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Clay catalyzed RNA synthesis under Martian conditions: Application for Mars return samples

Prakash C. Joshi*, Krishna Dubey, Michael F. Aldersley, Meaghen Sausville

The New York Center for Astrobiology, Department of Chemistry and Chemical Biology, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

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ABSTRACT

Catalysis by montmorillonites clay minerals is regarded as a feasible mechanism for the abiotic production and polymerization of key biomolecules on early Earth. We have investigated a montmorillonite-catalyzed reaction of the 5′-phosphorimidazolide of nucleosides as a model to probe prebiotic synthesis of RNA-type oligomers. Here we show that this model is specific for the generation of RNA oligomers despite deoxy-mononucleotides adsorbing equally well onto the montmorillonite catalytic surfaces. Optimum catalytic activity was observed over a range of pH (6–9) and salinity (1 ± 0.2 M NaCl). When the weathering steps of early Earth that generated catalytic montmorillonite were modified to meet Martian soil conditions, the catalytic activity remained intact without altering the surface layer charge. Additionally, the formation of oligomers up to tetramer was detected using as little as 0.1 mg of Na^+-montmorillonite, suggesting that the catalytic activity of a Martian clay return sample can be investigated with sub-milligram scale samples.

1. Introduction

The formation of complex prebiotic molecules on the early Earth is likely to have involved a component of mineral catalysis [1–3]. Amongst the variety of clay minerals that have been investigated for their ability to catalyze the formation of RNA on Earth are montmorillonites [1]. The “RNA World” is one of the principal paradigms for the origins of life [4–6] suggesting that simple RNAs formed by abiotic processes evolved into the more complex RNA structures that eventually led to the present DNA-protein world. The catalytic montmorillonites are 2:1 layer silicates with a wide range of chemical compositions [7]. They are commonly, but not exclusively, produced by the weathering of silicic volcanic ashes to form Bentonites. Once formed, they gradually transform to Illites at a modest pressure and temperature [8]. It is considered likely that the catalytic Na-rich montmorillonites are distinctive, not because of the composition of the original volcanic ash, but rather due to the shallow brackish waters into which the volcanic ash was deposited and subsequently altered during shallow burial. Our studies have shown that certain montmorillonites upon initially conversion to the Na^+-form by the procedure of Banin [9] are excellent catalysts for RNA oligomer synthesis [2,10–12]. In this process, native montmorillonite is treated with hydrochloric acid to exchange the interlayer cations for protons and then the acid form of the mineral is neutralized with an alkali. Those clay minerals that have been observed to be excellent catalysts come from a restricted range of elemental compositions [13]. The extent of catalysis is dependent upon the magnitude of the surface layer charge on the montmorillonite and the number of cations associated with it. The catalytic montmorillonites not only produced RNA oligomers but also facilitated homochiral selection [14,15]. The 2:1 phyllosilicates that includes montmorillonite, kaolinite, smectite and other clay minerals have been tentatively inferred to exist on Mars [16–20]. Could montmorillonites, if actually present on Mars become catalytically active via a natural Banin procedure in sulfuric acid rich brines inferred by the Opportunity rover at Meridiani Plenum [21–23]? Here we show that terrestrial montmorillonite can become catalytically active following a Banin-like process involving sulfuric acid, which is the primary source of acidity on Mars. Furthermore, the quantities of montmorillonite necessary for detectable catalysis will shape the limits for Mars return samples that could be used for testing catalytic activity.

* Corresponding author. Fax: +1 518 276 4887.
E-mail address: joship2@rpi.edu (P.C. Joshi).

