Amino Acid Specific Nonenzymatic Montmorillonite-Promoted RNA Polymerization

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Amino Acid Specific Nonenzymatic Montmorillonite-Promoted RNA Polymerization

Trishool Namani,[a] Savannah Snyder,[b] James M. Eagan,[a] Philip C. Bevilacqua,[c] Chrys Wesdemiotis,[b] and Nita Sahai*[a]

Understanding prebiotic RNA synthesis is essential to both the RNA world and RNA-protein co-evolution theories of the origin of life. Nonenzymatic templated RNA synthesis occurs in solution or by montmorillonite clay heterogenous catalysis but the high magnesium concentrations required are deleterious to protocell membranes. Here, we explore a multicomponent environmental system consisting of amino acids, RNA mononucleotides and montmorillonite at various Mg^{2+} concentrations. We show that specific alpha amino acids, especially those that were prebiotically most relevant, act as prebiotic coenzymes and further enhance montmorillonite-catalyzed polymerization in a cooperative mechanism to produce even longer RNA oligomers. Significantly, and different from template-directed nonenzymatic RNA polymerization by primer extension, added Mg^{2+} is not required for montmorillonite-catalyzed polymerization, especially as enhanced by specific amino acids. Thus amino acid specific montmorillonite-catalyzed RNA polymerization is compatible with protocell membranes and could occur in a wider variety of geochemical environments of various Mg^{2+} concentrations.

1. Introduction

One of greatest arguments in the origins of life field is the question of which biopolymer evolved first, protein or RNA. Synthesis of amino acids under a variety of prebiotic conditions suggests that amino acids were ubiquitous on the early Earth[1] and even short peptides can show catalytic properties,[2] which supports the argument that proteins evolved first. However, proteins cannot carry information to support replication. Hence, the "RNA world" hypothesis gained significance as RNA can both carry information and show catalytic properties.[3] In biology, proteins are synthesized by RNA and RNA is produced by proteins. Therefore, rather than the evolution of one polymer before the other, there might have been a mutual coevolution of these biopolymers.[4] Peptides can be synthesized by wetting drying cycles and other prebiotically plausible reactions.[5] The question is still unclear as to how the first RNA molecules emerged. The Richert group has achieved polymerization of unactivated RNA monomers in the presence carbodiimide as condensing agent, ethylimidazole as catalyst and $MgCl_2$ with and without amino acids present to obtain RNA oligomers and peptidoRNAs.[6]

The nonenzymatic synthesis of RNA long enough to exhibit functionality is a challenge.[7] The condensation reactions needed for the formation of RNA phosphodiester bonds are not thermodynamically favorable in aqueous solutions. To circumvent this, microenvironments, such as mineral surfaces,[8] lipid surfaces[9] or water-ice eutectic phases[10] have been used to facilitate condensation reactions.[11,12] RNA polymerization by nonenzymatic template-directed primer extension has been achieved but requires high (~80 mM) concentrations of Mg^{2+} and the question remains as to where the primer or template came from.

Montmorillonite clay facilitates the non-enzymatic polymerization of activated mononucleotides.[8,12–17] Effects of various salt-exchanged clays, background electrolyte ions, pH values, minerals, nucleotides and activating groups were examined on the efficiency,[15,18–22] regioselectivity[15,23–25] and chiral selectivity[26] of the polymerization reaction.

