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STUDIES IN THE MINERAL AND SALT-CATALYZED FORMATION OF RNA OLIGOMERS

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Abstract. Activated mononucleotides oligomerize in the presence of montmorillonite clay to form RNA oligomers. In the present study, effects of salts, temperature and pH on the clay-catalyzed synthesis of RNA oligomers were investigated. This reaction is favored by relatively high concentration of salts, such as 1 M NaCl. It was shown that the presence of divalent cations was not required for this reaction. High concentrations of NH_4^+ and HCO_3^- and 0.01 M HPO_4^{2-} inhibit the reaction. The yields of RNA oligomers decreased as the temperature was raised from 4 °C to 50 °C. $A^{5'}$ ppA was the major product at pH's below 6. The catalytic activity of a variety of minerals and three meteorites were investigated but none of them except galena catalyzed the oligomerization. ATP was generated from ADP but it was due to the presence of HEPES buffer and not due to the minerals. Meteorites catalyzed the hydrolysis of the pyrophosphate bonds of ATP. The results suggest that oligomers of RNA could have formed in pH 7–9 solutions of alkali metal salts in the presence of montmorillonite clay.

Keywords: RNA oligomers, catalysis, origins of life, montmorillonite, meteorite, mineral, ATP

1. Introduction

Recent studies on the montmorillonite catalysis have emphasized the formation of longer RNA oligomers. The reaction proceeds with imidazole (Weimann *et al.*, 1968) and 1- or 3-methyladenine activating groups (Figure 1) (Prabahar and Ferris, 1997; Huang and Ferris, 2003) in a reaction solution containing 0.1 M buffer, 0.075 M $MgCl_2$ and 0.2 M NaCl and at a temperature of 25 °C. The reaction proceeds equally as well with purine or pyrimidine nucleotides with a 3′, 5′-phosphodiester bonds forming 67% of the time with purine and 20% of the time with pyrimidine nucleotides (Ferris and Ertem, 1993; Ding *et al*., 1996; Prabahar and Ferris, 1997).

Not all montmorillonites catalyze the oligomerization reaction. The more effective catalysts include SPV-200 Volclay, a commercial product from the American Colloid Company, montmorillonite 22-A from Wards Scientific, Belle Fourche, North Dakota H-27 (Kerr *et al.*, 1951) and SWA-1, a sample from the Clay

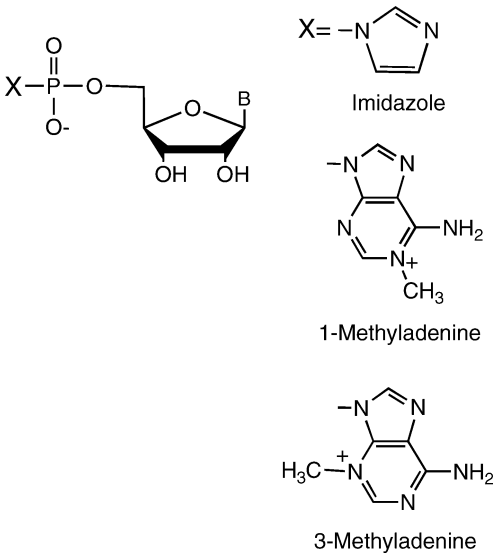

Figure 1. Activated RNA monomers. B= adenine, guanine, hypoxanthine, cytosine and uracil.

Minerals Society. A sample from Japan is somewhat less active than those mentioned above (Kawamura and Ferris, 1994) while montmorillonites with either very low or very high iron content (nontronites) have little or no catalytic activity. Most montmorillonites have to be converted to a homoionic form in a procedure that uses a cold acid wash and then adjusting the pH 6-7 before they exhibit catalytic activity (Banin 1973; Banin *et al.*, 1985). Catalytic activity is observed when the exchangeable cations are alkali or alkaline earth cations with the one exception being Mg^{2+} (Ferris and Ertem, 1992). The catalytic activity of montmorillonite is retained when ammonium ion is the exchangeable cation (Ferris and Ertem, 1992).

