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Mechanism of Montmorillonite Catalysis in the Formation of RNA Oligomers

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Abstract: The montmorillonite clay-catalyzed reactions of nucleotides generate oligomers as long as 50-mers. The extent of catalysis depends on the magnitude of the negative charge on the montmorillonite lattice and the number of cations associated with it. When cations in raw montmorillonites are replaced by sodium ions, the resulting Na^+-montmorillonite does not catalyze oligomer formation because they saturate the interlayers between the platelets of montmorillonites, which blocks the binding of the activated monomers. Treating the montmorillonite with dilute hydrochloric acid replaces the cations on the raw montmorillonite with protons. The protonated montmorillonite, titrated to pH 6−7, serves as a catalyst for the formation of RNA oligomers. The titration does not add sufficient sodium ions to the interlayers of the montmorillonite platelets to prevent the activated monomer from entering. It was noted that noncatalytic montmorillonites have a higher negative charge on their platelets that is due mainly to the natural substitution of the tetravalent and trivalent elements in the montmorillonite lattice with trivalent and divalent metal ions, respectively. The larger negative charge on these montmorillonites was demonstrated by the almost 2-fold greater amounts of sodium hydroxide needed to titrate noncatalytic montmorillonites as compared to the catalytic montmorillonites. Adsorption isotherms established that the equilibrium binding is strongest for ImpA and weakest for ImpU. Of the 22 montmorillonites investigated, 12 were catalysts. This research provides insight into the mechanism of the catalytic process.

Introduction

The RNA world proposal for the origin of life is where RNA both stores genetic information and catalyzes chemical reactions. The DNA protein world evolved from the RNA world. Our recent findings emphasize the synthesis and properties of the RNAs formed by montmorillonite catalysis. The montmorillonite catalyzed reaction of the 5′-phosphorimidazolides of nucleosides (Figure 1a) yielded 10-mers when analyzed by HPLC.[1] It was possible to detect 35-mers in the same reaction products when analyzed by MALDI mass spectra.[2] Elongation of a decameric primer by montmorillonite catalysis of the reaction of the 5′-phosphorimidazolides of nucleosides yielded 30−50-mers when analyzed by gel electrophoresis.[3,4] These long oligomers were also formed in the montmorillonite-catalyzed reaction of the 5′-(1-methyladenine) derivatives of nucleosides (Figure 1b).[5,6] The oligomers formed exhibit sequence and phosphodiester bond selectivity.[7] These findings lead to the proposal that the minerals or metal ions initiated the formation of the RNA world on the early Earth.[8,9] The presence of montmorillonite on Mars suggests that these processes may also have taken place on Mars.[10,11]

The formation of the Earth's metallic core and the impact of a Mars-sized object onto the proto-Earth[12] to form the Moon-forming event both occurred by ∼4.54 Ga ago[13] (Ga = 10^9 years). These extreme processes would have created environments too hostile to the formation of life. Geochemical and isotopic data acquired on individual crystals of zircon with ages of up to ∼4.40 Ga[14,15] from Western Australia continue to furnish information about the early Earth suggesting that more moderate environments than had previously been assumed were present. The ages of the ancient zircons are determined using the uranium−lead isotopic method.[14,15] Ratios of stable isotopes[14,15] and the abundances of trace elements like

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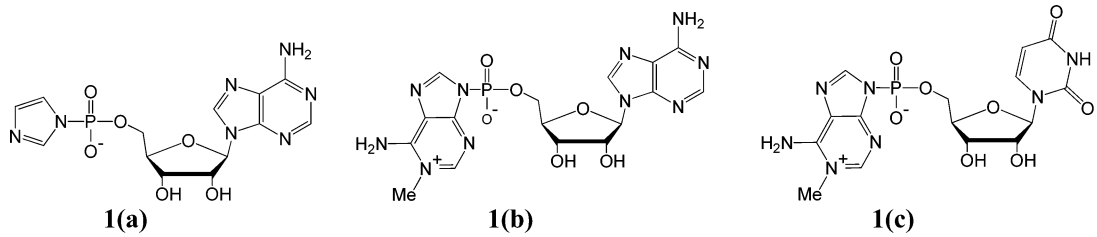

Figure 1. (a) Anion of adenosine-5′-phosphorimidazolide (ImpA). (b) Adenosine 5′-phosphoro-1-methyladeninium (MeadpA). (c) Uridine-5′-phosphoro-1-methyladeninium (MeadpU).