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2. Materials and methods

2.1. Chemicals

Adenosine-5′-monophosphate (AMP), anhydrous sodium perchlorate ($NaClO_4$); 2,2′-dithiodipyridine, imidazole, sodium phosphate monobasic (NaH_2PO_4), uridine-5′-monophosphate (UMP), trifluoroacetic acid (TFA), triphenylphosphine, triethylamine (TEA) and Trizma base (Tris) were obtained from Sigma. Perchloric acid ($HClO_4$) was purchased from Aldrich. N,N-Dimethyl-formamide (DMF), dimethyl sulfoxide (DMSO), acetonitrile (CH_3CN), ether, sulfuric acid (H_2SO_4) and acetone were obtained from Mallinckrodt. Anion exchange resin (IONAC®NA-38, OH^- Form, type 1, Beads 16-50 Mesh) and sodium chloride (NaCl) were procured from J.T. Baker. Sodium hydroxide (NaOH) was obtained from Macron Fine Chemicals, PA. Hydrochloric acid (HCl) and HPLC grade sodium perchlorate ($NaClO_4$) were purchased from Fisher. Ultrapure molecular biology grad nuclease-free water was obtained from USB Corp., Cleveland, OH. HPLC analysis was performed on a Hitachi L-6200A intelligent pump system equipped with a Hitachi L-4000 UV detector operating at 260 nm. The oligomers as anions were separated on a Dionex DNAPac®-100 (4.0 × 250 mm) analytical anion exchange column from Dionex Corporation, Sunnyvale, CA, using a gradient of 0–0.4 M $NaClO_4$ with 2 mM Tris at pH 8. The purity of 5′-phosphorimidazolide of adenosine (ImpA) and 5′-phosphorimidazolide of uridine (ImpU) were examined on a reverse phase Alltima C-18, 5μ (4.6 mm × 250 mm) column (Grace) using a gradient of 0.02 M NaH_2PO_4 with 0.2% TFA (pH 2.5) and 30% CH_3CN in H_2O with 0.2% TFA (pH 2.5).

2.2. Preparation of catalytic montmorillonite

Using a raw montmorillonite sample obtained from the Pembina Hill region, 2 km south of Treherne, southwestern Manitoba, Canada, its acidic form (H^+-montmorillonite) was prepared as follows: 12 g was treated with 50 mL of 0.5 M HCl (Earth simulation conditions) or with 0.25 M H_2SO_4 (Martian simulation conditions) by constant stirring (30 min) at varying temperature (4°, 25°, 50°, 75° and 100 °C). At the end of each treatment, excess acid was removed by centrifugation (3500 rpm) and decanting the supernatant. Fresh acid (50 mL) was added to the montmorillonite pellet and the treatment was repeated twice more. H^+-montmorillonite was washed with 100 mL deionized water maintained at 4°, 25°, 50°, 75° and 100 °C for 30 min with constant stirring. At the end of the washing, excess water was separated by centrifugation (3500 rpm) and decanting of the supernatant. Washing with water was repeated three times. The H^+-montmorillonite slurry was added to water (1000 mL) and to this was added 45 mL of wet anion exchange resin to remove traces of the residual acid. The mixture was stirred for 30 min, the pH was measured (3.00 ± 0.2), the anion exchange resin was removed by filtration using a stainless steel sieve (115 mesh) and the H^+-montmorillonite obtained by centrifugation was freeze dried. One gram of H^+-montmorillonite was dispersed in 100 mL of deionized water and was titrated with 0.02 M aqueous NaOH to pH 7. The water was separated by centrifugation (3500 rpm) and the Na^+-montmorillonite pellet was freeze-dried. To provide montmorillonite samples across the full range of pH studied, 1 g of H^+-montmorillonite was suspended in 100 mL of water and was titrated with 0.02 M aqueous NaOH. At the appropriate pH, the titration was terminated, the sample was centrifuged, the supernatant discarded and the montmorillonite was freeze-dried.

2.3. Preparation of the activated nucleotides

A mixture of a mononucleotide (AMP or UMP, 1.76 mmol) and imidazole (22 mmol) was added to DMF (10 mL) in a 50-mL flask and the solvent was evaporated to dryness at reduced pressure. The evaporation was repeated twice with DMF (2 × 10 mL) to remove residual water. The residue was dissolved in a mixture of DMF (10 mL) with DMSO (10 mL) and stirred with 2,2′-dithiodipyridine (6.5 mmol), triphenylphosphine (6.36 mmol), and TEA (6.5 mmol) for 2 h. The resulting product was recovered from the clear yellow reaction mixture as a precipitate by adding the reaction mixture drop wise to a solution of anhydrous $NaClO_4$ (3.5 g) in a mixture of ether (100 mL), acetone (100 mL) and TEA (20 mL) with stirring. The stirring was continued for 30 min (to ensure complete formation of the Na^+ salt) and a colorless, flocculent solid was precipitated which was allowed to settle (30 min). The supernatant is decanted and the remaining reaction mixture was centrifuged. The resulting colorless pellet was washed twice with a mixture of ether (100 mL) and acetone (100 mL) and then with ether (100 mL) and dried overnight in a vacuum desiccator. The purity of ImpA and ImpU as determined by reverse phase HPLC was >99.5%.