Oligomers as long as 40–50 monomer units (Ferris *et al.*, 1996; Ferris, 2002; Huang and Ferris, 2003), and an excess of homochiral products from D, L-mixtures are formed by montmorillonite catalysis (Joshi *et al.*, 2000). The oligomers formed by the reaction of a mixture of ImpA with ImpC exhibit sequence selectivity (Ertem and Ferris, 2000; Miyakawa and Ferris, 2003).

The scope of the montmorillonite catalyzed formation of RNA oligomers was reported previously (Ferris and Ertem, 1992). These studies have been extended in the present research where a variety of reaction conditions were examined to determine the limits under which oligomers form. The effect of ionic strength, temperature, and pH were investigated. Catalysis by other minerals and meteorites was also investigated as well as nucleotides with activating groups other than imidazole or adenine derivatives.

2. Experimental

2.1. MATERIALS

Adenosine, AMP, 2′,3′-cyclic AMP, 3′,5′-cyclic AMP, ADP, ATP, NH_2pA, $A^{5'}$ppA, $A^{2'}$pA, $A^{3'}$pA and buffers were obtained from Sigma. Alkaline phosphatase and phosphodiesterase I were obtained from Worthington Biochemical and ribonuclease T_2 was from Sigma. Montmorillonite [Volclay SPV-200, (Al, $Fe_{1.67}$, $Mg_{0.33}$)$Si_4O_{10}(OH_2)Na^+Ca^{2+}{}_{0.33}$] was a gift from the American Colloid Company. Murchison meteorite, which is a CM2 type carbonaceous chondrite, was a gift from Dr. S. Pizzarello in Arizona State University. The Murchison meteorite collected in Australia in 1969 is known to contain many types of bioorganic compounds (Cronin and Chang, 1993; Gilmour, 2003). Yamato-791717 and Yamato-86751 were obtained from the Antarctic Meteorite Research Center, National Institute of Polar Research in Japan. These Antarctic meteorites are CO3 and CV3 type carbonaceous chondrites, respectively (Kaiden *et al.*, 1997; Murakami and Ikeda 1994). Olivine ([Mg,Fe_2]SiO_4), galena (PbS), calcite ($CaCO_3$), magnetite (Fe_3O_4), rhodochrosite ($MnCO_3$), sphalerite (ZnS), magnesite ($MgCO_3$), siderite ($FeCO_3$), hematite (Fe_2O_3), brucite ($Mg(OH)_2$, chalcosite (Cu_2S), talc ($Mg_3[Si_4O_{10}][OH]_2$) and dolomite ($CaMg(CO_3)_2$), were obtained from Dr. M. J. Gaffey's collection and pyrrhotite (Fe_7S_8) and pyrite (FeS_2) were purchased from Alfa Aesar.

2.2. GENERAL PROCEDURES

An aqueous solution containing 0.015 M ImpA, salts and 0.1 M MOBS or HEPES buffer was kept in the presence of Na^+-montmorillonite clays, minerals or meteorites for several days at room temperature. ImpA was prepared following the literature procedure (Joyce *et al.*, 1984). The purity of ImpA was checked by anion-exchange HPLC prior to starting reactions to make sure it was higher than 95% and the $A^{5'}$ ppA content was lower than 1%. pA and $A^{5'}$ ppA may form during storage of ImpA. Since $A^{5'}$ ppA is a good initiator of oligomer formation (Ferris, and Ertem 1993), care was taken to be sure it was not present as an impurity in the activated monomers. The Na^+-montmorillonite was prepared from Volclay by titration method (Banin *et al.*, 1985). MOBS was favored as a buffer (Figure 2) because it

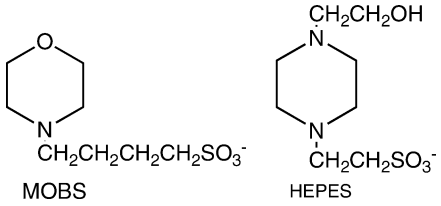

Figure 2. Structures MOBS and HEPES.