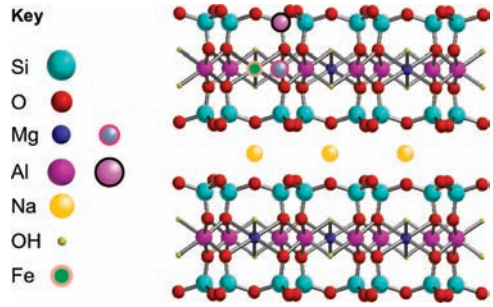

Figure 2. Unit cell of montmorillonite based upon Grim.[20]

titanium[16,17] demonstrate that water was present in the Earth's crust,[16] perhaps even oceans[14,15] up to ~4.40 Ga ago. The magmas that crystallized to form those ancient zircons are now known to have been essentially 'granitic' in composition',[16] which is a major component of the Earth's current landmasses. The bombardment rate of large meteorites would also have important effects on environments of the early Earth'.[18] One view on the time-dependent flux of meteorites is that it was high, but declined steadily, for the first ~0.6-Ga of Earth history. Another emerging view suggests that the bombardment rate was modest during much of that time, and was punctuated by a brief spike of increased flux at ~3.90 Ga.[19] Although the near-surface environments on the early Earth would have been quite different in those two competing scenarios, life may have been sustainable[18] in both.

Objectives. We are investigating the mechanism by which montmorillonite catalyzes the formation of RNA to gain insight into how a catalyst would initiate the formation of RNA. Here we report our studies on the montmorillonite catalysis with the objectives of:

1. Determining why the interlayer metal cations must be removed and replaced with alkali metal cations before the mineral has catalytic activity. Homoionic montmorillonite is required for catalytic activity.

2. Determining why only certain montmorillonites catalyze RNA formation.

3. Determining the reaction pathway for RNA synthesis on montmorillonite.

The unit cell of montmorillonite is composed of silicon, aluminum and oxygen together with smaller amounts of other cations (Figure 2). These other cations were present in the volcanic ash or in the water where the montmorillonite was formed and some were incorporated into the lattice. The cations incorporated are usually smaller than the cations they replace. Silicon is replaced mainly by aluminum and aluminum is replaced mainly by iron and magnesium. Aluminum may also be replaced by chromium, zinc and lithium.[20]

Table 1. Types of Selected Montmorillonites[21]

type	interlayer charge range	mean
Wyoming	0.66−0.84	0.76
Otay	0.84−0.97	0.91
Chambers	0.85−0.98	0.94

There are many montmorillonites that differ in the extent of their isomorphic substitution. The Wyoming (Volclay), Otay and Chambers montmorillonites will be emphasized in this study. The negative charges on the Otay and Chambers classes of montmorillonites are proposed to be similar to one another but are greater than those of the Wyoming montmorillonites (Table 1).

Results and Discussion

As noted above, montmorillonites have negative charges on their lattice structures that are due to the incorporation of lower valence cations in their tetrahedral and octahedral layers. This negative charge is neutralized by the ionic binding of cations (Figure 2). Since montmorillonites are formed by the reaction of volcanic ash with water, the cations present in the ash or in the water may be incorporated into the clay lattice. It is known that some metal ions catalyze the formation of RNA oligomers[22−25] so it is important to replace the cations in the raw montmorillonite with sodium ions, or other suitable species, for catalytic activity studies.

