and **2** at room temperature.

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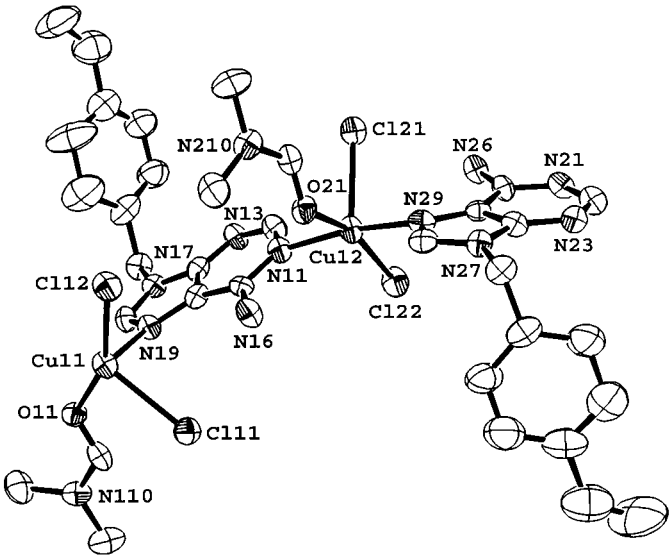

Figure 2. POVRAY-rendered ORTEP diagram showing the asymmetric unit of 9VBA-Cu complex. Hydrogen atoms are excluded for clarity.

Table 1. Important bond lengths [Å] and angles [°] for 9VBA-Cu complex.[a]

Cu11–O11	2.000(5)	N11–Cu12	2.027(6)
Cu11–N19	2.026(6)	Cu12–O21	2.014(5)
Cu11–N21$^{\#1}$	2.037(6)	Cu12–N29	2.015(6)
Cu11–Cl12	2.274(2)	Cu12–Cl22	2.270(2)
Cu11–Cl11	2.572(2)	Cu12–Cl21	2.576(2)
O11-Cu11-N19	87.8(2)	O21-Cu12-N29	88.1(2)
O11-Cu11-N21$^{\#1}$	87.3(2)	O21-Cu12-N11	87.6(2)
N19-Cu11-N21$^{\#1}$	170.3(3)	N29-Cu12-N11	168.6(3)
O11-Cu11-Cl12	155.83(18)	O21-Cu12-Cl22	157.96(18)
N19-Cu11-Cl12	92.44(18)	N29-Cu12-Cl22	91.69(19)
N21$^{\#1}$-Cu11-Cl12	88.63(19)	N11-Cu12-Cl22	88.38(19)
O11-Cu11-Cl11	95.20(17)	O21-Cu12-Cl21	94.86(18)
N19-Cu11-Cl11	94.40(19)	N29-Cu12-Cl21	95.34(18)
N21$^{\#1}$-Cu11-Cl11	94.3(2)	N11-Cu12-Cl21	95.5(2)
Cl12-Cu11-Cl11	108.86(8)	Cl22-Cu12-Cl21	107.09(8)

[a] Symmetry transformations used to generate equivalent atoms: #1: $x-1$, y, z; #2: $x+1$, y, z.

A notable feature of the 9-(4-vinylbenzyl)adenine–copper complex is that it extends into a coordinated polymeric array assisted by metal centers (Figure 3). Such an extended framework bears a striking resemblance to the periodicity of single-strand nucleic acids. In this case, modified adenine monomers appear to be tethered noncovalently through the participation of copper ions, and this assembly extends in the solid-state solely through coordination to copper ions, which act in a fashion analogous to the phosphodiester bridges in natural nucleic acids.

It is highly plausible that heterocyclic nucleobases, containing various metal ion coordination sites, could have been assembled in situ with the aid of metal ions, in the absence of *N*-glycosyl substituents and the phosphodiester backbone. Therefore, such nucleobase assemblies could have served the role of protonucleic acids to accomplish initial, primordial templating and catalytic reactions.

Furthermore, modified adenine has been cross-polymerized and post-synthetically metalated to yield metalated templates.[7] This modification was introduced to mimic adenine adsorbed on mineral surfaces, and it ensured that any resulting catalytic assistance was heterogeneous in nature. Analogously to mineral surfaces, the polymeric matrix provided a constellation of adenine residues and a synthetic scaffold for nucleobase/metal-ion coordination. All of the kinetic studies were performed with the metalated polymeric template to exploit their reusability[11] and are reported in subsequent sections.

