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Modeling prebiotic catalysis with nucleic acid-like polymers and its implications for the proposed RNA world*

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Abstract: The theory that RNA molecules played a pivotal role in the early evolution of life is now widely accepted. Studies related to this hypothetical "RNA world" include three major areas: the formation of precursors for the first RNA molecules, the polymerization process, and the potential of RNA to catalyze chemical and biochemical reactions. Several chemical and biochemical studies performed under simulated prebiotic conditions support the role of RNA as both genetic as well as catalytic material. However, owing to the lack of credible mechanism for de novo nucleic acid synthesis and the hydrolytic instability of RNA molecules, there has been some serious discussion of whether biopolymers that closely resembled nucleic acid preceded the "RNA world". In this context, an overview of prebiotic chemistry, the role of mineral surface, and the significance of studies related to RNA-like polymers in the origin of life are presented here.

INTRODUCTION

Though life that exists today and in the geological record is based on DNA genomes and protein enzymes, there is convincing evidence that hypothesizes the origin of life based on RNA [1]. This hypothetical primordial era is referred to as the "RNA world" [2]. The "RNA world" hypothesis assumes that all modern organisms are descendants of a prebiotic self-replicating moiety, which utilized RNA as both genetic as well as catalytic material. The discovery of catalytic RNA molecules (ribozymes) by Cech and Altman provided the impetus for an extensive discussion on the role of RNA, in the origin of life [3,4]. Over the years, scientists have been accumulating evidences in support of RNA-based early life. The isolation protocol, SELEX (systematic evolution of ligands by exponential enrichment) has been extensively employed in the in vitro selection of RNA sequences with precise molecular recognition and catalytic properties from a pool of randomized sequences [5–7].

Addressing questions regarding the sources of small organic molecules that composed the first self-replicating system and the mechanism of evolution of cell-like enclosure from an abiotic supply of biomonomers has been an important aspect of study on the origin of life [8,9]. Numerous chemical and biochemical studies pertaining to the chemical origin of life, performed under simulated prebiotic conditions, have appeared owing to pioneering efforts by Orgel, Ferris, Miller, and others [10–13]. One theme of these investigations relates to the preponderance of homogenous vs. heterogeneous catalysis in the primordial era. The aggregation of biological precursors into prebiotic biopolymers was puzzling until Bernal suggested that adsorption of organic molecules onto mineral surface would have catalytic relevance to the emergence of prototypical polymeric templates [14]. Studies related to

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the aggregation and entrapment of nucleobases on natural mineral surfaces reinforce the potential role of natural minerals acting as scaffolds and aiding in the catalysis of reactions necessary for the evolution of early life [15].

Although RNA molecules have been recognized to play an important role in contemporary biology, the difficulty finding a credible mechanism for prebiotic RNA synthesis has lead to the speculation of RNA as the first genetic material. Indeed, most researchers in this field propose that life processes would have originated from nucleic acid-like polymers possessing desired templating and catalytic features [16–18]. The era of these RNA-like polymers is sometimes also referred to as the "pre-RNA world", which probably gave rise to the "RNA world" (Fig. 1). Therefore, identification and construction of a system that closely resembles RNA, capable of self-replication and possessing catalytic domains, would shed more light on the origin of life processes. This approach has met with substantial progress in recent years and has led to systematic investigation of naturally occurring nucleic acid analogs containing various sugars and linkage isomers that potentially form intriguing pairing systems [19]. The most notable candidate of all is the threose nucleic acid (TNA) [20], which pairs with itself and with RNA. Other pre-RNA precursors are peptide nucleic acid (PNA), glycerol-derived nucleic acid analogs and pyranosyl RNA (p-RNA) [21–23]. Although several nucleic acid analogs with modified base, sugar, and phosphate backbone have been developed [24], fewer attempts have been made for the preparation of nucleic acid-like polymers devoid of sugar moiety and phosphodiester linkage [25]. We recently reported our attempt to model prebiotic catalysis using RNA-like nucleobase polymers devoid of phosphate-sugar backbone, instead possessing a nonhydrolyzable alkyl backbone and also containing a constellation of metal-coordination sites in the polymeric matrix. These metallated polymers displayed significant catalytic efficiency toward phosphate ester hydrolysis and oxygen insertion reactions [26].

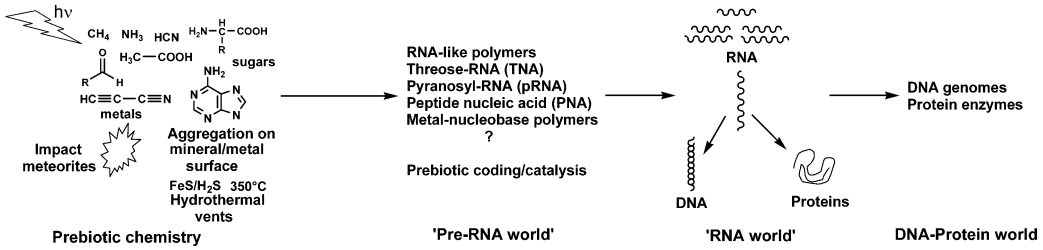

Fig. 1 Probable events related to the origin of life.