Clays Are Not Catalytic After Treatment by the Saturation Procedure. In our initial studies we used both the Banin[9] and the saturation procedure[26] to generate montmorillonites where sodium is the principal exchangeable cation. In the Banin procedure the montmorillonite is treated briefly with cold hydrochloric acid to exchange the interlayer cations with protons and then the montmorillonite is titrated to pH 6−7. In the saturation procedure the montmorillonite is treated with excess sodium chloride to replace the exchangeable cations with sodium ions. We were initially surprised to observe that when Volclay

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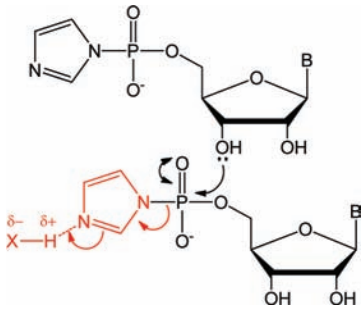

Figure 3. Proposed phosphodiester bond formation on montmorillonite. XH is an undifferentiated protic species within the clay galleries.

was treated by the Banin procedure it was catalytic while the Volclay treated by the saturation procedure was not catalytic. When a montmorillonite was treated first by the Banin procedure and then subjected to the saturation procedure it had no catalytic activity but Volclay treated first by the saturation procedure and subsequently treated by the Banin procedure was a catalyst.[27] This finding suggested that the Banin procedure was responsible for the catalytic activity of the montmorillonite. The montmorillonites described in subsequent research were all treated by the Banin procedure before being tested for catalytic activity.

The observation that montmorillonites that are catalytic when the interlayer cations are replaced by the Banin procedure and are not catalytic when the interlayer cations are removed by the saturation procedure suggested that something about the Banin procedure was the key to the answer of the catalysis. A second clue was the observation that the titration of Volclay by the Banin procedure to pH 11 instead of pH 7 yielded a montmorillonite that was not catalytic. This observation suggested that complete replacement of protons with sodium ions inhibited the catalysis. Sodium ion is an inhibitor if it blocks the access of the activated monomers into the interlayer between the clay platelets where catalysis occurs.[28] This finding is consistent with the absence of catalysis when the montmorillonite is not treated by the Banin procedure or is treated by the saturation procedure where most of the sites in the interlayer are occupied by cations.

The presence of protons or un-ionized hydroxyl groups within the clay galleries enables protonation of the phosphorimidazolide. The protonated form is an even more reactive species and phosphodiester bond formation is envisioned as shown (Figure 3).

Binding of Activated Monomers to Volclay, Chambers and Otay Montmorillonites. The binding of the activated monomers to three different montmorillonites was measured since the extent of binding may provide insight into their catalysis of RNA formation. The much stronger binding of ImpA on Volclay, compared to Otay and Chambers, is consistent with the proposed greater negative layer charge on the Otay and Chambers montmorillonites (Table 1). The greater layer charge requires more base to adjust the pH to 7 in the Banin procedure for the Otay and Chambers montmorillonites. The additional cations from the base bind in the interlayer between the platelets and prevent the activated nucleotides from entering.

ImpA (Figure 1a) binds more strongly to Volclay than ImpC and ImpU. We ascribe the strong binding of ImpA to a strong van der Waals interaction of ImpA to the interlayers of the clay