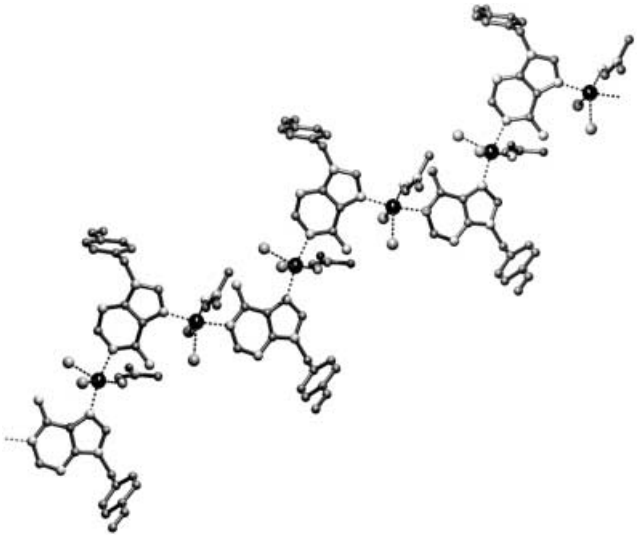

Figure 3. POVRAY diagram showing the polymeric structure of the 9VBA-Cu complex. Hydrogen atoms are omitted for clarity.

Efforts have been made to understand the coordination around copper in metalated templates, as compared with copper in the complex, by employing spectroscopic techniques. The solid-state EPR spectrum of the monomeric copper complex displayed an axial symmetry with $g_{\parallel} = 2.160$ and $g_{\perp} = 2.044$ (Figure 4), while both the polymeric templates displayed isotropic symmetry (Figure 1). Diffuse reflectance UV/visible spectra were recorded for the complex as well as for the templates **1** and **2**. The polymeric templates were found to follow similar UV/Vis patterns, with a d–d transition at 795 nm, ligand-to-metal charge transfer (LMTC) at 420 nm, and a $\pi-\pi^*$ transition at 282 nm. Though there was no change in LMTC and $\pi-\pi^*$ transitions for the copper–adenine complex, the d–d transition was blue-shifted (717 nm). This observation suggests a subtle difference between the coordination environments of copper at the monomer and at the polymer level, which is not quite clear in the latter case.

Kinetic investigation of phosphate ester hydrolysis: Kinetic parameters have been derived for the hydrolysis of activated phosphate esters, such as *p*-nitrophenyl phosphate (*p*NPP), bis(*p*-nitrophenyl) phosphate (bNPP) and 2-hydroxypropyl-*p*-nitrophenyl phosphate (hNPP), under catalysis by templates **1** and **2**. *p*NPP is an example of a phosphate monoester, bNPP is a phosphodiester, while hNPP is a phosphate diester that also serves as an RNA model, owing to the presence of an internal hydroxyl group. Hydrolytic reactions catalyzed by copper-adenylated templates were monitored at 400 nm as a function

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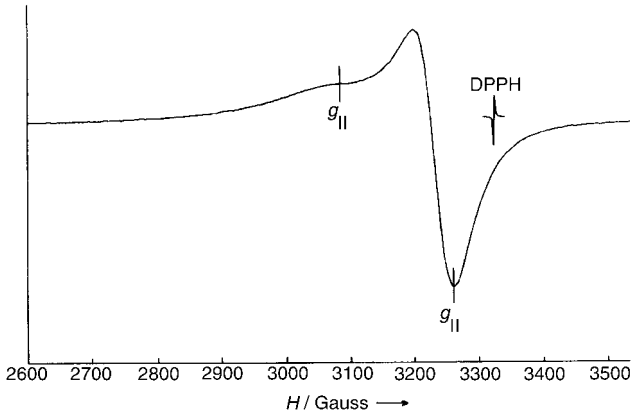


Figure 4. Solid-state EPR spectrum of the 9VBA-Cu complex.

of release of *p*-nitrophenolate anion with respect to time ($1.65 \times 10^4\,\text{M}^{-1}\,\text{cm}^{-1}$).

Significant rate acceleration was observed for the hydrolysis of activated phosphate esters when catalyzed by templates **1** and **2**, relative to uncatalyzed reaction. Pseudo-first-order rate constants (k_{obs}) for template-assisted hydrolysis were determined from plots of $\ln [A_\infty/(A_\infty - A_t)]$ versus time (Table 2). For *p*NPP hydrolysis, k_{obs} was found to be 3.52×10^{-4} and $5.11 \times 10^{-4}\,\text{min}^{-1}$ for templates **1** and **2**, corresponding to rate enhancements of 7.15×10^2- and 1.04×10^3-fold,

Table 2. Pseudo-first-order rate constants for templates **1** and **2**.[a]

Template	Substrate[b]	k_{obs} [min^{-1}]	k_{rel}[c]
1	*p*NPP	3.52×10^{-4}	7.15×10^2
	bNPP	1.23×10^{-4}	1.58×10^5
	hNPP	2.57×10^{-3}	1.30×10^3
2	*p*NPP	5.11×10^{-4}	1.04×10^3
	bNPP	1.68×10^{-4}	2.15×10^5
	hNPP	3.54×10^{-3}	1.79×10^3

[a] All hydrolytic reactions were performed in duplicate in 3 mL of 0.01 mM *N*-ethylmorpholine buffer in 10% aqueous methanol (pH 8.0, 30 °C). The reference cell contained substrate without polymer to correct for background hydrolysis. The concentration of templates **1** and **2**—pre-wetted with methanol (15 μL)—was 1 mg mL^{-1}. [b] Concentration of *p*NPP, bNPP, and hNPP was 5.0 mM, 5.0 mM, and 3.0 mM, respectively. [c] k_{rel} is with respect to the uncatalyzed reaction rate.