Table 1
Oligomerization of ImpA catalyzed by Na^+-montmorillonite prepared at varying temperature.

Treatment with	Total oligomer (%)	Percent distribution of the oligomers												
		II[a]	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
ImpA + Na^+-montmorillonite (4 °C)[b]														
HCl	21.9	60.8	24.0	10.0	3.57	1.15	0.35	0.08	0.04	0.01				
H_2SO_4	21.6	62.7	22.7	9.01	3.48	1.58	0.28	0.14	0.07	0.03	0.01			
ImpA + Na^+-montmorillonite (25 °C)														
HCl	20.3	62.7	21.7	8.37	4.16	1.36	0.86	0.48	0.21	0.11	0.04	0.01		
H_2SO_4	20.7	59.6	22.4	10.1	4.20	2.68	1.87	0.88	0.54	0.29	0.09	0.04	0.01	
ImpA + Na^+-montmorillonite (50 °C)														
HCl	19.3	60.3	21.1	8.77	3.45	3.23	1.83	1.22	0.1					
H_2SO_4	20.4	75.1	18.9	4.49	0.97	0.26	0.18	0.08	0.05	0.01				
ImpA + Na^+-montmorillonite (75 °C)														
HCl	18.5	74.5	20.0	4.72	0.52	0.20	0.05	0.01						
H_2SO_4	20.0	77.0	17.2	4.63	0.53	0.36	0.18	0.09	0.01					
ImpA + Na^+-montmorillonite (100 °C)														
HCl	6.70	80.9	16.4	1.81	0.75	0.12	0.02							
H_2SO_4	7.70	82.1	14.7	1.93	0.81	0.32	0.13	0.01						
ImpA + NaCl reagent only														
	0.10	99.9	0.10											

[a] Linear dimers. Other products of reaction are adenosine (2–3%), AMP (21–68%) and cyclic dimers of A (41–68%).
[b] Number in parentheses denotes the temperature at which acid treatment of the native montmorillonite (Treherne) was carried out with HCl (0.5 M) or H_2SO_4 (0.25 M).

Table 2
Oligomerization of ImpU catalyzed by Na^+-montmorillonite prepared at varying temperature.

Reaction mixture/Treatment with	Total oligomer (%)	Percent distribution of the oligomers												
		II[a]	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV
ImpU + Na^+-montmorillonite (4 °C)[b]														
HCl	30.3	29.0	32.7	14.9	9.62	5.03	3.37	2.09	0.91	0.78	0.68	0.51	0.34	0.17
H_2SO_4	27.7	34.3	36.2	14.9	7.75	4.37	1.33	0.55	0.30	0.19	0.07	0.04		
ImpU + Na^+-montmorillonite (25 °C)														
HCl	27.0	33.3	33.7	14.3	7.41	4.19	3.11	1.69	0.97	0.71	0.41	0.19	0.02	0.01
H_2SO_4	24.1	35.1	38.4	13.9	6.71	3.17	1.55	0.58	0.29	0.16	0.10	0.04		
ImpU + Na^+-montmorillonite (50 °C)														
HCl	23.7	36.9	32.5	15.6	6.52	3.96	2.73	1.32	0.31	0.13	0.03			
H_2SO_4	17.2	48.6	34.5	10.5	4.13	1.57	0.47	0.18	0.05					
ImpU + Na^+-montmorillonite (75 °C)														
HCl	20.4	40.0	34.6	13.7	5.14	3.23	2.06	1.03	0.17	0.04	0.03			
H_2SO_4	17.1	48.9	34.5	10.5	4.04	1.47	0.35	0.18	0.06					
ImpU + Na^+-montmorillonite (100 °C)														
HCl	18.1	50.0	34.9	10.3	3.35	0.87	0.49	0.06	0.02	0.01				
H_2SO_4	13.4	56.7	32.4	7.94	2.08	0.52	0.30	0.06						
ImpU + NaCl reagent only														
	0.30	99.9	0.10											

[a] Linear dimers. Other products of reaction are uridine (2–3%), UMP (41–71%) and cyclic dimes of U (14–27%).
[b] Number in parentheses denotes the temperature at which acid treatment of the native montmorillonite (Treherne) was carried out with HCl (0.5 M) or H_2SO_4 (0.25 M).