RNA in contemporary biology

The "RNA world" hypothesis hinges on the assumption that RNA first promoted the reactions required for the origin of early life with the help of nucleobases, amino acids, metals, and other cofactors. When the metabolism became more complex, RNA translated its genetic role to DNA, while its catalytic property was transferred to the proteins. However, RNA still retains some important role in contemporary biology. It acts as a primer in DNA replication, a messenger that carries genetic information to the translation machinery and a catalyst that directs the processing of the pre-mRNA. Catalytic RNAs (ribozymes) have been identified in RNA processing events and replication of viral genomes. Group I introns were the first catalytic RNA molecules to be discovered [3]. The fundamental function of group I and group II introns concerns the self-splicing of non-coding regions of pre-mRNA sequence to yield processed mRNA. Ribonuclease P RNA is an endonuclease RNA motif that specifically hydrolyzes phosphodiester bond of the precursor sequences from the 5'-pre-tRNAs, to produce mature 5'-termini tRNAs [4]. Unlike introns, it is truly catalytic exhibiting multiple turnovers in vivo and in vitro. Hammerhead ribozyme is the smallest catalytic RNA containing around 30 nucleotides, having the ability to cleave any target RNA molecule [27]. It catalyzes the hydrolysis of phosphodiester bond in a site-specific manner, forming a 5'-hydroxyl and a 2',3'-cyclic phosphate product.

In vitro selection of ribozyme

In recent years, biochemists have come to appreciate RNA as a versatile biocatalyst in view of its complex tertiary structure, which is analogous to structured proteins. In vitro RNA selection technique, which gives an opportunity to explore the repertoire of RNA-catalyzed reactions, has been successfully used in the isolation of many new ribozymes (Fig. 2). These include RNA motifs that catalyze nucleotide synthesis by glycosidic bond formation [28], RNA polymerization [29], aminoacylation of transfer RNA [30], peptide bond formation [31], Diels–Alder reaction [32,33], hydroxyl phosphorylation [34], and Michael reaction [35]. Virtually all redox reactions in modern organisms are catalyzed by protein enzymes. However, Suga and coworkers showed that RNA molecules are also able to promote such reactions by directed evolution of a NAD^+-dependent redox-active ribozyme mimicking alcohol dehydrogenase activity [36]. Such a redox ribozyme would have been extremely essential for RNA-based metabolism in the early emergence of life.

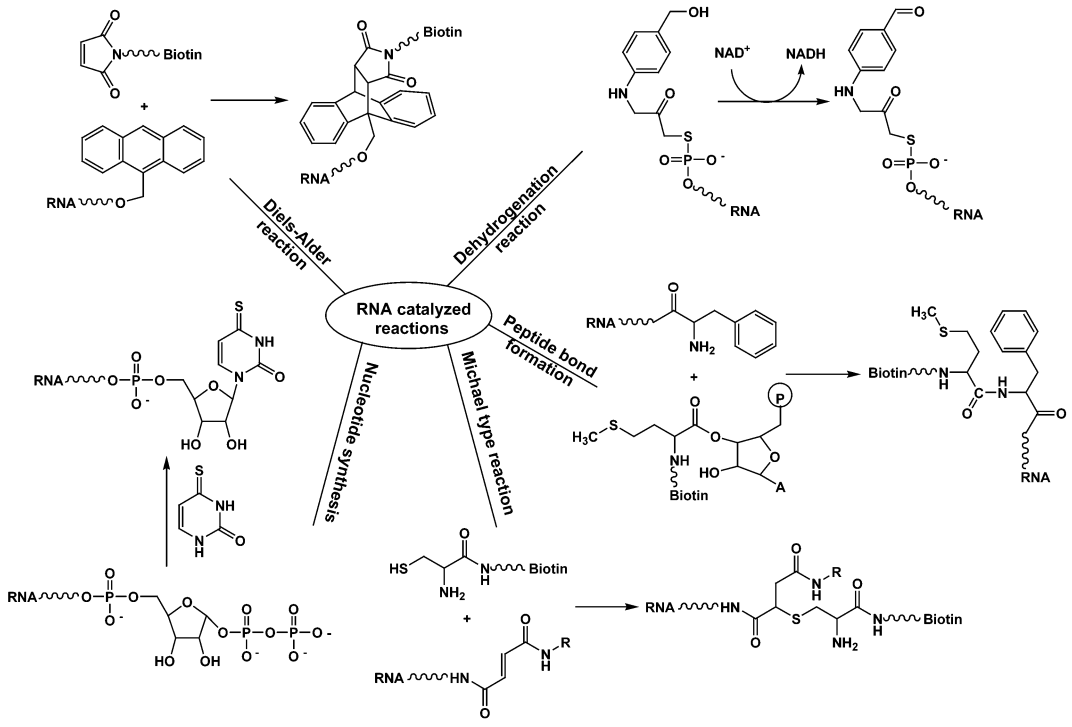

Fig. 2 Representative examples of in vitro selected ribozymes.