does not have a hydroxyl group that can react with ImpA. At the end of the reaction time, the mixture was centrifuged and the supernatants were withdrawn. The clay minerals and meteorites were extracted twice with a mixture of 0.1 M NaCl and 30% acetonitrile (Miyakawa and Ferris, 2003), 0.1 M, pH 9 pyrophosphate (Ferris 2002), or ammonium acetate to elute the oligomers from the montmorillonite. Ammonium acetate is less efficient than the other eluants in removing the cyclic dimers and long oligomers of AMP from montmorillonite. The supernatant and the extracts were combined and incubated at pH 4 and 37 °C for 4 h to hydrolyze the unreacted imidazole activating groups. Oligomers were analyzed by anion-exchange HPLC (Biosphere GMB 1000Q column, Melcor Tech. Inc.) at 260 nm with a 2 mM Tris buffer (pH 8) (Buffer A) and 2 mM Tris (pH 8) and 400 mM $NaClO_4$ (Buffer B) as eluants, and by reversed-phase HPLC (Alltima C18 column, Alltech) at 260 nm with a pH 2.7, 0.2% trifluoroacetic acid (Buffer A) and a pH 2.7, 0.2% trifluoroacetic acid and 30% acetonitrile (Buffer B) as eluants (Kanavarioti, 1997). Oligomers were identified by enzymatic hydrolysis with alkaline phosphatase and phosphodiesterase I or ribonuclease T_2 and by coelution on the HPLC with authentic samples. The hydrolysis details are described in Miyakawa and Ferris (2003). The yields of oligomers were calculated from the uncorrected peak areas of HPLC chromatograms following the procedure of Ferris and Ertem (1993). The length of oligomers was obtained by counting the number of peaks on the anion-exchange HPLC chromatograms (Ferris and Ertem, 1993).

2.3. Temperature variations

The pH 8 solutions containing 0.015 M ImpA, 0.2 M NaCl, 0.075 M $MgCl_2$, and 0.1 M HEPES were added to the montmorillonite clays and were kept at 50 °C for 1 day, at 37 °C for 2 days, at room temperature for 3 days, at 4 °C for 1 week. The oligomers formed were extracted with 0.1 M ammonium acetate and analyzed by HPLC using a Biosphere anion exchange column.

2.4. pH profile

The following buffers containing 0.35 M NaCl were used: hydrochloric acid for pH 0–1, sodium fomate for pH 2–5, MES for pH 6, MOBS for pH 7–9, CHES for pH 10, and sodium hydroxide for pH 13–14. The pH values were measured with a pH meter both before and after the reactions to make sure it did not drift. The buffers, with pHs that were lower than 2 and higher than 12, were carefully prepared and were not measured with the pH meter. ImpA was added to the buffers to give a final concentration of 0.015 M. These solutions were then added to the montmorillonites.

Since reaction products may hydrolyze if the incubation time is too long, different incubation times were used at different pHs. The incubation times were 1 day for pH 0–5, 7 days for pH 6–8 and 13–14, and 14 days for pH 9–10. The formation

of $A^{5'}$ ppA was monitored at a variety of times at pH 2.8 and 4.5. The yields were maximum and nearly constant between 10 and 70 hours. The formation of $pA^{3'}pA$ was monitored at a variety of times at pH 6 and 8. The yields were maximum and nearly constant between 1 and 7 days. The yields of $pA^{3'}pA$ and $pA^{2'}pA$ at pH 10 were not much different from 14 days to 50 days. Unreacted ImpA (26%)was detected after 50 days at pH 10.

2.5. Meteorites and minerals

Meteorites and minerals were powders that were washed with distilled water before use. In different runs, meteorites were washed with ethanol, benzene or hexane, but no difference in the reaction products was observed. In reactions, a pH 8 solution containing 0.015 M activated AMP, 0.2 M NaCl, 0.075 M $MgCl_2$, and 0.1 M HEPES was kept in the presence of meteorites or minerals for 1 week. ImpA, ATP, ADP and cyclic AMP were used as the activated AMP. The products were extracted with 0.1 M ammonium acetate or a mixture solution of 0.1 M NaCl and 30% acetonitrile, and analyzed by HPLC.