2.4. Montmorillonite-catalyzed oligomerization of activated nucleotides

The stock solution of activated mononucleotide (15 mM) was prepared in 1 M NaCl. To a 200-μL reaction mixture was added 10 mg Na^+-montmorillonite, the suspension was vortexed and allowed to stand at 24 °C for 72 h. After the completion of reaction, the supernatant was collected from the reaction mixtures by centrifugation at 13,200 rpm/16,100 × g (6 min). The reaction products were desorbed from Na^+-montmorillonite by extracting four times (2 × 1 h, 1 × overnight, and 1 × 1 h) with 200 μL of 30% CH_3CN in 0.1 M NaCl elution reagent. Each extract was collected by centrifugation and combined with the supernatant to give 0.9 mL of combined extracts. The combined extract was diluted to 1.0 mL, filtered with an Alltima 0.45 μm nylon syringe filter, adjusted to pH 4 with 1% $HClO_4$ and incubated at 40 °C for 2.5 h to cleave any unreacted imidazole groups from the activated nucleotides. The samples were diluted (0.1 mL/mL) and 50 μL aliquots were analyzed by HPLC using an ion exchange column. The percentage yield of products given in Tables 1 and 2 are calculated from an average of three independent determinations. Experimental outcomes were always within 5% of one another. For the extraction of RNA oligomers from the reactions with smaller quantities of Na^+-montmorillonite (Table 3), the extraction reagent (0.8 mL) was added directly to the reaction mixture following the completion of 72 h reaction. After an overnight (16 h) desorption of oligomers from the Na^+-montmorillonite, the mixture (1 mL) was centrifuged (13,600 rpm; 8 min). The transparent liquid sample was filtered with an Alltima 0.45μ nylon syringe filter, adjusted to pH 4 with 1 M $HClO_4$ and incubated at 37 °C (2.5 h) to cleave the unreacted imidazolide groups from the reaction products as well as the unreacted activated mononucleotide.

Table 3
Determination of minimum level of Na^+-Montmorillonite (H_2SO_4 treated Treherne clay at 25 °C) required for examining the catalytic activity of a clay mineral.

Na^+-Montmorillonite	Chain length[a] of oligomers with		
	ImpA	ImpU	ImpA + ImpU
10.0 mg	13	12	11
5.00 mg	11	11	10
4.00 mg	10	10	9
3.00 mg	8	8	7
2.00 mg	7	6	6
1.00 mg	5	5	5
0.50 mg	4	4	4
0.25 mg	4	4	4
0.10 mg	3	4	3

Reaction mixture: Activated nucleotide (15 mM) + Na^+-montmorillonite + 1 M NaCl (200 μL, 72 h, 25 °C).
[a] As detected by ion exchange HPLC.

2.5. Reaction and analysis in the absence of minerals

Control samples of activated nucleotides (15 mM) were prepared in 1 M NaCl (200 μL) without Na^+-montmorillonite. They were allowed to stand at 24 °C for 3 days. After the completion of reaction, the mixtures were diluted to 1.0 mL with the 30% CH_3CN in 0.1 M NaCl reagent, adjusted to pH 4 with 1 M $HClO_4$, and incubated at 37 °C for 2.5 h to cleave any unreacted imidazole groups from the reaction products and the residual activated nucleotides.