Prebiotic chemistry

The scientific community has a divided opinion on the composition of the primitive atmosphere that led to the formation of the prebiotic soup of biomonomers, varying from strongly reducing conditions to near neutral conditions. Many theories with conflicting evidence have appeared in the search for the source of the abiotic supply of small biomonomers that made up the first self-replicating system. A proposed theory of origin of early life from the cosmic kitchen elaborates that the soup of organic monomers was formed by the action of light on the reducing atmosphere. Attempts to simulate prebiotic chemistry resulted in the much celebrated "rock-of-faith" experiment of amino acid synthesis by Miller by the action of electric discharge [37,38]. Urey–Miller experiments led the way for some intense exploration toward the synthesis of biological precursors under prebiotic conditions. Interestingly, Oro and Kimball synthesized adenine from hydrogen cyanide and ammonia [39]. Later, Orgel and

coworkers showed that cyanoacetylene, a potential precursor for pyrimidine bases was identified upon action of electric discharge on a mixture of methane and nitrogen [40]. Recently, Zubay and Mui showed that adenine could be synthesized by using ammonium formate instead of hydrogen cyanide, making the conditions more prebiotic [41].

Atmospheric chemists, who dismiss the possibility of reducing environment, believe that the bulk of the organic monomers were brought to the earth by meteorites and comets [42,43]. This theory is interesting because meteorites and comets contain substantial amount of polycyclic aromatic hydrocarbons, carboxylic acids, amino acids, and nitrogen heterocycles [38]. It was hard to imagine life deep beneath the sea, where there is no source of energy in the form of light, until the discovery of hydrothermal vents in the late 1970s. The existence of an ecosystem under such extremely hostile conditions (extremophiles) [44] led to the proposition that life probably originated from deep sea hydrothermal vents [45]. However, chemically, the chances of life originating at deep sea thermal vents are unlikely. It is known that organic molecules are unstable at high temperatures, and are destroyed as quickly as they are produced [46]. Nevertheless, supporters of this theory claim that the organic molecules at the thermal vents were not formed in 300 °C temperatures, but rather in a gradient formed between the hydrothermal vent and the extremely cold water, which surrounds the vent at the bottom of the ocean [47]. In a nutshell, there are three popular theories of prebiotic origin of biomonomers—cosmic theory, impact theory, and vent theory—but none is entirely convincing. It is more likely that more than one source of organic molecules would have assisted in the emergence of the first coding system.

Role of minerals in prebiotic chemistry

The organization of biomonomers into polymers capable of self-replication relies on the mechanism of concentrating the basic ingredients from vastly diluted early oceans. Long ago, Bernal suggested that polymerization may have taken place with the aid of clay minerals, which would have provided a surface for the adsorption of organic molecules [14]. He argued that clay minerals would have played a vital role in the synthesis of large prebiotic molecules by providing a means of concentrating the monomers on a heterogeneous surface. Toward this end, the high affinity of purine and pyrimidine bases for mineral surfaces like graphite [48], clay [49], zeolites, and dealuminated feldspars have been documented [15].

It is believed that polymers with lengths in the range of 30–60 monomers are required for a viable genetic as well as catalytic system [50]. Ferris and Ertem came up with regiospecific oligoribonucleotide synthesis on montmorillonite clay [51]. The condensation reaction of 5'-phosphorimidazolide-activated adenosine in the presence of Na^+-montmorillonite resulted in the formation of oligomers up to decamer. The montmorillonite-catalyzed reaction was highly specific, with 85 % of the oligomer containing 3',5'-linkage. Attempts were also made to synthesize longer oligomers by successive feeding with the monomer. In this strategy, a radio-labeled decanucleotide, $[^{32}P]dA(pdA)_8pA$ adsorbed on Na^+-montmorillonite, was periodically fed with activated adenosine. Polyadenylates containing more than 20 nucleotides were formed after two feedings, while polyadenylates of more than 50 monomers were formed after 14 feedings, with major oligomeric products being 20–40 units long [52]. However, in all of these experiments, adsorbed oligomers did not show replicating ability. Nevertheless, this feeding protocol models prebiotic polymerization, wherein the mineral surface experiences the sporadic input of low concentrations of monomers. Ertem and Ferris also demonstrated template-directed synthesis of heterogeneous oligonucleotide on the montmorillonite, but it was not regiospecific [53,54].

Porter and coworkers invoked metal-clay synergism to investigate the oligomerization of amino acids and activated nucleotides on clay mineral Cu(II)-exchanged hertorite by using scanning force microscopy [55]. Under simulated prebiotic heating and wetting cycles, the clay absorbs, concentrates, and subsequently catalyzes the polymerization of monomers into polypeptides and oligonucleotides. The presence of Cu(II) cations within the clay cavities was found to be crucial for the observed reaction.

Compartmentalization of replicating nucleic acid is very important to enable Darwinian evolution [56]. Szostak and coworkers reported unusual interaction behavior between mineral surfaces and membrane-forming amphiphiles. They demonstrated that montmorillonite clay markedly accelerates the conversion of fatty acid micelles into vesicles [57]. Interestingly, these clay particles were found to be encapsulated in the so-formed vesicles, thereby setting a pathway for the prebiotic encapsulation of catalytically active surfaces within membrane vesicles. This observation was further strengthened when fluorescently labeled RNA adsorbed on montmorillonite particles was added to micelles, the RNA-loaded particles were found to be encapsulated inside the resulting vesicles. Going further, Szostak and his colleagues showed that the vesicles formed could grow by incorporating fatty acids supplied as micelles and also could divide when forced through small-pore membrane filters. These results in principle demonstrate that vesicle growth and division can result from simple physicochemical interactions, without any complex biochemical machinery, and, hence, a similar scenario of rudimentary compartmentalization and propagation can be envisioned in the primordial era.