respectively, relative to the uncatalyzed reaction.[17] The values of k_{obs} for the hydrolysis of bNPP by **1** and **2** were found to be 1.23×10^{-4} and $1.68 \times 10^{-4}\,\text{min}^{-1}$, respectively, with relative rate enhancements relative to the uncatalyzed reaction of 1.58×10^5- and 2.15×10^5-fold, respectively.[17] Similarly, the k_{obs} values for the hydrolysis of hNPP with catalysis by templates **1** and **2** were determined to be 2.57×10^{-3} and $3.54 \times 10^{-3}\,\text{min}^{-1}$, respectively, corresponding to rate enhancements relative to the uncatalyzed reaction of 1.30×10^3- and 1.79×10^3-fold.[18] The higher rate enhancement observed with template **2** than with **1** may be attributed to higher copper loading in template **2**.

Both of the templates followed Michaelis–Menten saturation kinetics, as was confirmed by plotting substrate concentration versus initial velocity (Figure 5). Templates **1**

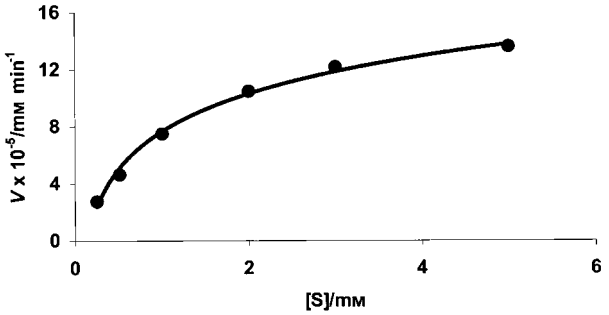


Figure 5. Michaelis–Menten saturation kinetics for bNPP hydrolysis catalyzed by template **2**.

and **2** were subjected to thorough kinetic analysis in order to determine kinetic parameters for the hydrolysis of activated substrates (Table 3, Figure 6), by means of Lineweaver–Burk plots ($1/V$ against 1/[S]). For *p*NPP hydrolysis catalyzed by

Table 3. Kinetic parameters for templates **1** and **2**.[a]

	Substrate	K_m [mM]	V_{max} [mM min^{-1}]	k_{cat} [min^{-1}]	$(k_{cat}/K_m)/k_{uncat}$ [M^{-1}]
1[b]	*p*NPP	1.18	4.01×10^{-5}	5.51×10^{-5}	9.49×10^4
	bNPP	0.62	9.79×10^{-5}	1.34×10^{-4}	2.77×10^8
	hNPP	2.95	1.71×10^{-3}	2.34×10^{-3}	4.01×10^5
2[c]	*p*NPP	1.42	6.23×10^{-5}	6.29×10^{-5}	9.00×10^4
	bNPP	1.33	1.72×10^{-4}	1.75×10^{-4}	1.69×10^8
	hNPP	1.12	8.70×10^{-4}	8.79×10^{-4}	3.96×10^5

[a] All hydrolytic reactions were performed in duplicate in 3 mL of 0.01 mM *N*-ethylmorpholine buffer in 10% aqueous methanol (pH 8.0, 30 °C). The reference cell contained substrate without polymer to correct for background hydrolysis. Template concentrations were 1 mg mL^{-1}, corresponding to 0.73 and 0.99 mM of copper if the polymeric templates **1** and **2** were completely soluble in the buffer. [b] [S] concentration ranges of *p*NPP, bNPP, and hNPP for **1** were 1.0–5.0 mM, 0.50–4.0 mM, and 0.20–1.0 mM, respectively. [c] [S] concentration ranges of pNPP, bNPP, and hNPP for **2** were 1.50–3.0 mM, 0.25–3.0 mM, and 0.80–1.60 mM, respectively.

template **1**, K_m, V_{max}, and k_{cat} were found to be 1.18 mM, 4.01×10^{-5} mM min^{-1}, and $5.51 \times 10^{-5}\,\text{min}^{-1}$, while with **2** the parameters were 1.42 mM, 6.23×10^{-5} mM min^{-1}, and $6.29 \times 10^{-5}\,\text{min}^{-1}$, respectively. Similarly, K_m, V_{max}, and k_{cat} values for the hydrolysis of bNPP by **1** and **2** were found to be 0.62 mM, 9.79×10^{-5} mM min^{-1}, and $1.34 \times 10^{-4}\,\text{min}^{-1}$ for **1** and 1.33 mM, 1.72×10^{-4} mM min^{-1}, and $1.75 \times 10^{-4}\,\text{min}^{-1}$ for **2**. These parameters were also determined for the hydrolysis of RNA model substrate hNPP with catalysis by templates **1** and **2** to give 2.95 mM, 1.71×10^{-3} mM min^{-1}, and $2.34 \times 10^{-3}\,\text{min}^{-1}$ for **1**, and 1.12 mM, 8.70×10^{-4} mM min^{-1}, and $8.79 \times 10^{-4}\,\text{min}^{-1}$ for **2**.