3. Results and discussion

The oligomerization reactions of the representative purine and pyrimidine activated nucleotides (ImpA and ImpU) with Na^+-montmorillonite were found to be pH dependent (Fig. 1). At pH below 6, montmorillonite produced largely the pyrophosphates and mononucleotides. At pH above 9, montmorillonite produced predominantly the mononucleotides by hydrolysis. Optimum catalytic activity of montmorillonite was observed at pH between 6 and 9 with 1 ± 0.2 M sodium chloride (Fig. 2A and B). This salt concentration resembled that of ancient oceans [24,25]. In the absence of any salts, Na^+-montmorillonite (pH 7) was only a poor catalyst for the synthesis of RNA oligomers from these activated mononucleotides (Fig. 2 B). So, longer oligomers were only detected within these pH constraints. Additionally, the Na^+-montmorillonite-catalyzed reaction was only viable for the formation of RNA oligonucleotides. When the ribonucleotides were replaced by the 5′-phosphorimidazoilide of deoxy-adenosine or deoxy-uridine, their adsorption to montmorillonite (Fig. 3A) remained comparable, but no long chain DNA-type oligomers were detected (Fig. 3 B). The specificity of RNA synthesis by mineral catalysis further supports the “RNA World” hypothesis proposed by Woese [6] and Gilbert [4], which suggests that the early life was based on RNA, and DNA and protein evolved from it [5].