RNA-LIKE POLYMERS

Owing to the lack of a credible mechanism for de novo nucleic acid template synthesis, difficulty in forming the glycosidic bond for the nucleotide synthesis under prebiotic conditions, and inherent instability of RNA molecules, it is more likely that biopolymers closely resembling RNA and with relatively simple chemistry would have dominated the early Earth [16–18]. Eschenmoser and coworkers systematically studied the properties of nucleic acid analogs in which the sugar backbone was replaced by pyranose (p-RNA) or threose form (TNA, Fig. 3) [19]. β-hexopyranosyl-(6',4')-linked oligonucleotide analogs synthesized from the hexose sugars such as allose, altrose, and glucose displayed inferior base-pairing as compared to natural RNA. This observation was attributed to the steric encumbrance offered by the fully hydroxylated hexopyranosyl sugar units [58]. Consequently, Eschenmoser's group turned their focus toward sterically less hindered pentopyranose sugars. Interestingly, diastereomeric pentopyranosyl-(4',2')-linked oligonucleotide systems formed Watson–Crick paired double he-

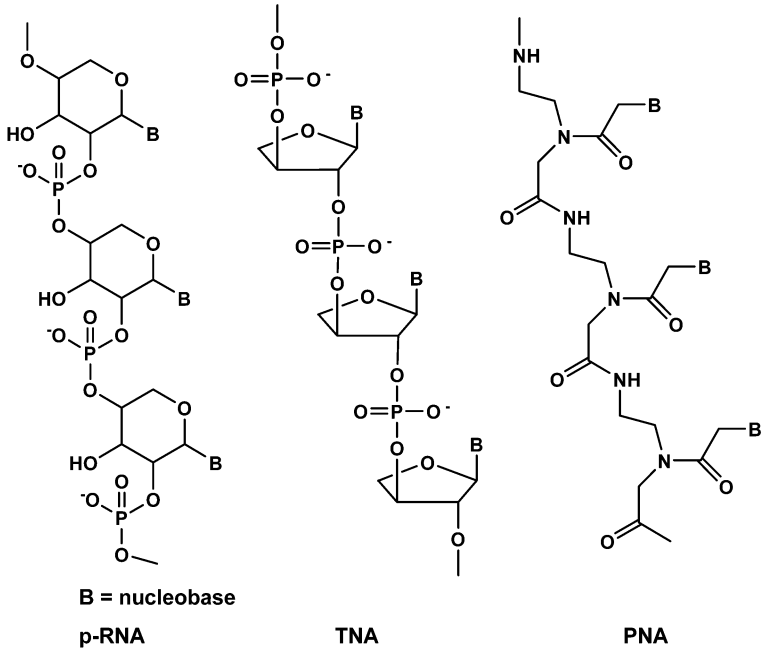

Fig. 3 Nucleic acid analogs.

lices that were more stable than RNA [59]. The threose form, α-threofuranosyl oligonucleotides (TNAs) containing vicinally connected 3',2'-phosphodiester bond display efficient base-pairing, which is similar to that of p-RNA [60]. Strikingly, TNA oligonucleotides were found to cross-pair with RNA and DNA and were also hydrolytically much more stable than RNA. Therefore, TNA could potentially serve as a template in nonenzymatic template-directed oligomerization of RNA, although this is yet to be tested. This behavior of TNA would mean that a transition from a TNA world to an "RNA world" is possible. Though prebiotic synthesis of nucleic acid analogs is not likely going to be easier, TNA can be conceivably a potential primitive replicating system with relatively simple chemistry and also containing hydrolytically stable phosphodiester backbone.

Peptide nucleic acid (PNA) is another potential predecessor of RNA [61]. PNA is an uncharged, achiral analog of RNA or DNA in which the ribose-phosphate backbone is replaced by aminoethyl glycine units (Fig. 3). PNA forms stable double helices with RNA or DNA [62,63], and information can be transferred from PNA to RNA in a template-directed fashion [64]. However, PNA monomers are susceptible to intramolecular *N*-acyl transfer reaction, which would make oligomer formation difficult under prebiotic conditions [65]. Considering the chemical nature of PNA monomers, it is unlikely that PNA would have been very important in the early existence of life. It is also important to mention that the catalytic potential of PNA, TNA, and other possible RNA analogs has not yet been explored in detail.