The catalytic proficiency ($[k_{cat}/K_m]/k_{uncat}$), which is a measure of an enzyme's ability to lower the activation energy of the reaction, was also calculated for these templates for the hydrolysis of activated substrates (Table 3).[19] These values indicate that both templates have high affinities for the substrates *p*NPP, bNPP, and hNPP, at concentrations of 10^{-3}, 10^{-7}, and 10^{-4} M, respectively.

As in our previous studies with allyl polymers, the hydrolytic activity of templates **1** and **2** was found to be pH dependent, with hydrolytic rates peaking at nearly pH 8.3

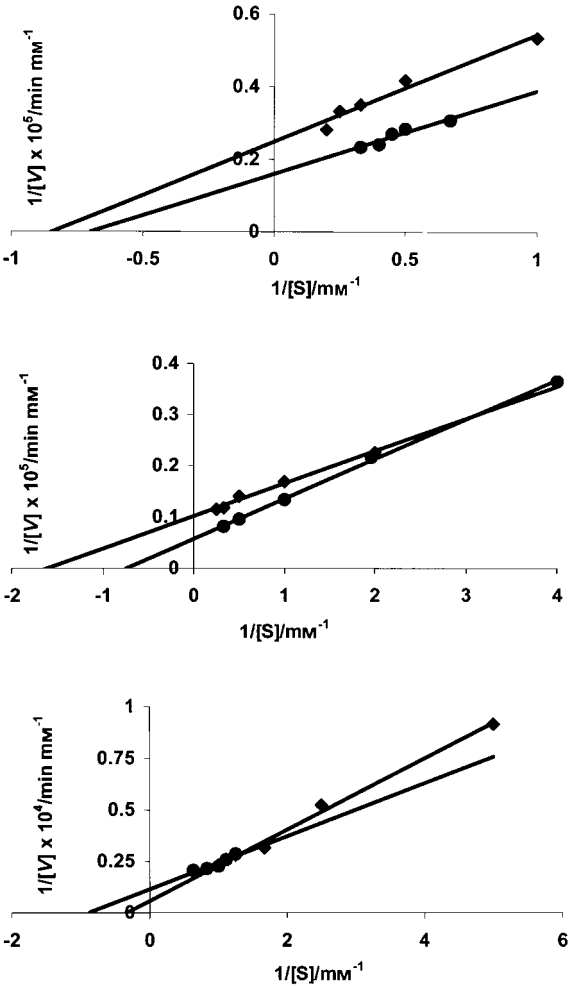
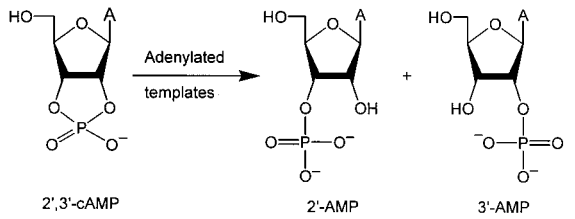


Figure 6. Lineweaver–Burk plots for hydrolysis of *p*NPP (top), bNPP (middle), and hNPP (bottom) by templates **1** (◆) and **2** (●).

(data not shown). While solvent studies confirmed preferential involvement of metal–hydroxy intermediates in phosphate ester hydrolysis, a linear dependence of assisted hydrolysis rate with respect to temperature revealed the high thermal stability and activity of these templates (data not shown).

Cleavage of 2′,3′-cAMP: We have employed 2′,3′-cAMP to evaluate the catalytic potential of our templates toward natural, unactivated substrate (Scheme 2). Adenylated templates **1** and **2** catalyzed the hydrolysis of 2′,3′-cAMP with appreciable rate enhancement and regioselectivity. Hydrolytic reactions were performed in *N*-ethylmorpholine buffer (pH 8.0) at 30 °C, and the appearance of product was monitored by C-18 reversed-phase HPLC (Figure 7).[20a]



Scheme 2.

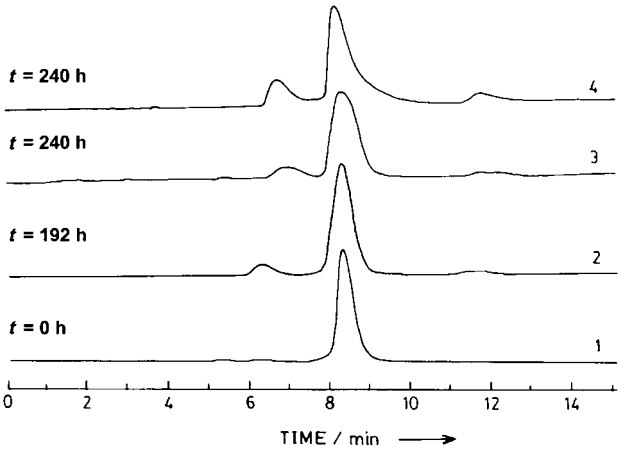


Figure 7. HPLC traces for the cleavage of 2′,3′-cAMP at pH 8.0 and 30 °C. 1: 2′,3′-cAMP; 2: cleavage of 2′,3′-cAMP by template **2**; 3: cleavage of 2′,3′-cAMP by template **1**; 4: recycled template **1**. Retention times: 3′-AMP 6.91 min; 2′,3′-cAMP 8.33 min; 2′AMP 11.95 min.