Another key component of protocell emergence is the formation of membranes. The high concentrations of Mg^{2+} required for template-directed RNA polymerization and also utilized for montmorillonite-catalyzed RNA polymerization destabilizes model protocell membranes composed of fatty acids.[27] Thus, RNA formation with stable protocell membrane self-assembly is a challenge in the origins of life field. Here we focus on the role of amino acids in montmorillonite-promoted polymerization of the activated nucleotide, 2-MeImpA (Figure 1a), at neutral pH and various Mg^{2+} concentrations. The goals of this study are to explore the possibility of cooperation between biomolecules, such as amino acids and nucleotides, and minerals in the evolution of biopolymers, to explore a wider range of geochemical environments for RNA synthesis and coexistence of RNA with stable protocell membranes. To

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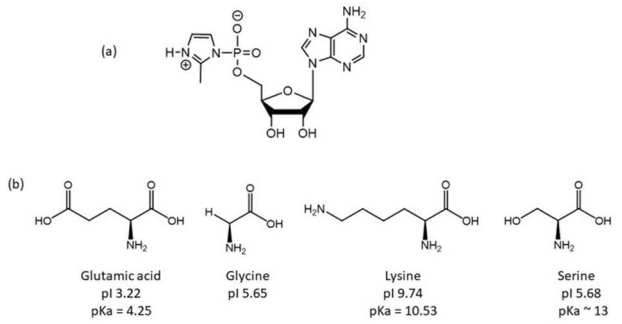

Figure 1. Chemical structure of adenosine 5′-phospho-2-methylimidazolide (2-MeImPA) (a), and specific amino acids (b) with corresponding pI and side chain pK_a values. Amino acids functional groups are shown in their neutral states.

the best of our knowledge, this study reports for the first time the impact of amino acids on the nonenzymatic polymerization of activated mononucleotide. We also show that montmorillonite-promoted RNA polymerization does not require Mg^{2+}, especially in the presence of specific amino acids, thus allowing for protocell membrane self-assembly.

2. Results and Discussion

The reference system consists of 15 mM 2-MeImPA, 50 mg.mL^{-1} montmorillonite clay, 0.2 M NaCl and 0.075 M $MgCl_2$, incubated at room temperature for 4 days. Oligomers up to 8-mers were obtained in the reference system as shown by HPLC and MALDI-MS analyses (Table 1; Figure 2a and Figure 2f; Table S1). Most ions observed were of the $[M-H]^-$ ion type representing linear oligomers formed by a dehydration reaction (e.g., Figure S2b), indicated by a_n, where n is the oligomer number (Figure 2 and Table S1). The maximum length of oligomers achieved is referred to here as degree of polymerization. The % yield of oligomers is very small (Table S2) but is consistent with previous reports in the literature.[13–19] Some signals indicative of cyclization by additional dehydration were also observed resulting in ($[M-H_2O-H]^-$ ions, indicated by a_n* (e.g., Figure S2c, d). Dephosphorylated oligomers forming $[M-PO_4CH_2-H]^-$ type ions as indicated by a_n^+ (Figure S2e) were also observed. Note that the oligomer structures drawn in Figure S2 are shown with 3′,5′-regiospecificity, even though the products may be expected to be a mixture of 3′, 5′- and 2′, 5′-oligomers. This is because montmorillonite-catalyzed RNA polymerization is predominantly (80–90%) 3′, 5′-regiospecific.[13,15,23,24]

Table 1. Degree of polymerization (n) of 2-MeImPA on montmorillonite in the presence of various amino acids (75 mM), 75 mM $MgCl_2$ and 200 mM NaCl observed from HPLC and MALDI-MS analysis. Also see Table S2.

Amino Acid	Isoelectric Point pI	Side Chain pK_a	*n*
Reference System (no amino acid)	N.A.	N.A.	8
Glu	3.22	4.25	10
Asp	2.77	3.65	9
Gly	5.65	–	10
Ala	6.00	–	9
Val	5.96	–	9
Leu	5.98	–	9
Lys	9.74	10.53	5
Orn	9.74	10.53	6
Dab	9.74	10.53	5
Arg	10.76	12.48	3
His	7.59	6.00	4
Ser	5.68	~13.00	4
Cys	5.07	8.50	3