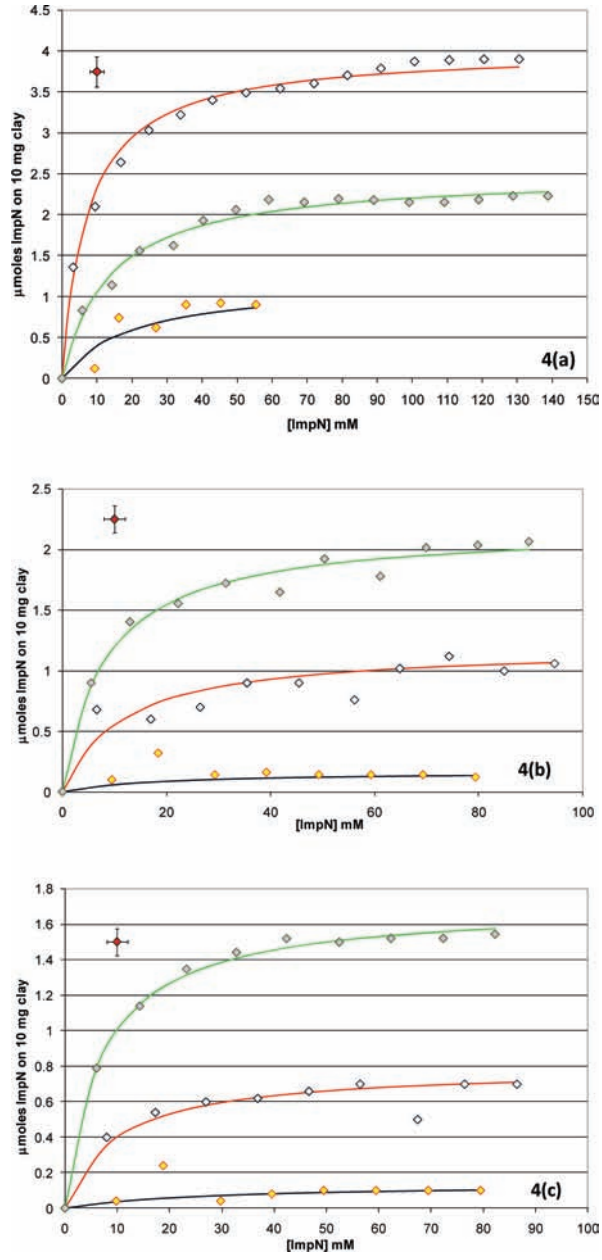

Figure 4. Binding of activated monomers to montmorillonite with (a) Volclay, (b) Otay and (c) Chambers. The red line through the experimental points is ImpA, the green line is ImpC and the black line is ImpU. The lines are the calculated best fit to the experimental data. The error limits are shown in the upper left-hand corner of each plot.

platelets of Volclay.[29,30] ImpU is the weakest binder of the three montmorillonites. It binds more strongly to Volclay than it does to the Otay and Chambers montmorillonites. This is the same relative order of ImpA binding to the three montmorillonites.

ImpC also binds more strongly to Volclay than it does to the other two montmorillonites. This reflects a weaker van der Waals interaction of ImpC with the montmorillonite than that of ImpA. The greater binding of ImpC than ImpU to Otay and Chambers reflects the greater van der Waals forces of the clay-cytosine interaction compared with that of the uracil ring of ImpU.

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Table 2. Values of K_L and a_s[a] from Binding Studies at 4°C

		Otay	Chambers	Volclay
K_L	ImpA	101	87	137
a_s	ImpA	7.91×10^{-07}	1.19×10^{-06}	4.02×10^{-06}
K_L	ImpU	36	54	50
a_s	ImpU	1.39×10^{-07}	1.67×10^{-07}	1.18×10^{-06}
K_L	ImpC	142	121	73
a_s	ImpC	1.71×10^{-06}	2.18×10^{-06}	2.51×10^{-06}

[a] The units of a_s are μmol/10 mg clay.

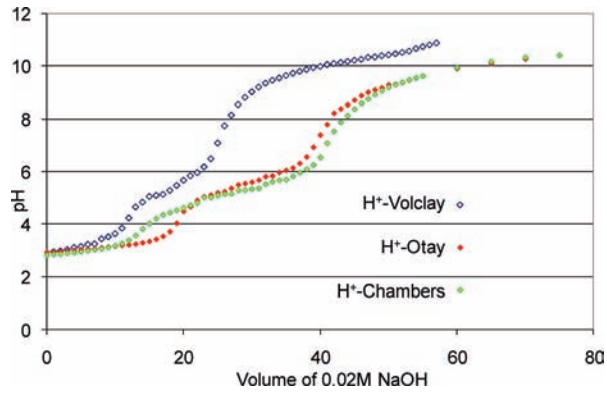

Figure 5. Titration plot of the acid forms of three montmorillonites: protonated Volclay, protonated Otay and protonated Chambers.