First-order rate constants (k_{obs}) for templates **1** and **2** were 2.43×10^{-6} and $4.06 \times 10^{-7}\ s^{-1}$, respectively, which corresponded to enhancements of ≈1600- and 270-fold relative to the uncatalyzed reaction.[20b] Furthermore, the template-assisted hydrolytic reaction led to preferential formation of 3′-AMP as the major regioisomer, in 70% yield.

All cleavage reactions were performed under heterogeneous conditions, since the polymers are insoluble in aqueous buffer. Consequently, attempts were made to recycle the polymeric template for subsequent cleavage reactions. In a simple procedure, after one complete cleavage reaction, the polymer was filtered, washed with aqueous buffer, methanol, and acetone, and air-dried. In one such recycling experiment, template **1** was reused for 2′,3′-cAMP hydrolysis and, interestingly, the extent of cleavage for the recycled template remain unchanged when compared to the fresh catalyst (Figure 7). The amounts of copper in **1** and **2** after one cycle of hydrolytic cleavage of 2′,3′-cAMP were found to be 37.0 and 42.0 mg per gram of polymer, respectively. Atomic absorption spectroscopy estimated similar copper contents for fresh and recycled polymer, suggesting a lack of change in copper-ion content during hydrolytic reactions. This observation was further reinforced by arrest experiments as detailed below.

The reusability of the polymeric templates indicates that such constructs act as rugged, insoluble scaffolds for holding multiple adenine residues and copper ions. The rate enhancements obtained with our polymers are comparable to those given in recent reports,[20a,c] except for dinuclear and trinuclear homogeneous copper complexes, for which relatively higher rate accelerations have been achieved.[20b,c] However, the rare reusability feature of these templates clearly suggests a rugged and catalytic nature of our systems.

^{31}P NMR spectroscopy: Efforts were made to establish a working mechanism for these templates by study of assisted hydrolysis of hNPP. This reaction could yield either a monophosphorylated product, through an intermolecular nucleophilic attack, or a cyclic phosphate, through an intramolecular attack. It has already been shown for certain artificial systems

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that metal-ion-promoted hNPP hydrolysis proceeds through an intramolecular pathway to yield a cyclic phosphate.[21] Exclusive, time-dependent formation of a cyclic phosphate product as a result of template-mediated hNPP hydrolysis was detected in this study, thus suggesting the activation of the internal hydroxyl group by metalated templates for transesterification (Figure 8). However, in the absence of an

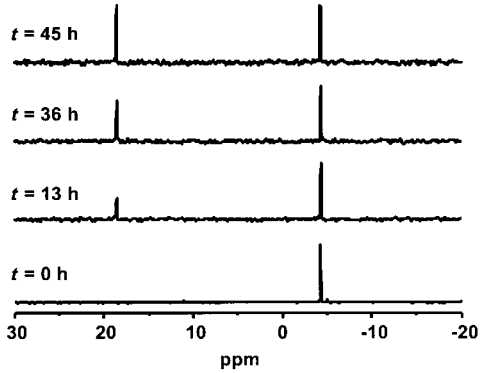

Figure 8. ^{31}P NMR spectra for the hydrolysis of hNPP by template **2**. Chemical shifts for hNPP and for the cyclic phosphate product are -4.32 ppm and 18.58 ppm, respectively.[21d] The chemical shifts are with respect to 85 % H_3PO_4 as external reference.

internal nucleophile for 2′,3′-cAMP, an intermolecular attack by metal-bound hydroxyl seems a probable mechanism. A Fenton-type, free-radical-mediated cleavage of activated non-natural phosphate esters has already been ruled out for such polymeric templates.[11a] However, it is worth mentioning that these templates might display an alternative mechanism of action while interacting with natural substrates.

Arrest experiments: Implications for a crucial role of coordinated copper ions was obtained from "reaction arrest" experiments. In this assay, **2** was incubated with bis(*p*-nitrophenyl) phosphate (bNPP) as a model phosphodiester substrate for 6 h, followed by its removal by filtration. This resulted in the complete abrogation of bNPP hydrolysis when observed over a time period of 75 h (Figure 9), thus indicating

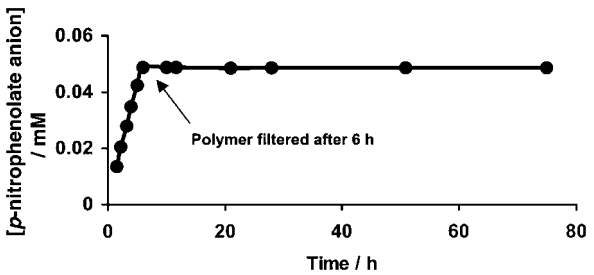

Figure 9. Reaction arrest experiment for bNPP hydrolysis by template **2**.

intricate involvement of *coordinated* copper ions, and not the leached out ions, for the catalytic activity of the polymeric templates. As control reactions, nonmetalated template or copper salt alone did not support 2′,3′-cAMP hydrolysis under the reaction conditions.