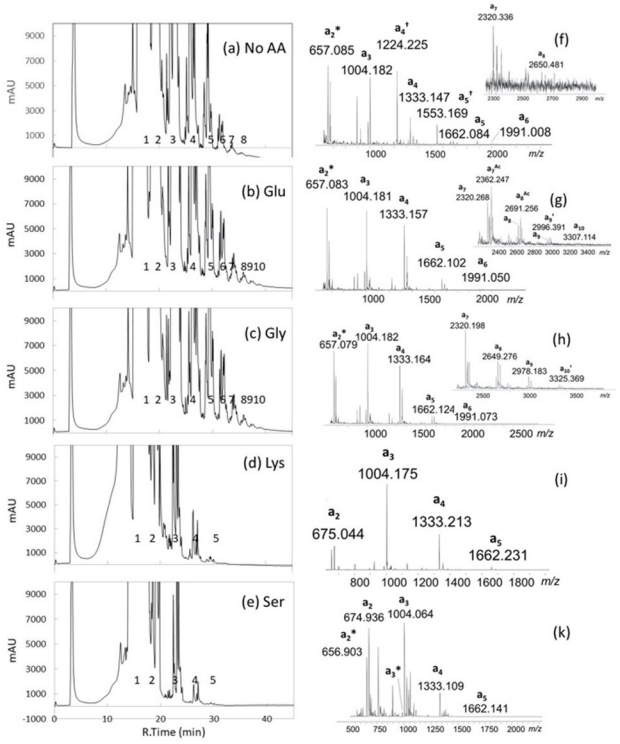

Figure 2. Polymerization of 2-MeImPA (15 mM) on montmorillonite clay in the presence of 0.2 M NaCl, 0.075 M $MgCl_2$, 0.1 M HEPES buffer, pH 7. Panels (a–e) are anion-exchange HPLC chromatograms, and panels (f–k) are the corresponding MALDI-MS spectra of the mixture with reference system (no amino acids) (a f); and in the presence of glutamic acid (b, g); glycine (c, h); lysine (d, i) and serine (e, k). The number on the HPLC chromatogram represents the oligomer length (n-mer). A superscripted *, ′, †, or Ac indicates additionally dehydrated, hydrated, dephosphorylated, or acetylated ions, respectively (see Table S1).

The effects of negatively charged amino acids, such as glutamic acid and aspartic acid, on the polymerization efficiencies of 2-MeImPA in the presence of montmorillonite were studied. Glutamic acid results are shown as representative of the acidic amino acids in Figure 2 (b, g) and aspartic acid results are shown in Figure S5 (a, e) (Table 1). Glutamic acid was observed to result in HPLC peaks at around 38 minutes (Figure 2b), which were confirmed to be 10-mer oligomers via MALDI-MS (Figure 2g). Also, the HPLC results show that a significantly greater amount of all oligomers were produced compared to the reference system (Table S2, Figure S3). As with

the reference system, most ions observed by MALDI-MS were linear oligomers (a_n); ions of a_n* type were also seen. In addition, peaks representing hydrated ion of the $[M+H_2O-H]^-$ ion type ($a_n^{'}$) (Figure S2f) and acetylated ions of the $[M+Ac-H]^-$ ion type (a_n^{Ac}) (Figure S2g) were also observed in the higher mass region (see legend of Figure 2 and Table S1). The origin of the acetyl groups is still under investigation. Thus, longer oligomers up to 10-mers were obtained in the presence of acidic amino acids compared to the reference system.

Polymerization in the presence of non-polar amino acids, such as glycine, alanine, valine, and leucine, also promoted longer oligomer formation up to 10-mers and a much greater concentration of all oligomers than the reference system (Table 1; Table S2; Figure S3). Glycine results are shown as representative of non-polar amino acids in Figure 2 (c, h) and the other non-polar amino acids are shown in Figure S5 (b–d, and f–h). In addition to the linear oligomers, other oligomer structures were also observed (Figure 2h; Figure S5f–h). We recognize that the oligomer length (10-mer) extension compared to the reference system (8-mer) is small in an absolute sense; however, the extension represents a 25% increase in a relative sense. Also, the yield of shorter oligomers increases in the presence of the promoting amino acids (Table S2, Figure S3). From the above experimental results, it is manifested that both the acidic and non-polar amino acids behave similarly and further facilitate 2-MeImPA polymerization, forming longer oligomers than the reference system.