Titration of the Acidic Form of the Montmorillonites. The data in Table 1 proposes that the Wyoming class of montmorillonites have lower layer charges than the Otay and Chambers classes. It is of particular interest that the negatively charged ImpNs (Figure 1a) bind to the Wyoming Montmorillonite even though the clay lattice also has a negative charge. Since we did not have a direct comparison between the layer charges of the three montmorillonites we measured them by the titration of the acid form of the montmorillonites with 0.0210 M NaOH (Figure 5). The acid form of the montmorillonites was prepared by the same method used in the Banin procedure given in the Experimental. The titration plots have two equivalence points at pH 4 and pH 7. As can be seen in Figure 5, the volume of NaOH required to titrate from the equivalence point at pH 4 to the one at pH 7 is much less for Volclay than for the Otay and Chambers montmorillonites. This observation confirms that the Otay and Chambers montmorillonites have a higher layer charge than does Volclay, a finding consistent with the proposal that the Otay and Chambers montmorillonites are not catalysts because the activated monomers are not able to bind in the galleries between the clay platelets. The interlayer is occupied by the cations needed to neutralize the layer charge so there is insufficient space for the activated monomers to fit.[28,31,32]

RNA oligomer formation proceeds in the interlayers between the montmorillonite because that is where the activated monomers will be held in close proximity to react with each other having lost most of their rotational and translational degrees of freedom. The reaction of the anion of uridine-5′-phosphorimidazolide, ImpU, in the presence of montmorillonite gives 20% 3′, 5′-linked oligomers.[33] When the activating group of the nucleotides is changed to 1-methyladenine as in MeadpU (Figure 1c), 61% of the oligomers have 3′, 5′-links.[6] It is likely that the increase in regiospecificity is due to the stronger binding of the neutral MeadpU to montmorillonite compared with the ImpU anion. The stronger binding may locate the 3′- hydroxyl group of one activated monomer proximate to the activated monomer of another nucleotide so that reaction with the 3′- hydroxyl group is favored. This outcome is consistent with the greater binding of the neutral MeadpU to the montmorillonite compared with that of the negatively charged ImpU.

Table 3. Yields from the HPLC Analyses Given in Figure 6[a]

	molar %		
oligomer length[b]	sample 1[c]	sample 2[d]	sample 3[e]
1	47.6	71.4	97.7
2*	38.0	20.8	2.28
2**	10.6	6.51	0.06
3	2.57	1.04	
4	0.77	0.19	
5	0.27	0.039	
6	0.10		
7	0.03		
8	0.008		
9	0.004		

[a] No hyperchromicity correction was made for the product oligomers, each of which is a complex mixture of isomers. [b] 2* cyclic dimers 2** linear dimers. [c] ImpA (15 mM) with Volclay. [d] ImpA (15 mM) with Little Rock. [e] ImpA (15 mM) with Chambers.

Table 4. Catalytic Activity of Selected Montmorillonites[a]

activity	clay/location
Excellent	Volclay-Supercol, WY
Excellent	H-27 Belle Fourche, SD
Excellent	H-25 Upton, WY
Excellent	H-26 Clay Spur, WY
Excellent	SWy-1, Crook City, WY
Good	Sodium ions-EconoPlug, WY
Good	JCSS-3102, Japan
Good	Dana 496, Osage, WY
Good	H-28, Little Rock, AR
Good	H-21, Polkville, MS
Good	H-22, Oxidizable Blue, Amory, MS
Good	H-22a, Yellow, Amory, MS
Poor	H-19, Polkville, MS
Poor	H-24, Otay, CA
Poor	H-31, Cameron, AZ
Poor	JCSS-3101, Tsukinuno, Japan
Poor	SAz-1 Cheto-Apache City, AZ
Poor	Bayard, NM
Poor	H-23, Chambers, AZ
Poor	S−Ca-1, San Diego, CA
Poor	Flat Creek #14, NY **ISOB**[b]
Poor	Millbrig K-Bentonite, KY **ISOB**[b]

[a] Clays are Cretaceous, that is, 140−65 million years old. [b] Illite/Smectite Ordovician Bentonite, that is, ~450 million years old.