SYNTHETIC NUCLEIC ACID-LIKE POLYMERS

Several nucleic acid analogs with modified base, sugar, and phosphate backbone have been developed [24]. However, relatively few attempts have been made for the preparation of nucleic acid-like polymers devoid of sugar moiety and phosphodiester linkage [25]. Pitha, Overberger, and other workers have reported the synthesis of several nucleic acid mimics containing nonhydrolyzable polymeric backbone for a variety of applications [66,67]. These include: interference with cell-free protein synthesis [68], antiviral activity [69], optically active polymers [70], and base-specific detection of oligonucleotides [71]. More recently, nucleobase-functionalized polythiophenes containing adenine and uracil have been synthesized by electrochemical oxidation and their molecular recognition process was studied [72]. However, the catalytic potential of these non-natural nucleobase polymers has not been investigated in detail. Komiyama and coworkers demonstrated ribonuclease activity of a vinyladenine and vinylamine copolymer [73]. The catalytic activity of the copolymer was considerably higher than poly(vinylamine), indicating a significant role for adenine residues in the polymer-assisted hydrolysis of poly(adenylic acid). Maurel and coworkers conjugated adenine residues to a polyallylamine backbone, followed by the evaluation of its catalytic ability for the hydrolysis of *p*-nitrophenyl acetate [74].

Nucleic acids can coordinate to metal ions through the participation of base keto oxygen atoms, heterocyclic ring nitrogen atoms, sugar hydroxyl groups, and phosphate oxygen atoms [75]. We have drawn inspiration from the nucleic acid analogues [73–75] and the known metal-ion coordination ability of purine nucleosides and nucleotides [76,77], in constructing nucleic acid-like homo- and cross-polymers capable of catalyzing phosphate ester hydrolysis and phenol oxidation reactions (Fig. 4).

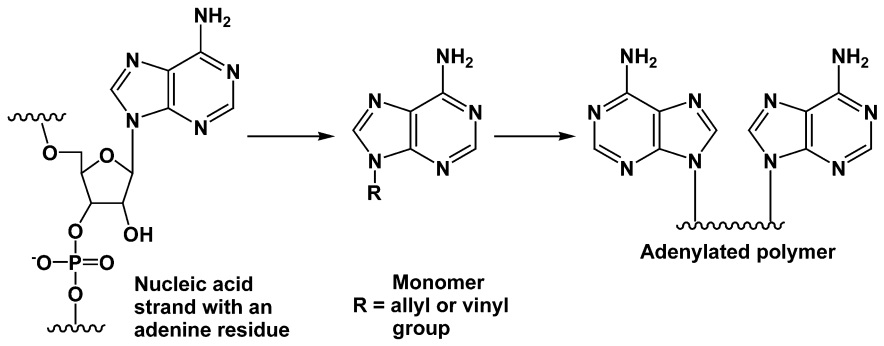

Fig. 4 Design strategy for polymeric matrices.

We replaced the sugar residue at N9 position by an allyl (**9AA**) [78] and vinylbenzyl (**9VBA**) [79] group to generate a molecular framework containing multiple adenine residues in the polymeric matrix, connected by a nonhydrolyzable backbone. Such polymeric matrices, devoid of sugar residue and phosphate backbone, are expected to be more stable in routinely used reaction media. Copper metalation afforded a constellation of metal centers within the polymeric matrix (Fig. 5) [80,81].

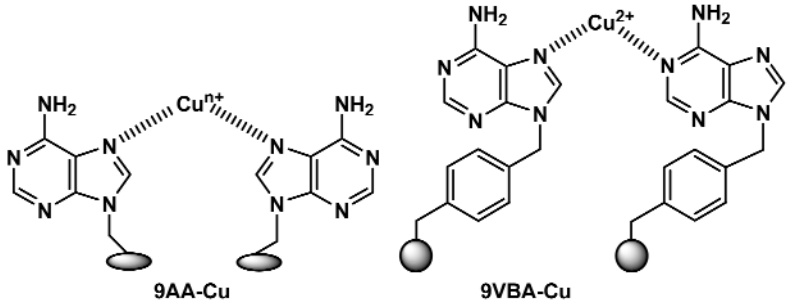

Fig. 5 Allyl and vinylbenzyl-modified adenine polymers

Several strategies pioneered by Lippert and others have used metal-nucleobase coordination complexes to generate novel coordination architectures [82]. X-ray crystal structure of **9AA-Cu** and **9VBA-Cu** monomer-metal complexes was determined to ascertain the coordination environment around the copper atom. Crystal structure of **9AA-Cu** revealed a dimeric copper complex where two allyladenine molecules were coordinated to copper through the N7 nitrogen atoms, while in **9VBA-Cu** complex copper was found to be coordinated to the monomer through both N1 and N7 nitrogen atoms. Simultaneous coordination to N1 and N7 has been observed before in Pt(II)-metallated modified purine crystal structures [83]. In addition, coordination to other nitrogen atoms has also been observed for various copper-metallated adenine crystal structures [84]. A notable feature of **9VBA-Cu** complex is that it extends as a one-dimensional coordinated polymeric array assisted by metal centers (Fig. 6) [85]. Such an extended framework bears a striking resemblance to the periodicity of single-stranded 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 by coordinating to the copper ions, which act in a fashion analogous to phosphodiester bridges in nucleic acids.