The presence of basic amino acids, such as lysine, ornithine, diaminobutyric acid, arginine and histidine, resulted in significant suppression of the degree of polymerization (Table 1; Table S1; Table S2, Figure S3). Results for lysine are shown as representative of basic amino acids in Figure 2 (d, i) and for the other basic amino acids in Figure S6.

The polar amino acids, serine and cysteine, exhibited inhibitory effects (Tables 1; Table S1; Table S2, Figure S3). Results for serine are shown as representative of polar amino acids in Figure 2 (e, k) and cysteine results are shown in Figure S7 (a, c). Interestingly, longer oligomers up to 10-mers are formed in the presence of anionic cysteic acid (Figure S4b; Figure S7b, d), similar to the acidic and non-polar amino acids.

Several dicarboxylic acids, such as succinic acid, glutaric acid and adipic acid (Figure S4b), were tested to explore the importance of the carboxylic acid group in promoting polymerization. These dicarboxylic acids produced 8-mers similar to the reference system (Figure S8). This result indicated that both carboxylate and ammonium functional groups are necessary to promote polymerization.

The polymerization reaction was also tested in the presence of β-alanine and 6-amino hexanoic acid (Figure S4b) in order to examine the importance of the position of the amine group with respect to the alpha carboxylic acid group. These two molecules have both amine and carboxylic acid functionalities similar to the non-polar amino acids, particularly to alanine, but possess different spacing between the amine and carboxylic acid functional groups. However, these two molecules showed no effect on polymerization and resulted in 8-mers similar to the reference system (Figure S9). These observations along with the previously discussed observation that the non-polar amino acids promote polymerization allows for the conclusion that the position of the amine group with respect to the carboxylic acid group has a significant role in promoting the polymerization of 2-MeImPA, i.e., both amine and carboxylate groups need to be at the C_α position.

We also tested diamines, 1,3-diaminopropane and 1,4-diamonobutane, to further explore ammonium group interactions (Figure S4b). These molecules inhibited the reaction, similar to the basic amino acids (Figure S9).

The effects of amino acid concentration, Mg^{2+} concentration and NH_4^+ on montmorillonite-promoted polymerization were investigated. A lower concentration of glutamic acid (15 mM) was as effective as 75 mM (Figure S10 and Table S3).

In the absence of Mg^{2+} and with 75 mM glutamic acid present, pentamers were observed by HPLC and heptamers by MS (Figure S10 and Table S3). At Mg^{2+} concentrations of 5, 10 and 15 mM, the oligomer length increased and did not increase from 75–150 mM Mg^{2+}. Thus, Mg^{2+} is not required for the formation of oligomers and oligomer length increases in the presence of Mg^{2+}, without or with amino acids present.

We further explored RNA polymerization with promoter and inhibitor amino acid combined together. The coincubation of glutamic acid, lysine and 2-MeImPA resulted in oligomers of the same length as the reference system (Figure S11 and Table S3).

Finally, the effect of sequential amino acid adsorption followed by polymerization was investigated. In one experiment, the amino acids were pre-incubated with montmorillonite clay before the addition of mononucleotide; in another experiment, the sequence was reversed. Regardless of the sequence, the results obtained by pre-incubation are the same (data not shown) as those for co-incubation of each amino acid with mononucleotide.

Having explored effects of various small molecules, concentrations and combinations, we sought to rationalize our results.

When 75 mM NH_4Cl was added to the reaction in the presence of 75 mM lysine, oligomerization was similar to the reference system (Figure S11 and Table S3). Thus, NH_4^+ "rescued" the inhibitory effect of the basic amino acids.