The Search for Catalytic Montmorillonites. The discovery that not all montmorillonites are catalysts prompted an evaluation of those montmorillonites provided mainly by Prof. Michael Gaffey at University of North Dakota. By determining the percentage of the montmorillonites that are catalysts, we suggest that catalytic montmorillonites may have been available on the early Earth to catalyze RNA formation (Table 3). The extent of catalytic activity was measured by the length of the oligomers formed from ImpA in a 3 day reaction as determined by HPLC: generating 7-mers or greater was designated as "excellent", 4−7-mers as "good" and less than 4-mers as "poor". We found that 12 of the 22 montmorillonites were either "excellent" or "good" catalysts (Table 4 and Figure 6).

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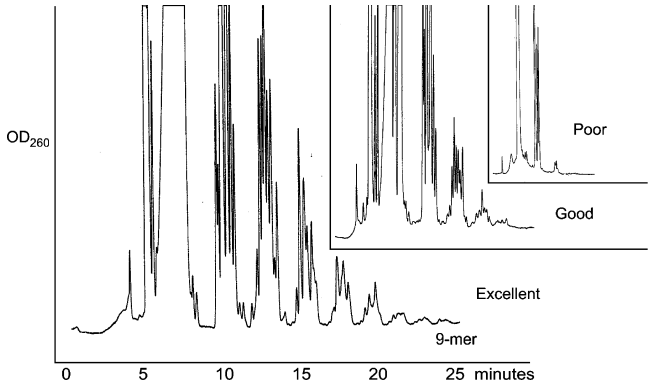

Figure 6. HPLC traces obtained for three representative types of montmorillonite. They are Volclay (an excellent catalyst), Little Rock (a good catalyst) and Chambers (a poor catalyst).

Conclusions

We succeeded in answering the three questions that were posed in the Objectives. The first question was why was it necessary to generate catalytic montmorillonites by the Banin procedure and not by the saturation procedure. The Banin procedure involves the conversion of the montmorillonite to its protonated form and then back-titration to pH 6−7 while the saturation procedure involves treatment of the montmorillonite with excess sodium chloride. The saturation procedure results in the replacement of cations in the montmorillonite interlayer with sodium ions while the Banin procedure only replaces those protons in the acidic montmorillonite with sodium ions required to reach pH 6−7. Elemental analyses (data not shown) confirm that there are fewer sodium ions in the interlayer of the Volclay when it is titrated to pH 7 than the Otay and Chambers montmorillonites when they are titrated to pH 6−7, consistent with the titration data (Figure 5). Titration to pH 6−7 results in the substitution of only some of the protons with sodium ions. If most of the sites in the interlayer are filled with sodium ions it is impossible for the activated monomers to intercalate between the montmorillonite platelets that catalyze the formation of RNA oligomers.

The second question is why do only some of the montmorillonites catalyze the oligomerization reactions even though they were treated by the Banin procedure? When the protonated montmorillonites of the Otay and Chambers class were titrated with sodium hydroxide it was observed that significantly larger volumes of alkali were required to titrate to the pH 7 equivalence points than was required for the Volclay montmorillonite. This established that the Otay and Chambers montmorillonites have more isomorphous substitution of metal ions in the montmorillonite lattice. Consequently there are more sodium ions in the interlayer between the platelets of the Otay and Chambers classes of montmorillonites than is present in the interlayer of the Volclay montmorillonites. The larger amount of cations also binds the platelets of the Otay and Chambers montmorillonites together more strongly than Volclay. This makes it impossible for the activated monomers to intercalate between the clay platelets so the Otay and Chambers montmorillonites are unable to catalyze the formation of RNA oligomers.[31]

The answers to questions 1 and 2 made it possible to determine the reaction pathway, the third question in the Objectives. The activated monomers react to form oligomers when they are intercalated between the platelets of the montmorillonite. The interlayer provides an environment where the activated monomers are proximate and can readily react to form phosphodiester bonds. The relative orientation of the activated monomers with RNAs determines whether 3′, 5′- or 2′, 5′-phosphodiester bonds are formed. The stronger binding of the activated monomers to the montmorillonite results in its more favorable orientation of the growing RNA for the formation of 3′, 5′ phosphodiester bonds.