Rate inhibition by vanadate ions: Phosphatases are inhibited by vanadate ions, as they mimic the transition state of phosphate ester hydrolysis.[22] We have studied the inhibitory effect of vanadate ion on phosphate ester hydrolysis catalyzed by our templates, and Lineweaver–Burk plots were consequently derived for bNPP hydrolysis catalyzed by **2** in the presence or absence of ammonium vanadate (0.10 mM) at pH 8.0 (Figure 10). The values of K_m and V_{max} in the presence

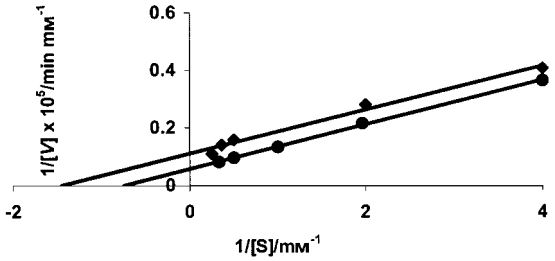

Figure 10. Lineweaver–Burk plots for bNPP hydrolysis by template **2**: (●) in absence of inhibitor; (◆) in the presence of inhibitor.

of inhibitor were found to be 0.69 mM and 8.99×10^{-5} mM min^{-1}, respectively, and in its absence 1.33 mM and 1.72×10^{-4} mM min^{-1}. The kinetic data obtained suggest an uncompetitive mode of inhibition by vanadate ions, with $K_i = 0.11$ mM. A precise explanation for this uncompetitive inhibition is not available at present.

Conclusion

Curiously, the crystal structure of metalated vinylbenzyl adenine monomer has the form of an extended strand-like structure, which bears a resemblance to single-strand nucleic acids. Copper ions act as tethers (or the backbone), in the absence of sugar residues and phosphodiester linkage, to stabilize the metalated assembly.

The adenylated polymeric templates described here are insoluble due to cross-linking, and act heterogeneously to accomplish non-natural and natural phosphate ester hydrolysis. Their prolonged and sustained reactivity, as demonstrated by recycling experiments and reaction arrest assay, indicate that they can retain metal-ion-dependent catalytic properties for long durations, without altered reaction rates. Hence, we propose that these insoluble templates, containing multiple adenine residues, mimic metal-coordinated adenine aggregates or primordial adenine-based oligomers adsorbed on mineral surfaces. Favorable Cu^{II} interactions observed in this study also allude to synergistic interactions between nucleobases and metal ions in the prebiotic era for catalyzing fundamental biochemical reactions.[23] Interestingly, a modified adenosine moiety has previously been examined for its role in catalyzing ester hydrolysis.[24]

In short, the metalated adenine-containing polymers described in this report combine a scaffold for substrate binding and a nucleobase matrix for copper-ion coordination, to produce a catalytically active, nucleic-acid-like polymeric template with a biological function. It is proposed that studies with such model constructs might provide further insight into

primitive organization of nucleic acid constituents and into their cooperative interaction with metal ions for prebiotic catalysis. Another intriguing possibility is that such metal–nucleobase assemblies could have functioned as short, prebiotic templates for recognition of other bases and amino acids, for subsequent primordial oligomerization reactions. We are currently evaluating these templates for nucleic acid modification, while template-assisted biosynthetic reactions have also been envisaged.

Experimental Section

Instrumentation: HPLC assay was performed on a Perkin–Elmer 785 A UV/Vis detector. ^{1}H and ^{31}P NMR spectra were recorded on a JEOL-JNM LAMBDA 400 model operating at 400 and 161.7 MHz, respectively. EPR spectra were recorded on a Varian 109E Line Century Series X-band spectrometer. The amount of copper in the metalated nucleobase polymer was determined by AAS spectrometry (GBC), at FEAT Laboratory, IIT-Kanpur. Elemental analyses and mass spectra were recorded at the Regional Sophisticated Instrumentation Centre, Lucknow (India). Reaction kinetics were examined on a Shimadzu UV-160 UV/Vis spectrophotometer.