2.1. Requirement for Both Carboxylate and Amine Functional Groups

Acidic amino acids (glutamic acid and aspartic acid) produced longer oligomers than the reference system and greater amount of 5- to 8-mers than the reference system (Figure 2b, g and Figure S5a, e). To test whether the side chain carboxylic acid of the negatively charged amino acids is sufficient to promote polymerization, we determined whether negatively charged carboxylic acid functional groups alone are capable of promoting the polymerization reaction. The reaction in the presence of dicarboxylic acids (succinic acid, glutaric acid, and adipic acid) showed no effect (Figure S8). This result indicates that both acid and amine functionalities are required for promoting polymerization. This conclusion is consistent with the behavior of the neutrally charged non-polar (glycine,

alanine, valine, and leucine) amino acids. However, positively charged basic amino acids, which also possess both carboxylate and amine functional groups inhibited the reaction. Our observation that amino acids promote polymerization, while amine-free small molecules (dicarboxylic acids) and carboxylate-free small molecules (diamines) do not, strongly supports the hypothesis that both carboxylate and amine functional groups are necessary to promote polymerization; an additional requirement is that the charge of the amino acid side-chain should be neutral or negative for the enhancement effect.

2.2. Requirement for C_α Position

We next examined the role of the amine position in the amino acids with respect to the carboxylic acid group. β-alanine and 6-amino hexanoic acid possess both amine and carboxylic acid functionalities, but the amine group is not in the C_α position (Figure S4b). These two molecules had no impact on 2-MeImPA polymerization (Figure S9). We interpret these results as evidence for a conformational requirement of the negatively charged carboxylate and positively charged ammonium groups, as found in α-amino acids, to be crucial for promoting polymerization.

Based on these observations, we propose that both acid and amine functional groups in the C_α position are required for promoting the polymerization reaction. This hypothesis is confirmed by the result obtained for cysteic acid, in which the thiol of cysteine is changed to sulfonic acid, and longer oligomers up to 10-mer were obtained (Figure S7b, d).

2.3. Proposed Mechanism of Alpha Amino Acid Mediated Promotion and Inhibition of RNA Oligomerization

The polymerization mechanism in the reference system has been explored previously.[18,28] An important question is the site of catalysis on the montmorillonite: does the activated monomer enter the interlayer sites or interact at the edge sites of montmorillonite? It was found that blocking the edge sites did not hinder the polymerization reaction whereas blocking the interlayer sites abolished the catalytic activity of the clay.[16] Furthermore, detailed XRD studies of montmorillonite interacted with activated monomers showed changes in domain size.[22] These results suggest that the activated monomer enters the interlayer space. However, the montmorillonite interlayer spacing increased when reacted with some activated monomers and reduced when reacted with others. Thus, it is not unambiguously determined whether the activated monomer interacts with the montmorillonite in the interlayer sites or on the edge sites. Furthermore, the protonated form of the activating nitrogen group was proposed to interact with negatively charged sites in the interlayer space. Protonation was proposed to make the activating group a better leaving group than the neutral form and its interaction with the negative interlayer sites to enhance the nucleophilic attack of the ribose.[24]

A potential mechanism is proposed below to rationalize the amino acid-specific montmorillonite-catalyzed polymerization observed in the present study (Figure 3). We begin by considering the activated monomer. The leaving group of 2-MeImPA is predominantly in its protonated form because the pKa of 2-methylimidazole (pKa = 7.85)[29] is greater than the experimental pH 7. Therefore, it is proposed that the protonated imidazolium form of the activated monomer interacts with the negatively charged sites on montmorillonite. However, we do not definitively propose whether the interaction of the activated monomer occurs in the interlayer sites or on the edge sites.