Experimental Section

General. Adenosine 5′-monophosphate (AMP), anhydrous $NaClO_4$, cytidine 5′-monophosphate (CMP), 2, 2′-dithiodipyridine, NaH_2PO_4, imidazole, potassium hydrogen phthalate (KHP), perchloric acid, Trizma base, uridine 5′-monophosphate (UMP) and trifluoroacetic acid (TFA), were obtained from Sigma. Acetonitrile (CH_3CN) and hydrochloric acid were obtained from Mallinckrodt. Spectra/Por membrane MWCO: 1000 was obtained from Spectrum Laboratories, Inc., CA. Anion exchanger resin (10NACNA-38, OH^--Form, type I, Beads 16−50 Mesh), sodium chloride and magnesium chloride were purchased from J.T. Baker. Montmorillonite H-23 bentonite (Chambers, AZ), H-24 bentonite (Otay, CA,) H-27 bentonite (Belle Fourche, SD) and H-28 bentonite (Little Rock, AR) were obtained from Ward's Natural Science Establishment, Inc., Rochester, NY, via the Department of Earth and Atmospheric Sciences at Rensselaer Polytechnic Institute.

HPLC analysis was performed on a Hitachi L-7100 pump system equipped with a Hitachi L-4200 UV−vis detector operating at 260 nm. The negatively charged products were separated on a Dionex DNA Pac-100 μm (4 × 250 mm) analytical anion exchange column from Dionex Corporation, Sunnyvale, California, USA using a gradient of 0 − 0.4 M $NaClO_4$ with 2 mM Tris (pH 8) at a flow rate of 1 mL/minute. The analysis of samples was also performed on a reverse phase Alltima C-18, 5 μ (4.6 mm × 250 mm) column (Alltech) using 3.6% CH_3CN in 0.02 M NaH_2PO_4 with 0.2% TFA, pH 2.5 (isocratic) as a mobile phase.

Banin Procedure for the Formation of Catalytic Montmorillonites.[9] Montmorillonite samples (12 g) were treated with 0.5 M hydrochloric acid (50 mL) by continuous stirring at 4 °C for 30 min. At the end of each treatment excess acid was removed by centrifugation at 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 of distilled water at 4 °C for 30 min with constant stirring. At the end of the washing, excess water was separated by centrifugation at 3500 rpm and decanting of supernatant. Washing with water (100 mL) was repeated three more 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 the residual hydrochloric acid. The mixture was stirred for 30 min, pH was measured (3.25 ± 0.05) and the anion exchange resin was removed by filtration. Fresh anion exchange resin was added to a slurry of the montmorillonite in a beaker of water and it was titrated with 1 M sodium chloride to pH 6−7 with stirring. The anion exchange resin was removed by filtration (125 μm) and the montmorillonite slurry was removed and freeze-dried.

The analytical behavior of the sodium montmorillonites when prepared by the two methods is not unique. For example, an analysis of a zinc Polkville montmorillonite revealed that the montmorillonite prepared by the saturation method had about 25% more Zn^{2+} than the montmorillonite prepared by the Banin procedure.[34]

Titration of H^+-montmorillonites. One gram of H^+-montmorillonite was suspended in 100 mL of water and was titrated with 0.021 M aqueous sodium hydroxide that had been standardized using KHP.

Preparation of Activated Nucleotides (ImpN). The phosphorimidazolides of the 5′-nucleotides were prepared as described

(34) Ferris, J. P.; Hagan, W. J., Jr. *Orig. Life Evol. Biosphere* **1986**, *17*, 69–84.

previously.[35] The purities of ImpN determined by reverse phase HPLC were: ImpA > 99.5%, ImpC, 100% and ImpU > 99.9%.