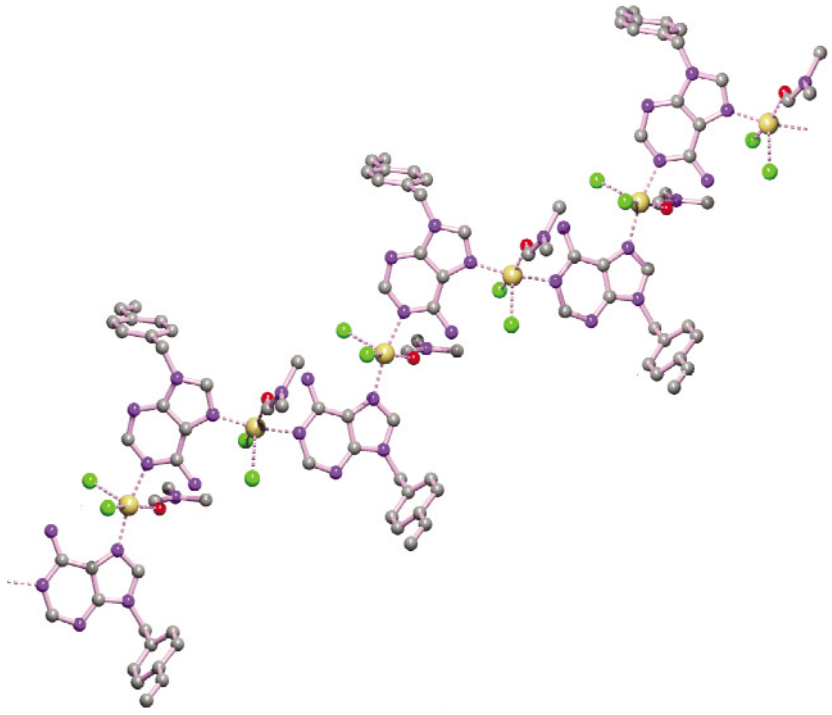

Fig. 6 Polymeric structure of **9VBA-Cu** complex. Gray: carbon, blue: nitrogen, green: chlorine, red: oxygen, copper: copper

MODELING PREBIOTIC CATALYSIS WITH ADENYLATED POLYMERS

Phosphate ester hydrolysis

Enzyme-catalyzed phosphate ester (P-ester) hydrolysis is of considerable importance owing to its central role in key cellular processes [86]. This reaction has acquired wider attention owing to its potential application in biotechnology and therapy [87]. Different classes of enzymes such as phosphatases, cyclic phosphodiesterases, nucleases, and ribozymes catalyze this reaction. Most phosphate ester cleaving enzymes are metalloenzymes and display remarkable dependence for a wide array of metal ion cofactors [88]. In general, magnesium ion predominates in natural nucleolytic enzymes probably due to its redox inertness, ligand exchange rates, and charge density [89]. However, metal ions, such as Zn^{2+}, Fe^{2+} and Ca^{2+}, and other multinuclear metal centers have also been identified in phosphate ester hydrolases.

In view of metal ion-dependent, phosphate ester-modifying activities, numerous artificial phosphatases and nucleases have been evaluated for possible biochemical applications ranging from chemotherapy to synthetic-restriction enzymes [90]. Phosphoesterase models employ a wide range of transition and inner-transition-metal ions coordinated to ligands of natural and synthetic origin and consequently, high rate accelerations for the cleavage of natural and non-natural phosphate ester substrates have been reported. Transition-metal ions and complexes of Cu, Zn, Fe, Ni, Co, Ru, and Pd, etc. demonstrate phosphoesterase [91,92] and nuclease [93,94] activity either in the absence or in the presence of activating agents. Several synthetic phosphate ester hydrolases based on inner-transition-metal ions, such as La^{3+}, Ce^{4+}, Eu^{3+}, Tm^{3+}, Yb^{3+}, Lu^{3+}, Th^{4+}, UO_2^{2+}, and their complexes display significant rate acceleration [95].

Although enzyme-like precise substrate recognition, efficiency, and high turnovers are difficult to accomplish, artificial systems provide a valuable insight for probing the role of metal ions and operative mechanisms for phosphate ester cleavage. Moreover, small molecule models are easily characterized by X-ray structure analysis and by spectroscopic techniques. Interestingly, many designed synthetic models emulating multiple metal ion coordination behavior of enzymes have proven to be more effective than corresponding mononuclear metal complexes [96]. Another focus of these endeavors is to develop artificial systems for sequence-specific hydrolysis of DNA and RNA phosphodiester bonds for possible application as synthetic nucleolytic enzymes and chemotherapeutic agents [97].

Most often, synthetic P-ester cleaving systems operate under homogeneous conditions. The heterogeneous nature of our polymeric system prompted us to explore its catalytic potential toward P-ester hydrolysis under heterogeneous conditions. We employed model phosphate ester substrates to evaluate P-ester hydrolase activity of copper-metallated adenylated polymers. Significant rate accelerations were observed for the hydrolysis of activated phosphate esters, catalyzed by the templates, as compared to the uncatalyzed reaction [80,81,85]. Two million-fold acceleration of bis(*p*-nitrophenyl) phosphate (bNPP) hydrolysis by one of the templates is at par with many previously reported rate enhancements for this substrate by employing metallated ligands or by the direct addition of metal ions [98]. Copper-metallated adenylated polymers also catalyzed the hydrolysis of unactivated phosphodiester substrate, adenosine 2',3'-cyclic monophosphate with appreciable rate enhancement and regioselectivity. We have also reported ruthenium and uranium containing adenylated polymers as synthetic dephosphorylation reagents using model phosphoesterase substrates [99,100]. Detailed mechanistic investigations revealed the involvement of metal-bound hydroxide, which probably acts as a general base in assisting the hydrolysis of phosphate esters.