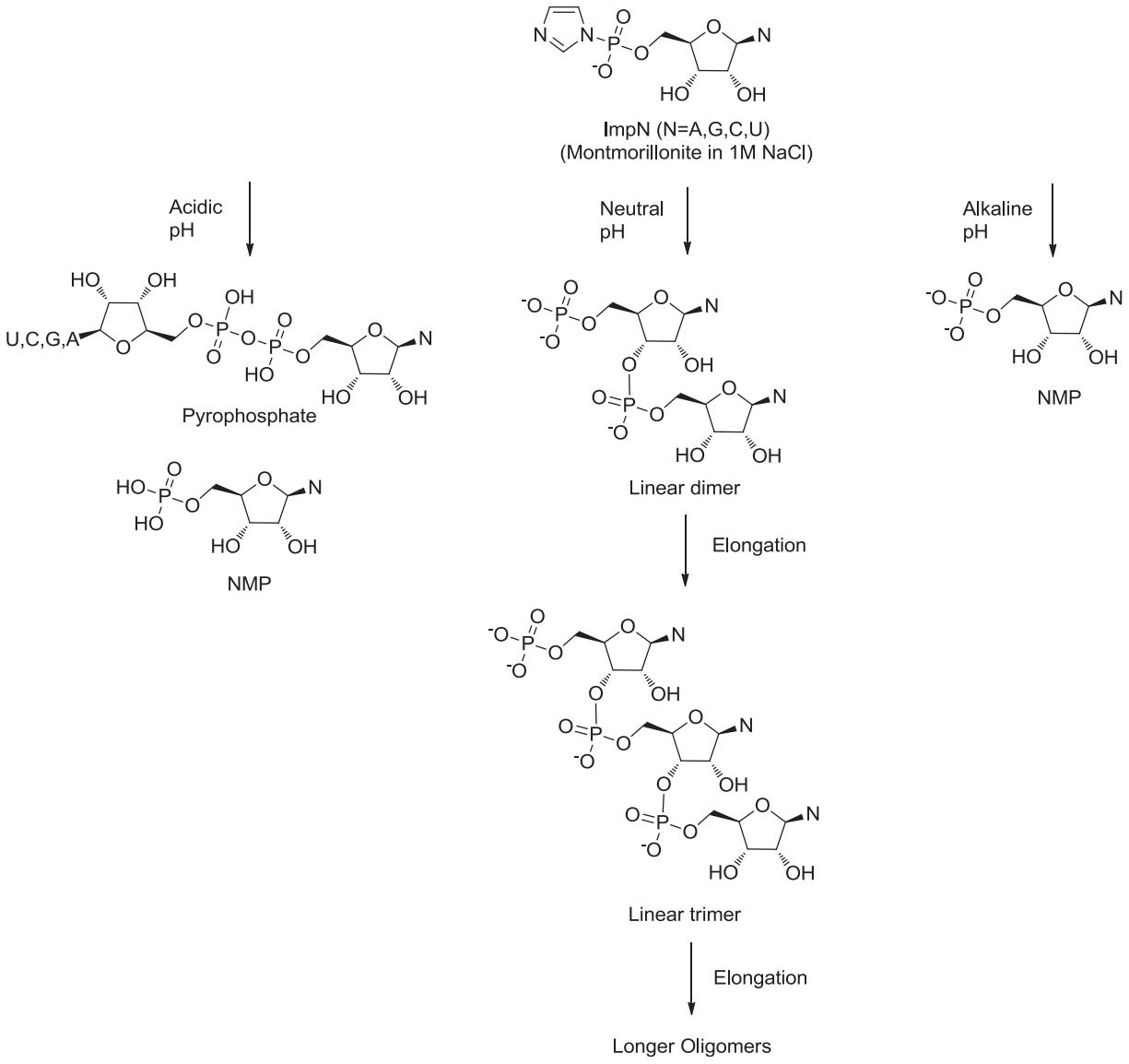

Fig. 1. pH dependent reactions of activated mononucleotides with saline Na^{+}-montmorillonite.

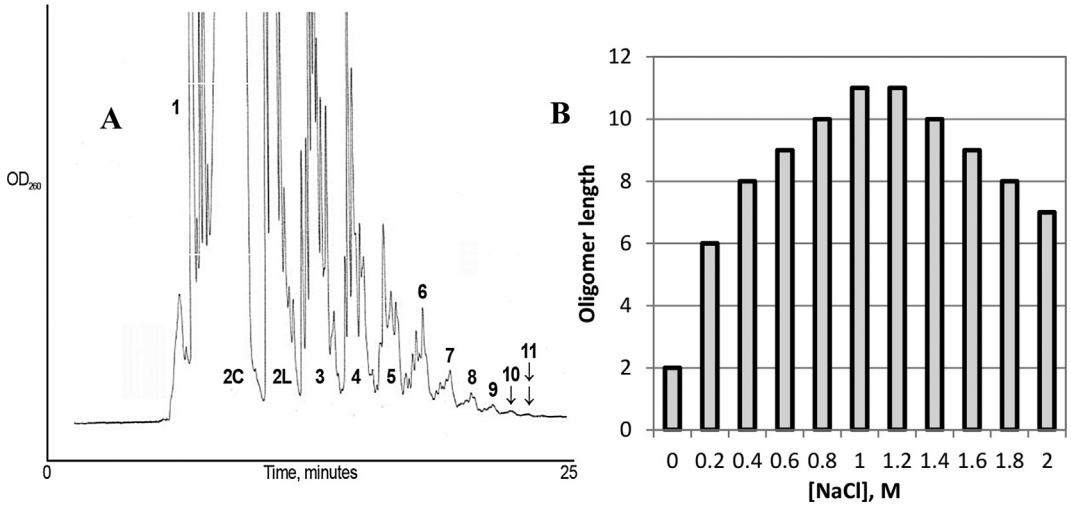

Fig. 2. Formation of RNA oligomers by Na^{+}-montmorillonite catalyzed reaction of 15 mM ImpA in saline water. A, Separation of RNA oligomers with increasing negative charges in an anion exchange HPLC column. Addition of each nucleotide to the monomer (−2 charges) adds up a negative charge and oligomers are eluted accordingly. B, Effect of varying concentrations of NaCl in the chain length of RNA oligomers.

The effect of temperature during acid treatment (weathering) upon the catalytic activity of Na^+-Montmorillonite was investigated by simulating both an early Earth and Martian soil conditions. The simulation of the former condition involved treatment of native montmorillonites with mild hydrochloric acid, following neutralization to pH 7 with NaOH [9]. Under Martian simulation conditions, the treatment of native montmorillonites was carried out with mild H_2SO_4 followed by neutralization to pH 7 with NaOH. The acid treatment of native montmorillonite was carried out at 5 different temperatures; 4°, 25°, 50°, 75° and 100 °C to examine any change in the surface charge and catalytic activity of the clay mineral with temperature. The catalytic properties of these ten acid treated samples of Na^+-montmorillonites were investigated by conducting two sets of experiments, one set involving ImpA (a purine nucleotide) and the other involving ImpU (a pyrimidine nucleotide). In the oligomerization reactions of ImpA with H_2SO_4 washed Na^+-Montmorillonite, the oligomer chain length were comparable to a similar experiments carried out with HCl washed montmorillonites (Table 1). Variation of temperature (4°-75 °C) in the preparation of H^+- montmorillonite had no effect in the catalytic activity. However, in the H_2SO_4 treated montmorillonite prepared at 100 °C, the total yield of oligomers was reduced from 21% to 8%, although catalytic activity was retained and oligomers up to 8-mers were still detected (Table 1). When the purine base (adenine) of the activated nucleotide (ImpA) was replaced by a pyrimidine (uracil) in ImpU, the net outcome of the oligomerization reactions (as determined by the comparison of chain length and relative yield of the oligomers) in both the acid treated montmorillonites was similar (Table 2). The control reactions carried out in the absence of Na^+-montmorillonite produced only dimers and traces of trimers (Tables 1 and 2).