Montmorillonite-catalyzed Oligomerization of Activated Nucleotides. A stock solution of activated mononucleotide (15 mM) was prepared in magnesium chloride (0.075M) and sodium chloride (0.2M). A 200 μL aliquot was added to 10 mg of montmorillonite; the suspension was vortexed and allowed to react at 24 °C for 3 days. The supernatant was separated from the reaction mixtures containing montmorillonite by centrifugation at 13,200 rpm for 10 min. The reaction products were extracted from montmorillonite with 200 μL of 0.1 M sodium chloride in 30% acetonitrile (twice for 2 h and then overnight and finally for 1 h.). Each extract was collected by centrifugation and combined with the supernatant to give the combined extracts. A combined extract was filtered through an Alltima 0.45 μm nylon syringe. The combined extract was adjusted to pH 4 with 1 M perchloric acid and incubated at 37 °C for 4 h to hydrolyze any unreacted ImpA. The products were analyzed on a Dionex ion exchange column.

Reaction and Analysis in the Absence of Minerals. Control solutions of ImpA (15 mM) were prepared in magnesium chloride (0.075M) and 0.2 M sodium chloride (200 μL) without montmorillonite. The mixtures were allowed to react at 24 °C for 3 days. At the end of reaction time, the mixture was then diluted to 0.5 mL with water, adjusted to pH 4 with 1 M perchloric acid and incubated at 37 °C for 4 h to hydrolyze any unreacted ImpA. The reaction products were analyzed on a Dionex ion exchange column.

Binding of Activated Monomers to Montmorillonite. An improved procedure was used that avoided the use of HEPES buffer.[36] Stock solutions of ImpN (150 mM) were prepared in an magnesium chloride (0.075M) and sodium chloride (0.2M) mixture at 4 °C. The solution was filtered through a 0.2-μm-nylon syringe filter (Alltech Associates, Inc., Deerfield, IL) and diluted to the desired molarity (10 mM − 140 mM) in the same reagent. For adsorption isotherm measurements, the binding of ImpN (200 μL) to the montmorillonite (10 mg) was determined after incubating the solution at 4 °C for 60 min. After the 60 min incubation, the sample was centrifuged at 13,200 rpm (10 min) and the supernatant was analyzed by HPLC on an Alltima C-18 column. No oligomer formation was ever detected in these binding studies. The stock solution that had not been mixed with montmorillonite was also analyzed by HPLC and the difference in the peak areas was used to measure the extent of binding. The sets of data were analyzed using a Langmuir isotherm model[37] although other isotherms have been used for comparison purposes.

The equilibrium under analysis is that between the activated nucleotide in aqueous solution and the same nucleotide species adsorbed onto the clay surface: viz.

$$\mathrm{ImpN}_{(aq)} + \mathrm{clay} \rightleftharpoons \mathrm{ImpN}_{(\mathrm{clay})}$$

Let:

1. K_L be the Langmuir equilibrium isotherm constant
2. a be the amount of activated nucleotide adsorbed on the 10 mg of clay, equivalent to a measure of [ImpN (clay)]
3. a_s be the saturation level of nucleotide adsorbed on the 10 mg sample of clay, equivalent to a measure of $[\mathrm{ImpN(clay)}]_{max}$
4. c be the concentration of activated nucleotide in solution, $[\mathrm{ImpN}_{(aq)}]$

Then at equilibrium,

$$K_L = \frac{a}{c(a_s - a)}$$

This expression can be rearranged to give:

$$\frac{a}{c} = K_L a_s - K_L a$$

whence a plot of a/c versus a gives a line of slope $-K_L$ and intercept $K_L a_s$, from which the value of a_s can be obtained.

A further rearrangement of the expression yields:

$$a = \frac{cK_L a_s}{1 + cK_L}$$

This enabled a theoretical curve of the adsorption to be drawn for each data set for which K_L and a_s are now known.

When necessary, numerical methods were used to minimize the residuals existing between the theoretical curve and the curve obtained by analysis of the experimental data set. The final correlation between the calculated points and the experimental data was always better than 0.95.

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Note Added after ASAP Publication. The heading of the first subsection in the Results and Discussion was corrected in the versions published September 16, 2009.

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