2.6. ATP hydrolysis by meteorites

Reactions containing 1 mM ATP and 10 mM MOBS were adjusted to pH 7 and were incubated at 37 °C in the presence of meteorites. Small amount of samples were withdrawn at various time intervals and the amounts of ATP left were determined by anion-exchange HPLC chromatography. The decomposition rate of ATP followed pseudo first-order kinetics. The pseudo first-order rate constants and the half-lives were calculated from the slopes of graphs of Log ($[ATP]_{initial}/[ATP]$) as a function of time.

2.7. Mineral catalysis of the reaction of ATP, ADP, 3′, 5′-cyclic AMP and 2′,3′-cyclic AMP

The mineral catalysis of RNA oligomer formation was investigated using ATP, ADP, 3′,5′-cyclic AMP and 2′,3′-cyclic AMP as starting materials. These reactions were performed under a variety of conditions: (1) at pH 8.0 using HEPES buffer and pH 5.8 using MES buffer (2) using the above buffers in the presence of 0.075 M $MgCl_2$ and 0.2 M NaCl. The reactions were performed in a 1 mL solution in the presence of 50 mg of powdered mineral at 21 °C and 37 °C for 7 days. The reaction mixtures were centrifuged and the supernatant was collected. The mineral was extracted with pH 9, 0.1 M $Na_4P_2O_7$ to recover oligomers bound to the mineral and the combined extracts were analyzed by anion-exchange HPLC. Some of the reaction products were characterized further by hydrolysis using alkaline phosphatase followed by HPLC analysis of the hydrolysis products on an Alltima reverse phase column.

Electrospray mass spectroscopy was used to confirm the formation of AMP and ATP from ADP. The product mixture obtained when talc was the mineral tested as a catalyst, was separated on an ion-exchange HPLC column using a 0–45% gradient of 2 mM ammonium acetate, pH 8 and a 2 M ammonium acetate, pH 8 in 0–40 min at 1 mL/min with an Agilent 1100 series LC/MSD-SL ion trap mass spectrometer: m/e 346 ($M-1$), and 348.1 ($m+1$) (calculated for $C_{10}H_{14}N_5O_7P$, 347.063), AMP; 426.0 ($m-1$), 428.0 ($M+1$) (calculated for $C_{10}H_{15}N_5O_{10}P_2$, 427.029) ADP; 505.9 ($m+1$) and 507.9 ($m-1$) (calculated for $C_{10}H_{16}N_5O_{13}P_3$, 506.996) ATP.

Experiments were performed in which no mineral was added and the reaction was performed with ADP and varying amounts of 0.2M NaCl and 0.075 M $MgCl_2$ with 0.1M HEPES. The reactions were carried out at 25 °C for 30, 38 and 63 days at pH 8. Ion exchange HPLC indicated the presence AMP, ADP and ATP by retention time and coinjection with authentic samples. The products formed from the reaction of 0.015 M ADP, 0.1 M HEPES, 0.2 M NaCl and 0.075 M $MgCl_2$ were separated by HPLC as described above and analyzed by electrospray mass spectrometry; m/e 345.9 ($M-1$) and 348 ($M+1$) (calculated 347.063) AMP; 425.9 ($M-1$) and 428 ($M+1$) (calculated 427.029) ADP; 505.9 ($M-1$) and 507.9 ($M+1$) (calculated 506.996) ATP.