The properties of montmorillonites obtained from different natural sources are known to differ in their catalytic activity, often due to differences in isomorphous substitutions [2,13]. Since every acid treated sample of montmorillonite from Treherne (Manitoba, Canada) retained their catalytic activity, their surface layer charges were determined for comparison with two non-catalytic montmorillonites obtained from Otay, CA (H-24) and Chambers, AZ (H-23), which have interlayer surface charges of 0.43, 0.45, respectively [13]. The titration plots of the acid forms of these montmorillonites with 0.02 M NaOH showed two distinctive equivalence points at about pH 4 and pH 7 (Fig. 4A and B). The volume of NaOH required to titrate the H^+-montmorillonite (prepared by acid treatment at 4°, 25°, 50°, 75°and 100 °C) to pH 7 was very similar for all ten catalytic montmorillonites. In contrast, the volume of NaOH required to titrate the non-catalytic H^+-montmorillonite to pH 7 was roughly 42 ± 1% higher (Fig. 4 C), which confirmed that these non-catalytic montmorillonites have lower layer charges (Treherne, 0.31) [2,26]. This finding is consistent with the proposal that the Otay and Chambers montmorillonites are not catalytic because the activated monomers are not able to bind together in the galleries between the clay platelets. Furthermore, the interlayer is occupied by the cations needed to neutralize the layer charge so there is insufficient space for the activated monomers to be adsorbed [27–29].

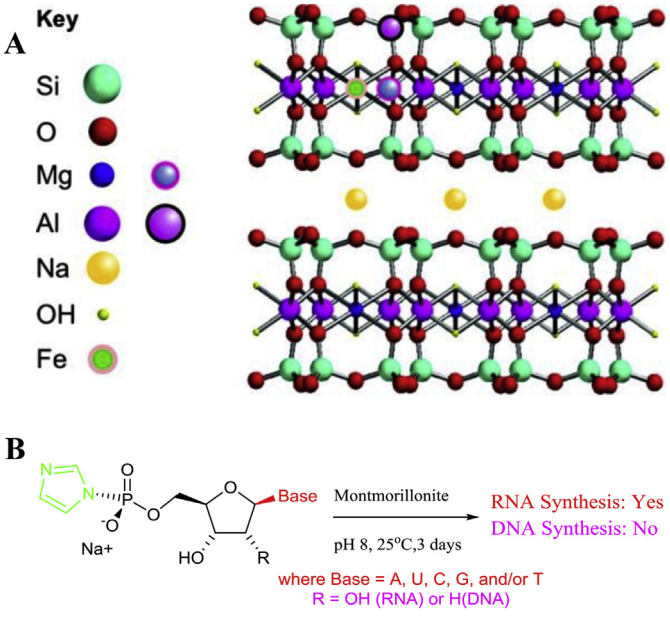

Fig. 3. A, The structure of montmorillonite based upon Grim [31]. B, Model reaction of the 5′-phosphorimidazolide of a nucleoside on Na^+-montmorillonite.