In our proposed mechanism, the zwitterionic form of the amino acids is proposed to play a role in promoting the reaction. The positively charged C_α ammonium of the acidic and neutral non-polar amino acids anchors to the negatively charged montmorillonite surface site and the C_α carboxylate group of the amino acids interacts with the cationic methlyimidazolium ring of 2-MeImPA to interact through H-bonding and/or electrostatics (Figure 3). The α-amino acids, including cysteic acid, provide the correct conformation (orientation and spacing) of the ammonium and carboxylate groups to facilitate the polymerization reaction. Molecules such as β-alanine and 6-amino hexanoic acid, which possess both ammonium and carboxylate groups, however, do not promote polymerization presumably because they do not provide the proper conformation. The dicarboxylic acids are also ineffective presumably because their lack of an ammonium group does not allow them to anchor to the negatively charged clay surface site.

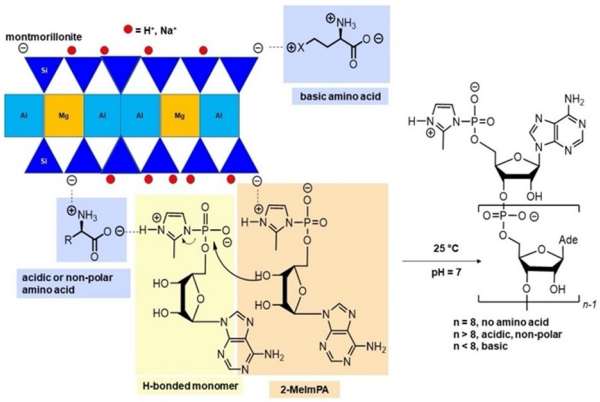

Figure 3. Proposed mechanism for the interaction of amino acids with 2-MeImPA and montmorillonite. 2-MeImPA interacts via the positively charged methylimidazolium moiety group with negatively charged montmorillonite surface sites. For acidic and non-polar amino acids, which promote the reaction, the C_α ammonium group is anchored to the clay surface and the C_α carboxylate group of the amino acid interacts in the appropriate geometry with the imidazolium moiety of the leaving group. The basic amino acid and polar amino acids inhibit the reaction (see text for explanation). Note that the exact site of interaction is unclear and may occur at the interlayer sites or at deprotonated silanol edge sites. "n" refers to the oligomer number. Dark blue triangles represent silicate tetrahedra; light blue squares represent aluminum octahedra; yellow squares represent magnesium octahedra; red spheres represent interlayer or edge H^+ or Na^+ ions; X^+ represents basic amino acid side chain, either ammonium or imidazolium group.

It is found that a lower concentration of glutamic acid is just as effective as the higher concentration. This result suggests Michaelis-Menten kinetics, where the reaction rate does not increase after a finite number of sites on the montmorillonite surface are saturated by glutamate.

Oligomerization was obtained even in the total absence of Mg^{2+} in the reference system;[12] importantly, longer oligomers were obtained in the presence of glutamate at $[Mg^{2+}]=0$ mM. Oligomerization efficiency improved with addition of Mg^{2+}. Therefore, it is proposed that Mg^{2+} plays the role of a co-catalyst to the montmorillonite and amino acids, potentially by providing charge screening to the growing oligomer chain.

Positively charged, basic amino acids are proposed to interact with the montmorillonite via their basic side chains, thus competing with the monomer for the active sites on montmorillonite. The diamines behave similarly. The addition of NH_4^+ to the reaction rescued the inhibitory effect of lysine, which shows that ammonium successfully competes with the basic amino acids. Furthermore, the basic amino acids are proposed to adsorb in a conformation that does not provide the ideal conformation for the alpha carboxylate to interact with the leaving group of the monomer. Together, these effects by the basic amino acids inhibit polymerization.