3. Results and Discussion

3.1. Effect of added salts

3.1.1. *NaCl*

In the past the standard reaction mixture for oligomer formation was 0.2 NaCl, 0.075 M $MgCl_2$. and 0.1 M buffer. Our previous results indicted that Mg^{2+} was essential for reaction since the oligomers did not grow as long in its absence (Kawamura and Ferris, 1994). However, investigation of ImpA oligomerization in the presence of varying concentrations of NaCl revealed that oligomer formation did occur in the presence of high concentrations of NaCl (Table I). A 1 M concentration of NaCl resulted in the formation of oligomers as long as 10 mers, an amount comparable to that observed when our standard buffer electrolyte (0.2 M NaCl and 0.075 M $MgCl_2$) and 0.1 M HEPES was used. A 0.1 M NaCl solution gave 6 mers. These studies establish that ionic strength and not coordination by Mg^{2+} is the more important element in the reaction pathway.

3.1.2. *Buffer Structure and Concentration*

In previous studies PIPES and HEPES (Good and Izawa, 1972) were used as buffers. HEPES contains a hydroxyl groups that may form phosphoester bonds by reaction with the activated monomers (Kanavarioti, 1997). 3-(*N*-Morpholino)butanesulfonic acid (MOBS) (Thiel *et al.*, 1998) was evaluated as an alternative buffer since it does not contain a hydroxyl group (Huang and Ferris, 2003). The variation of oligomer chain length with the concentration of

TABLE I

Effects of cations and anions on the montmorillonite-catalyzed synthesis of RNA oligomers

Solutions	pH	Ionic Strength	Yields of RNA oligomers (%)[a] 1	2	3	4	5	6	7	8	9	10 (mers)
1M NaCl	7.9	1.1	9.3	57	14	11	3.4	1.8	1.2	0.53	0.29	0.22
0.35M NaCl	7.9	0.42	11	66	13	7.4	1.6	0.61	0.39			
0.1M NaCl	7.9	0.17	15	66	12	5.0	0.84	0.41				
0.01M NaCl	7.9	0.077	24	64	8.3	2.2	0.25					
0.075M $MgCl_2$ + 0.2M NaCl	7.9	0.49	11 ± 0	50 ± 0	14 ± 0	10 ± 1	5.2 ± 0.1	3.2 ± 0.4	2.0 ± 0	1.0 ± 0	0.61 ± 0.08	
0.075M $CaCl_2$ + 0.2M NaCl	8.0	0.50	28 ± 2	45 ± 1	14 ± 1	6.9 ± 0.2	2.7 ± 0.1	1.5 ± 0.1	0.43 ± 0.07	0.16 ± 0.06		
0.35M KCl	7.9	0.42	30	46	16	5.7	1.6	0.49	0.26			
0.001M NH_4Cl + 0.075M $MgCl_2$ + 0.2M NaCl	8.0	0.50	13	49	14	9.9	5.0	3.2	1.9	1.3	0.71	
0.01M NH_4Cl + 0.075M $MgCl_2$ + 0.2M NaCl	8.0	0.51	14	48	14	10	4.8	3.0	1.9 1.0 0.71			
0.35M NH_4Cl[b]	7.8	0.41	59	31	6.2	2.0	0.33					
0.35M HCOONa	7.8	0.41	16 ± 1	60 ± 0	16 ± 1	6.6 ± 0.2	1.6 ± 0.1	0.36 ± 0.1				
0.35M CH_3COONa	7.9	0.42	20	62	12	5.1	0.58					
0.35M $NaNO_3$	7.9	0.42	12	68	12	6.1	1.2	0.30				
0.001M $NaHCO_3$ + 0.075M $MgCl_2$ + 0.2M NaCl	8.0	0.50	14	49	14	9.5	5.1	2.8	2.0 0.98			
0.01M $NaHCO_3$ + 0.075M $MgCl_2$ + 0.2M NaCl	8.0	0.51	21	47	14	8.1	3.8	1.9	1.0			

(*Continued on next page*)

TABLE I
(*Continued*)