Nuclease activity

The extraordinary stability of phosphodiester bonds in nucleic acids is implicated for the preservation of genetic information. Despite estimated half-lives of 200×10^6 and 800 years, for DNA and RNA phosphodiester bonds, respectively [93], nucleic acids are effectively cleaved in the presence of protein enzymes. Nucleases hydrolyze the phosphodiester linkage present in the nucleic acids. Nonenzymatic cleavage of DNA is usually achieved by oxidative damage at the deoxyribose moiety or at the nucleobase. Various reactive radical species generated in the presence of transition-metal ion catalysts and redox reagents induce strand scission at very high rates. Such modifications at sugar moieties and nucleobases have been discussed in the review by Burrows [101] and Tullius [102]. Few chemical systems are known to hydrolytically cleave DNA [93,95], where the products formed can be used for further enzymatic manipulations. Among transition-metal ions, copper-based artificial systems have received considerable focus owing to their dual mode of action: they can modify nucleic acids either through oxidative scission [103] or via hydrolytic pathway [104]. The former activity is attributed to redox cycling of copper in the presence of exogenously added oxidants or reductants, which leads to in situ formation of reactive species. Cu^{2+} is a substitutionally labile, strong Lewis acid as evident from its high charge density and ionization potential [105]. Copper complexes afford facile nucleic acid strand scission in the presence of exogenously added oxidizing and reducing agents. Hydrogen peroxide and peracids like oxone and magnesium monoperoxyphthalate (MMPP) are commonly employed oxidants. In the presence of oxidants, Cu(II) is oxidized to higher oxidation state leading to the generation of reactive copper-oxo species; however, formation of diffusible hydroxyl radicals is also a likely scenario [101]. Ascorbate and thiols reduce Cu(II) to Cu(I), and in situ generated Cu(I) complex could further react with molecular oxygen, resulting in the formation of reactive oxygen species culminating in oxidative scission [106,107]. Oxidative damage by free radicals is extensively utilized in DNA footprinting [108], for identifying base mismatches and loop regions [109], and for probing nucleic acid conformations [110], among other applications.

We evaluated the nuclease activity of the constructs using supercoiled plasmid DNA as a natural macromolecular substrate to extend the catalytic scope of our polymeric constructs. Cleavage of supercoiled plasmid DNA (pBR322) assisted by polymeric matrices was studied both in the absence and in the presence of exogenously added oxidants and reductants. The interaction of supercoiled plasmid DNA (pBR322) and **9AA-Cu(I)** polymer containing divinylbenzene cross-linker revealed a complete conversion of supercoiled plasmid DNA (Form I) to nicked DNA (Form II) in the absence of added activating reagent [111], while **9VBA-Cu(II)** polymers required exogenously added oxidants or reductants (thiols) to show cleavage activity. Preliminary mechanistic studies using radical scavengers indicated a possible role of copper-bound nondiffusible oxo species in the plasmid scission reaction [112]. Recently, Madhavaiah and Verma reported photonuclease activity of uranyl-impregnated adenylated polymer [113], thus suggesting an interesting possibility of light-induced transformations in the prebiotic era using coordinated, photoactive metal ions as catalytically competent units.

Most of the phosphate ester cleaving synthetic systems are active under homogeneous reaction conditions, leaving no scope for the catalyst recovery. Menger and coworkers demonstrated the phosphate ester hydrolyzing activity of copper-loaded polystyrene, which displayed multiple turnovers and was also active at elevated temperatures [114]. The catalytic activity of copper-containing cross-linked polymeric matrix derived from *N*-(4-vinylbenzyl)ethylenediamine, toward *p*-nitrophenyl phosphate (pNPP) hydrolysis has been reported [115]. Recently, Burstyn and coworker reported silica-bound $(Cu[9]aneN_3)^{2+}$ for bNPP hydrolysis and also demonstrated recycling [116].

The unique feature of our polymeric matrices is their facile recovery and reusability. The rarity of heterogeneous nucleolytic reagents and their possible reusability prompted us to explore our polymers for multiple nucleic acid cleavage reactions. The adenylated polymers were recycled for several consecutive reactions, and each time there was complete nicking of the supercoiled DNA. Their prolonged and sustained reactivity, as demonstrated by recycle experiments, indicate that they can retain metal-ion dependent catalytic property for long duration, without altered activity and a property that would be of functional importance in the case of prebiotic catalytic systems.

9AA-Cu and **9VBA-Cu** complexes also displayed nucleolytic activity similar to that of polymeric templates in the presence of exogenously added oxidants and reductants [117]. From the prebiotic catalysis viewpoint, it could be reasoned that such nucleobase-metal ion complexes could have been harnessed for nucleic acid processing in the absence of protein enzymes. Such complexes could have assembled subsequent to the abiotic synthesis of nucleobases and could have used existing metal ions present in surrounding minerals. Under optimal circumstances, such activity could be manifested in the presence or absence of oxidative conditions.