When combined together, the acidic (glutamate) and basic (lysine) amino acids have no net effect and the extent of oligomerization is the same as for the reference system. One possible explanation for this behavior is that some phenomenon prevents the amino acids from interacting with the montmorillonite. One potential phenomenon is liquid-liquid phase separation; however, no turbidity is seen in the solution, so this phenomenon is unlikely. Alternatively, the glutamate side chain and lysine side chain may interact electrostatically. Finally, the sequential adsorption reactions showed no difference compared to the effects of amino acids when co-incubated with 2-MeImPA. This indicates that the interaction of the amino acids with 2-MeImpA is fast and under thermodynamic-control, and is not path-dependent.

Based on the mechanism proposed above for the acidic and non-polar amino acids, the polar amino acids (serine and cysteine), should promote polymerization, yet they inhibit the reaction (Figure 2e, k and Figure S7a, c). This may be because the polar side chains (–OH and –SH, respectively) interact with the phosphate group of the 2-MeImPA monomers, similar to the –OH of ribose in a normal polymerization step. Indeed, the primary –OH and –SH groups, respectively, of serine and cysteine are more nucleophilic than the secondary –OH group of the ribose. Thus, the former can effectively promote the methyl imidazole to leave, such that the monomer becomes deactivated and polymerization is inhibited. Alternatively, the –OH and –SH side chains of these amino acids may H-bond to the montmorillonite surface preventing the alpha carboxylate group from interacting with the methyl imidazolium moiety of the leaving group.

The mechanism proposed here for amino acid-specific montmorillonite-catalyzed polymerization of activated monomers can be compared to the mechanism of nonenzymatic RNA-templated polymerization by primer extension. An imidazolium-bridged dinucleotide intermediate (Figure S12a) is involved in the reaction mechanism at pH 8.[30] We observe peaks corresponding to an imidazolium-bridged dinucleotide in the MALDI-MS spectra of some of our systems but not in others at pH 7 (Figure S12 and Table S4). Thus, the reaction in our system provides no support for this intermediate, although it is possible that it does not build up and so is below our detection limit. Additionally, we do not have a nucleic acid template for the monomer, which may obviate the need for a dinucleotide.

2.4. Relevance to Prebiotic Chemistry

The amino acid inventory on early Earth had both exogenous sources, such as carbonaceous chondrite meteorites, comet and interplanetary dust particles, as well as endogenous sources, such as lightning in reducing atmospheres, bolide impacts, hydrothermal vents, atmospheric photochemistry and aqueous reactions catalyzed by Fe^{2+} ions and hydroxylamine.[31,32] Based on the results of these experiments, it is generally accepted that the most plausible prebiotic alpha amino acids were glycine, alanine, valine, leucine, glutamic acid, aspartic acid, and serine.[31] It is interesting that the acidic amino acids and non-polar amino acids with simple –H or methyl side chains (as opposed to non-polar amino acids with bulky side chains), which were found in the present study to act as prebiotic coenzymes and promote mononucleotide polymerization, are also considered among the most prebiotically plausible alpha amino acids. The basic amino acids lysine and arginine; cysteine; and the amino acids with bulky side chains, such as tryptophan, tyrosine, proline and phenylalanine, are generally not considered to be prebiotically plausible.[31] If these amino acids were not present or were present only in low abundances on early Earth, then their inhibitory effect on montmorillonite-promoted mononucleotide polymerization may not have played a significant role, which is consistent with the results of our experiments in which acidic and basic amino acids were co-incubated with the monomer. We note, however, that some workers have argued that at least the shorter chain versions of lysine, such as ornithine and diaminobutyric acid, may have been present prebiotically,[33] and methionine and methyl cysteine were identified in H_2S-rich Miller-Urey spark discharge experiments.[34] The effectiveness of acidic amino acids in promoting polymerization even at lower concentrations suggests that they may have played a role under prebiotic conditions.

An important result is that Mg^{2+} is not necessary to promote montmorillonite-catalyzed oligomers of short to intermediate length, such as pentamers or heptamers, respectively, in the absence or presence of acidic amino acids. Therefore, Mg^{2+} is proposed to act as a co-catalyst to montmorillonite. Thus, montmorillonite-promoted RNA oligomerization could proceed even in geochemical environments with low Mg^{2+} concentrations. This broadens the range of geochemical conditions under which RNA synthesis may have been possible.