Solutions	pH	Ionic Strength	Yields of RNA oligomers (%)[a] 1	2	3	4	5	6	7	8	9	10 (mers)
0.35M $NaHCO_3$[c]	8.1	0.43	100									
0.175M Na_2SO_4	8.0	0.60	14	67	12	5.5	0.85					
0.001M Na_2HPO_4 + 0.075M $MgCl_2$ + 0.2M NaCl	8.0	0.50	49	36	9.4	3.1	1.7	0.72				
0.01M Na_2HPO_4 + 0.075M $MgCl_2$ + 0.2M NaCl	8.0	0.53	85	14								
0.175M Na_2HPO_4[d]	8.0	0.60	100									

Reaction: 0.015 M ImpA + Salts + 0.1 M MOBS + Volclay, 3 days.
[a]Yields were obtained from the peak area of HPLC chromatograms.
[b]About 20% of NH_2pA was detectable both in the presence and absence of montmorillonite.
[c]More than 60% of ImpA was detectable.
[d]About 20% of ADP and 20% of ImpA were detectable.

TABLE II
Effects of MOBS on the clay-catalyzed synthesis of RNA

MOBS (mol/L)	pH[a]	Ionic Strength	Yields of RNA oligomers (%)[b]			
			1	2[c]	3	4 (mers)
0	7.4	0	53 ± 1	46 ± 1	1.2 ± 0.1	0.14 ± 0.03
0.001	7.4	0.00038	53 ± 4	45 ± 4	1.3 ± 0.1	0.14 ± 0.03
0.01	7.7	0.0056	47	50	1.9	0.39
0.1	7.9	0.067	29	62	7.3	1.6

Reaction: 0.015M ImpA + MOBS + Volclay, 3 days.
[a]The value of pH is an average number measured before and after the reaction. The pH drifted in the reactions with low concentrations of MOBS.
[b]Yields were obtained from the peak areas of HPLC chromatograms.
[c]The most dominant dimer is cyclic $A^{3'}pA^{3'}p$.

MOBS had chain lengths comparable to those expected for a variation with ionic strength (Table II). The chain lengths were comparable to those observed using 0.1 M PIPES or HEPES in the presence of 0.075 M $MgCl_2$ and 0.2 M NaCl indicating that there is little reaction between the activated monomers and the buffers that contain hydroxyl groups.

3.1.3. *Some Soluble Salts That May Have Been Present on the Primitive Earth*
The presence of other soluble compounds on the primitive Earth that could have inhibited the oligomerization reactions by binding competitively to the montmorillonite (Wang and Ferris, 2001) or by reacting with the activated monomers was investigated. The effect of a series of simple inorganic compounds on the oligomerization reaction was investigated (NaCl, $MgCl_2$, $CaCl_2$, KCl, NH_4Cl, HCOONa, CH_3COONa, $NaNO_3$, $NaHCO_3$, Na_2SO_4, Na_2HPO_4) (Table I). Substitution of 0.075 M $CaCl_2$ for $MgCl_2$ in reaction solutions containing 0.2 M NaCl gave slightly lower yields of 4–8 mers than did $MgCl_2$ even though the ionic strengths of these solutions were the same (0.5).

The chain length of oligomers resulting from reactant solutions with ionic strengths of 0.4 of NaCl, KCl, HCOONa, and $NaNO_3$ were essentially the same (Table I). Some inhibition was observed with NH_4Cl and CH_3COONa. Total inhibition was observed with $NaHCO_3$ and Na_2HPO_4. The partial inhibition by NH_4Cl was due to the conversion of ImpA to a 20% yield of NH_2pA by displacement of the imidazole group with NH_3. This displacement occurred in the presence and absence of montmorillonite suggesting that the reaction took place in the aqueous phase and not on the clay surface. It has been observed that NH_2pA does not react on montmorillonite to form oligomers (Prabahar *et al*., 1994; Miyakawa and Ferris, 2003). The inhibitory effect of $NaHCO_3$ is apparently due to the binding of the HCO_3^- to montmorillonite as suggested by the presence of more than 60% of the starting ImpA remaining at the end of the reaction time. The inhibitory effect

of NaH_2PO_4 was due in part to the reaction of ImpA with $HPO_4^{2-}$to give a 20% yield of ADP. The recovery of 20% ImpA at the end of the reaction time suggests that Na_2HPO_4 may have also inactivated the clay catalyst.