Phenol oxidation

Copper-containing metalloproteins are involved in diverse array of oxidation reactions in biological systems [118,119]. There is considerable interest in the modeling of copper oxidase functions in order to understand the mechanism and role of copper for catalytic activity [120]. Numerous synthetic dinuclear copper systems have been designed, and studies using these test systems have provided valuable information regarding the binding of oxygen to the active site and subsequent oxidation reaction [119]. As adenylated polymeric matrices contain multiple copper residues, we became interested in evaluating tyrosinase-like activity of these templates toward phenol oxidation. Such studies were also intended to determine a functional role of adenine-copper complexes in acting as prebiotic oxidases, albeit in the absence of redox cofactors used by the protein enzymes. Interestingly, **9AA-Cu(I)** adenylated polymer exhibited both monophenolase and diphenolase activity (Fig. 7) under Michaelis–Menten conditions with significant rate enhancement over uncatalyzed reaction for catechol, 4-*t*-butylcatechol, and 4-hydroxyanisole [121]. Phenol oxidase activity of adenylated polymer suggests for an adenine-based prebiotic oxidase, which could have been used for the biosynthesis of hydroxylated amino acids and catecholamine neurotransmitters. There is ample evidence for diverse functional roles for adenine,

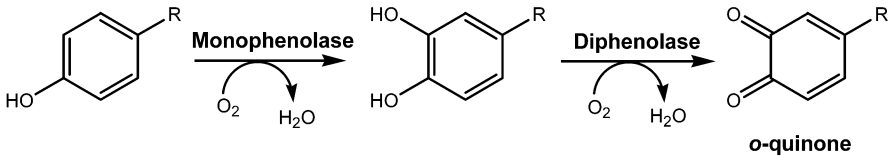

Fig. 7 Monophenolase and diphenolase activity.

adenosine, and other analogs in biological milieu, other than acting as a complementary base against thymine and uracil. Adenine holds a special significance in this context as it remains as a key component of several redox cofactors such as NAD^+, NADH, FAD, $FADH_2$, etc. In addition, coordination through the imidazole ring of adenine is also reminiscent of the histidine imidazole ring coordination of copper ions in several protein copper oxidases [118,122].

Our cross-linked polymeric systems can be considered as potential mimics for the natural scaffolds employed for prebiotic organization and catalysis. Analogous to purine-adsorbed mineral surfaces [123], these polymeric matrices provided a constellation of adenine residues and an insoluble scaffold for nucleobase-metal ion coordination. It is evident from the X-ray structures that these modified adenine monomers [78,79] and its copper complexes [85] can invoke multiple hydrogen-bonding events, which could be crucial for molecular recognition. In this context, Sowerby and coworkers have recently shown that adenine residues adsorbed on graphite surface display strong affinity for aspartic and glutamic acid [124]. Another intriguing possibility is that such metal-nucleobase assemblies could have functioned as short, prebiotic protonucleic acid templates for the recognition of other nucleobases and amino acids (Fig. 8), for subsequent primordial oligomerization reactions. Our results reinforce previously reported catalytic activities of adenine [125], *N*-6-ribosyladenine [126], and related polymers [127] and their potential role in prebiotic chemistry.

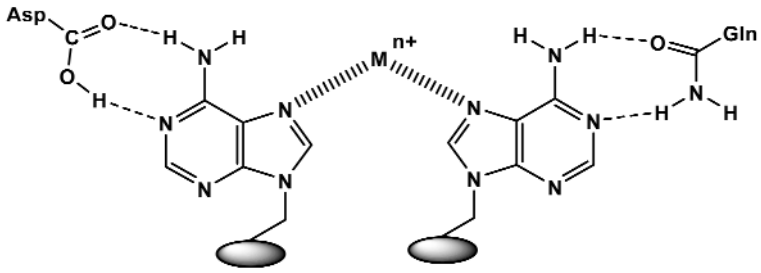

Fig. 8 Possible amino acid recognition by adenylated polymers.

OUTLOOK

Insight into RNA-based origin of life is largely inferential based on known chemical and biochemical properties of RNA. Innovative experimental approaches have demonstrated that it is possible to synthesize long oligomers under possible prebiotic conditions. However, regioselective polymerization to produce longer RNA molecules at a rate exceeding the rate of decomposition of the parental RNA has remained elusive. Studies related to prebiotic chemistry of biomonomers, origin of chirality, aggregation of biomolecules, and compartmentalization of replicating moiety along with biochemical analysis would further sharpen the picture of RNA-based life. Our results with metallated polymeric templates and nucleobase monomer-metal complexes suggest that simple synergism between metal ions and small biogenic molecules could have led to initial catalytic reactions in the primordial world. It is expected that studies with protonucleic acid constructs might provide further insight into primitive organization of nucleic acid constituents and to their cooperative interaction with metal ions for prebiotic catalysis.

Moreover, judging from the progress in ribozyme engineering in recent years, it seems likely that new types of RNA catalysts are on the anvil and may provide crucial insight not only for catalysis, but also for self-organization and recognition in primordial biotic life.

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