Chemicals and Reagents: 4-Vinylbenzyl chloride, ethyleneglycol dimethacrylate (EGDMA), and 1,4-divinylbenzene (DVB) were purchased from Fluka (Switzerland). 2′,3′-cAMP was purchased form Sigma. Azobis(isobutyronitrile) (AIBN; Ajax Chemicals, India) was recrystallized from methanol. Copper chloride·2H$_2$O (Merck, India), ammonium vanadate (S.D. Fine Chemicals, India) and *p*NPP (SRL, Mumbai) were used as supplied. Potassium carbonate was purchased from Lancaster (England). The sodium salt of bNPP (Fluka, Switzerland) was prepared from the commercial sample and used for kinetic assays. *N*-Ethylmorpholine, methanol (spectroscopic grade), and ethyleneglycol were from S.D. Fine Chemicals (India) and were distilled prior to use. 2-Hydroxypropyl-*p*-nitrophenyl phosphate (hNPP) was synthesized by the literature procedure.[25] Triply distilled water was used in all assays, and HPLC grade solvents were used for HPLC analysis. All solvents were distilled or dried when necessary by general procedures.

9-(4-Vinylbenzyl)adenine (9-VBA): 4-Vinylbenzyl chloride (0.5 mL, 3.5 mmol, 0.9 equiv) and K_2CO_3 (0.61 g, 4.4 mmol, 1.2 equiv) were stirred in anhydrous DMSO (15 mL) at 30 °C for 5 h, under a nitrogen atmosphere. The solvent was evaporated under reduced pressure, and silica gel column chromatography afforded the pure compound [$R_f = 0.6$ (ethyl acetate/methanol 9:1)] in 71% yield. M.p. 202 °C; ^{1}H NMR (400 MHz, [D$_6$]DMSO, 25 °C, TMS): $\delta = 5.2$ (d, ^3J(H,H) = 11 Hz, 1H; C–H), 5.4 (s, 2H; CH_2), 5.8 (d, ^3J(H,H) = 18 Hz, 1H; C–H), 6.7 (dd, ^3J(H,H) = 18, 11 Hz, 1H; C–H), 7.3 (d, ^2J(H,H) = 8 Hz, 2H; ArC–H), 7.4 (d, ^2J(H,H) = 8 Hz, 2H; ArC–H), 8.1 (s, 1H; ArC–H), 8.2 (s, 1H; ArC–H), 7.2 ppm (br; NH_2); ^{13}C NMR: δ = 45.8, 114.5, 118.6, 126.3, 127.8, 136, 136.5, 136.6, 140.7, 149.4, 152.5, 155.9 ppm; MS [EI]: *m*/*z* (%): 251 (10) [M^+], 250 (54) [$M^+ - H$].

9VBA-Cu complex: 9VBA (0.10 g, 0.4 mmol, 1.0 equiv) was dissolved in methanol/chloroform (4:1; 5.0 mL), and $CuCl_2 \cdot 2H_2O$ (0.035 g, 0.20 mmol, 0.5 equiv) was added to this solution. The reaction mixture was stirred for 2 h at room temperature. A brownish-green precipitate was formed, and this was isolated by filtration and washed with methanol (25 mL), chloroform (25 mL), and acetone (25 mL). The complex was dried under vacuum. Crystals suitable for X-ray studies were grown in *N*,*N*-dimethylformamide by diffusion. A green plate crystal was coated with Paratone 8277 oil (Exxon) and mounted on a glass fiber. All measurements were made on a Nonius Kappa CCD diffractometer with graphite monochromated Mo-Kα radiation.

Crystal data for the 9VBA-Cu complex: $C_{40}H_{54}Cl_4Cu_2N_{14}O_4$: $M_r = 1063.85$, triclinic, space group $P\bar{1}$, $a = 13.255(7)$, $b = 13.680(5)$, $c = 14.500(8)$ Å, $\alpha = 67.16(2)$, $\beta = 89.80(2)$, $\gamma = 88.46(3)^\circ$, $V = 2422.1(2)$ Å^3, $\rho_{calcd} = 1.459$ Mg m^{-3}, $T = 173(2)$ K, $Z = 2$, $\mu(Mo_{K\alpha}) = 1.15$ mm^{-1}, 20508 reflections measured, 10984 unique ($R_{int} = 0.13$), final $R1 = 0.091$, $wR2 = 0.212$ [$I > 2\sigma(I)$], $R1 = 0.223$ and $wR2 = 0.262$ (all data). The structure was solved with SIR92 and expanded by Fourier techniques. Non-hydrogen atoms were refined anisotropically, while hydrogen atoms were included at geometrically idealized positions. The final cycle of full-matrix, least-squares refinement by use of SHELXL97[26] converged with unweighted and weighted agreement factors, $R = 0.091$ and $wR = 0.262$ (all data), respectively, and goodness of fit, $S = 0.998$.

CCDC 181927 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via www.ccdc.cam.ac.uk/conts/retrieving.html (or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336033; or deposit@ccdc.cam.uk).

Cross-polymers of 9VBA with DVB and EGDMA: AIBN-initiated free-radical polymerization was employed for the synthesis of nucleobase cross-polymers. 9-VBA (1.0 g, 4.0 mmol, 1 equiv), DVB (0.23 mL, 0.8 mmol, 0.2 equiv) or EGDMA (0.15 mL, 0.8 mmol, 0.2 equiv), and AIBN (15 mg) were taken up in dry DMSO (8.0 mL) and the reaction mixture was purged with oxygen-free N_2 gas for 45 min. Polymerization was performed at 80 °C for 18 h in the presence of DVB and 6 h in the presence of EGDMA. A white gel was formed in both cases, and this was filtered, washed with hot DMSO (5 × 20 mL), methanol (5 × 20 mL), and acetone (3 × 20 mL), and dried under vacuum. The amounts of nucleobase cross-polymer obtained with DVB and with EGDMA were 0.85 g and 1.05 g, respectively.