Furthermore, Mg^{2+} concentrations greater than about 5 mM are deleterious to model protocell membranes, that are widely

believed to have been composed of fatty acids.[27,35] The present results show that protocell membrane self-assembly could have occurred in the same low-Mg^{2+} environments as acidic or non-polar amino acid specific montmorillonite-catalyzed RNA polymerization. This is different from the mechanism of non-enzymatic RNA-templated polymerization by primer extension, where ~80–100 mM Mg^{2+} or a complexing agent, such as citrate, is essential to promote the reaction.[30,36] Interestingly, RNA oligomers formed by the montmorillonite-catalyzed mechanism catalysis, when extracted from the surface, were capable of templating a complementary oligoRNA strand in homogenous solution.[37] Thus, montmorillonite-catalyzed RNA oligomers could have provided sufficiently long templates for further elongation by primer extension.

We have previously shown that minerals can promote model protocell self-assembly, with protocell formation rates related to the surface charge (isoelectric point) of the minerals.[38] Photocatalytic minerals can also promote a transmembrane redox reaction coupled to NAD reduction to NADH and the generation of a transmembrane pH gradient.[39] Thus, minerals may have supported the earliest stages of protometabolism. Reactive oxygen species associated with mineral surfaces could affect the prebiotic organic inventory, for example, promote protocell membrane peroxidation or aminoamide formation.[40] The present results show that minerals are also capable of RNA polymerization, which is enhanced in the presence of acidic and non-polar amino acids. Thus, minerals could have supported the prebiotic synthesis and self-assembly of all three major classes of biomolecules, namely, membranes, proteins and RNA.

3. Conclusion

The results presented here support the view of the development of biochemistry in the earlier stages of life based on the co-interaction of α-amino acids, nucleotides, and minerals, rather than independent emergence of proteins and nucleotides. The non-enzymatic polymerization of 2-MeImpA by montmorillonite was promoted further in the presence of acidic and non-polar alpha amino acids. Both carboxylic acid and amine functional group must be present and must be in the C_α position. Interestingly, the promoting effect on RNA polymerization was exhibited by those alpha amino acids considered to be the most prebiotically plausible, whereas the inhibitory effects were shown by the amino acids that are prebiotically less plausible. Montmorillonite-catalyzed RNA polymerization, especially aided by acidic and non-polar amino acids, proceeds even in the absence or at low concentrations of Mg^{2+}, which permits for RNA polymerization in a wider range of geochemical environments and is also compatible with protocell fatty acid membrane formation. Montmorillonite-promoted RNA oligomers could have provided the template for primer extension. Taken with our previous work, the present work provides evidence for the potential role of minerals in the origins of life.

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Conflict of Interest

The authors declare no conflict of interest.

Keywords: amino acids · cocatalyst · co-evolution · montmorillonite · polymerization · RNA

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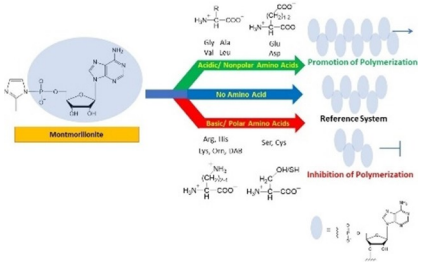
ARTICLES

Let's work together: A multicomponent environmental system consisting of amino acids, RNA mononucleotides and montmorillonite at various Mg^{2+} concentrations is reported. We show that specific alpha amino acids, especially those that were prebiotically most relevant, act as prebiotic coenzymes and further enhance montmorillonite-catalyzed polymerization in a cooperative mechanism to produce even longer RNA oligomers than the clay alone.



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Amino Acid Specific Nonenzymatic Montmorillonite-Promoted RNA Polymerization