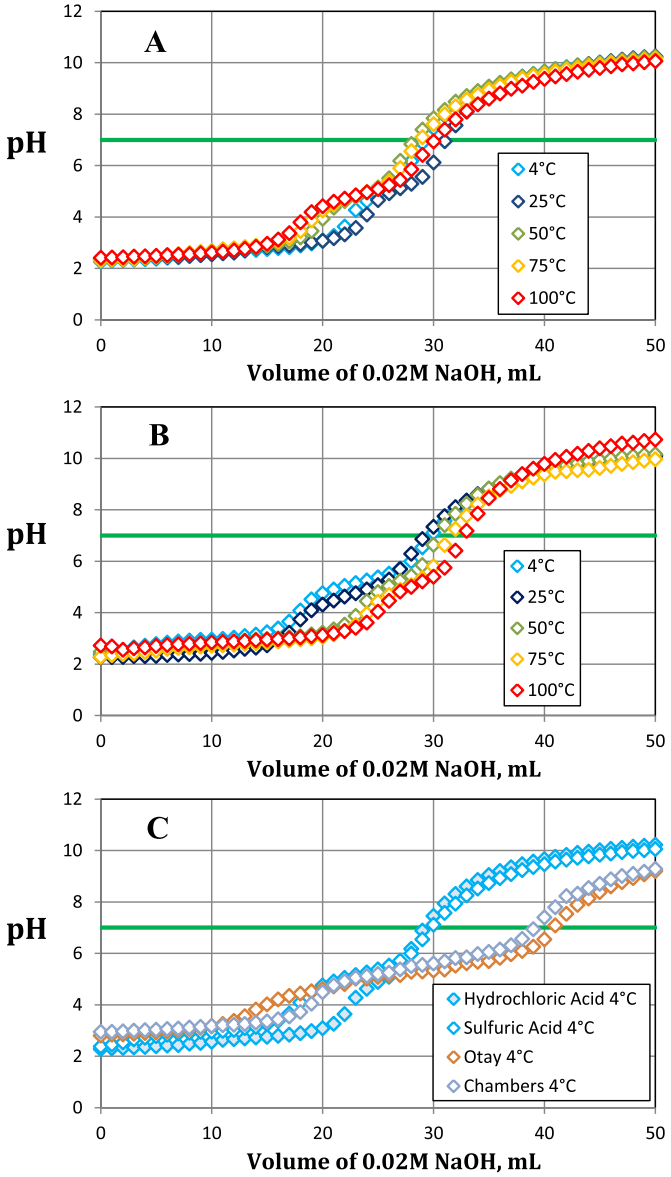

Fig. 4. Titration plots of the protonated forms of montmorillonites. A, HCl washed montmorillonite (Treherne, Manitoba, Canada) at varying temperature. B, H_2SO_4 washed montmorillonite (Treherne, Manitoba, Canada) at varying temperature. C, comparison of representative HCl and H_2SO_4 treated montmorillonite with two non-catalytic clays obtained from Otay (California, USA) and Chambers (Arizona, USA).

This study has direct relevance to the Mars sample return (MSR) mission which is formulating a new rover mission for an eventual sample return in the year 2020 [30]. To identify the minimum amount of Mars return material necessary to determine catalytic activity, three sets of reactions were conducted. Independent studies for the oligomerization of ImpA, ImpU and an equimolar mixture of ImpA and ImpU were carried out with varying amounts of Na^+-montmorillonite (0.1–10 mg) in 1 M NaCl. The Na^+-montmorillonite prepared by H_2SO_4 treatment at 25 °C was used as a catalyst in these model experiments. Maximum chain length of RNA oligomers (10-13 mers) was detected by HPLC with 10 mg Na^+-montmorillonite (Table 3). Catalytic activity was still maintained at 0.10 mg Na^+-montmorillonite. The difference in the results at 0.10 mg of catalyst was that the chain length was reduced to trimers and tetramers. The results suggested that the catalytic activity of MSR material can be investigated with sub-milligram scale samples.

The montmorillonite prepared under early Earth conditions retained its catalytic activity when weathered under Martian conditions. Optimum catalytic activity was observed within a narrow range of pH and salinity. The model reaction is exclusive for the formation of RNA oligomers thus further supporting the “RNA World” hypothesis. A protocol has been developed to analyze potential MSR materials for catalytic activity at sub-milligram level.

Author contributions

PCJ conceived the idea and wrote the manuscript. MFA helped with the discussions, editing of the manuscript and preparation of figures. PCJ and KD performed the experiments and carried out HPLC analysis. MS performed titrations of clay samples. All authors read the manuscript and approved the content.

Conflict of interest

None.

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Transparency document

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