Copper-metalated cross-polymer of 9VBA and DVB (template 1): Unmetalated template **1** (0.75 g) and $CuCl_2 \cdot 2H_2O$ (0.51 g) were taken up in methanol (25 mL). The reaction mixture was allowed to stir at room temperature for 24 h. Metalated polymer was isolated by filtration, washed with methanol (4 × 25 mL) and acetone (3 × 10 mL), powdered, and dried under vacuum. Template **1** was obtained as a green, amorphous powder (0.84 g) and was found to be insoluble in common solvents.

Copper-metalated cross-polymer of 9VBA and EGDMA (template 2): Unmetalated template **2** (0.80 g) and $CuCl_2 \cdot 2H_2O$ (0.543 g) were taken up in methanol (25 mL). The reaction mixture was allowed to stir at room temperature for 24 h. Metalated polymer was isolated by filtration, washed with methanol (4 × 25 mL) and acetone (3 × 10 mL), powdered, and dried under vacuum. Template **2** was obtained as a green, amorphous powder (0.99 g) and was found to be insoluble in common solvents.

Characterization of templates 1 and 2: Powder EPR spectra at room temperature indicated the presence of isotropic symmetry for templates **1** and **2**, with $g_{iso} = 2.112$ and 2.120, respectively. Elemental analyses found (%): Template **1**: C 61.11, H 9.85, N 17.01; Template **2**: C 53.29, H 13.52, N 16.26. It is thus determined from elemental analyses that every gram of templates **1** and **2** contains 610 mg (2.43 mmol) and 583 mg (2.32 mmol) of monomer, respectively. Copper in the polymeric matrices was estimated by AAS and was found to be 46.3 and 63.1 mg per gram of polymer for **1** and **2**, respectively.

General assay for the hydrolysis of *p*NPP, bNPP, and hNPP by 1 and 2 (spectrophotometric method): All hydrolytic reactions were performed in duplicate in centrifuge tubes thermostatted at 30 °C. The assay mixture contained 3 mL of substrate solution of appropriate concentration, prepared in 0.01 M *N*-ethylmorpholine buffer (pH 8.0) in 10% aqueous methanol. The amount of polymer was 1 mg mL^{-1} of buffered substrate solution, and the concentration of the catalyst corresponded to the amount of copper present in the polymeric matrix. The polymers were pre-wetted with methanol (15 μL). Initial velocities were determined as a function of time-dependent release of *p*-nitrophenolate anion ($\varepsilon_{400} = 1.65 \times 10^4$ M^{-1}cm^{-1}). Michaelis–Menten parameters were calculated from corresponding Lineweaver–Burk plots. Pseudo-first order rate constants were derived from $\ln [A_\infty/(A_\infty - A_t)]$ against time plots, where A_∞ and A_t are the absorbances at infinite time and at time *t*. All hydrolytic reactions were performed over at least four half-lives of each substrate.

^{31}P NMR experiment for hydrolysis of hNPP by 1 and 2: Hydrolytic reactions were performed in 1.0 mL *N*-ethylmorpholine buffer in 10% aqueous methanol (0.01 M, pH 8.0, 30 °C). The concentration of the substrate, hNPP, was 5 mM and the polymer weight was 3 mg mL^{-1} for **1** and **2**. ^{31}P NMR spectra were recorded in solutions of reaction mixture and D_2O (2:1) at appropriate time intervals, and the reported chemical shifts are with respect to 85% H_3PO_4 (40 mM) standard, in the same buffer.

Cleavage of 2′,3′-cAMP: Cleavage of cyclic phosphate catalyzed by templates **1** and **2** was monitored by HPLC. In a typical procedure, 2′,3′-cAMP solution (1.0 mM, 1.0 mL) in *N*-ethylmorpholine buffer (0.01 M in 10% aqueous methanol, pH 8.0), was stirred in an Eppendorf tube at 30 °C.

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Polymer weights for **1** and **2** were 5 mg mL^{-1} and 3 mg mL^{-1} of buffer, respectively. The reaction mixture was centrifuged and filtered to remove any particulates. Samples (20 μL) were analyzed on a C-18 RP-HPLC column (100 mm × 4.6 mm, Brownlee). An isocratic gradient of 95% A and 5% B [A = KH_2PO_4 (10 mM); B = methanol/water (3:2)] was used for elution and the eluents were monitored at 260 nm. First-order rate constants were obtained as the slopes of plots of ln (A_0/A_t) against time, where A_0 and A_t are the integrations of the area of the HPLC peak for 2′,3′-cAMP at time = 0 and t, respectively.

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