The effect of salt concentration on the inhibitory effect was explored further using inhibitor concentrations varying from 0.001 to 0.35 M (Table I). The ionic strength was adjusted with $MgCl_2$ and NaCl. No inhibition was observed using 0.001 and 0.01 but was observed using 0.35 M NH_4Cl. $NaHCO_3$ concentrations of 0.001 and 0.01 M resulted in the detection of 8 mer and 7 mers respectively but total inhibition was observed with a 0.35 M solution. Use of 0.001 and 0.01 M Na_2HPO_4 resulted in the formation of 6 and 2 mers respectively and no oligomers were formed with a 0.175 M solution.

3.1.4. *NH_4^+, HCO_3^- and HPO_4^{2-} on the Primitive Earth*

It is postulated that the highest concentration of NH_4^+ in the modern oceans is less than 0.01 M (Bada and Miller, 1968). Ammonia could have been formed by the hydrolysis of HCN and other nitrogen-containing organic compounds. It would have been destroyed by solar UV radiation at wavelengths less than 200 nm (Ferris and Nicodem, 1972) so that it is unlikely that there was sufficient NH_4^+ in the primitive oceans to inhibit the reactions of activated RNA monomers.

There are large differences in the proposed concentrations of HCO_3^- in the primitive oceans because there are large differences in the amounts of CO_2 proposed to be in the atmosphere. It has been suggested that if the atmospheric pressure of CO_2 was 0.3 atm. the concentration of HCO_3^- was 0.07 M (Grotzinger and Kasting 1993). Others have proposed a CO_2 partial pressure of $\leq$10 bars (Walker, 1985). Since there are little data to constrain the partial pressure of CO_2 in the atmosphere of the primitive Earth, it is not possible to determine if enough HCO_3^- was present to inhibit the montmorillonite-catalyzed formation of RNA oligomers.

Soluble phosphate may have had a central role in the prebiotic formation of genetic material but at the same time it could have inhibited RNA oligomers formation. While the concentration of phosphate in the ocean today is 10^{-6} M because of its biological conversion to the mineral apatite, this would not have occurred on the primitive ocean since apatite only forms at a pH $\geq$ 8.5 in the absence of life. The formation of the minerals whilockite and magnesium phosphate occurs in the pH range 7–9 and brushite at pH 6–7 (Gedulin and Arrhenius, 1994). These minerals are 100 times more soluble than apatite and that would have led to a phosphate concentration of 10^{-4} M, a concentration too low to inhibit the montmorillonite-catalyzed formation of RNA oligomers.

3.2. Temperature effects

Reactions run at 4 and 25 °C yielded 8 mers while 7 and 6 mers were formed at temperatures of 37 and 50 °C, respectively. The cause of the decrease in chain length

TABLE III

Effects of temperatures on the clay-catalyzed synthesis of RNA

Temp (°C)	Yields of RNA oligomers (%)[a]							
	1	2	3	4	5	6	7	8 (mers)
4	19 ± 3.0	47 ± 2.0	11 ± 4.0	6.0 ± 0.70	2.4 ± 0.70	1.9 ± 1 .0	1.0 ± 0.51	0.48 ± 0.33
25	26 ± 4.0	45 ± 10	9.0 ± 0.40	5.7 ± 1.1	2.2 ± 0.30	0.85 ± 0.55	0.31 ± 0.31	0.15 ± 0.15
37	28 ± 4.0	43 ± 3.0	9.0 ± 1.6	4.3 ± 1.5	2.9 ± 0.5	1.6 ± 0.50	1.0 ± 0.50	
50	55 ± 10	25 ± 5.0	4.9 ± 2.9	1.3 ± 0.90	0.78 ± 0.81	0.18 ± 0.18		

Reaction: 0.015M ImpA + 0.2M NaCl + 0.075M $MgCl_2$ + 0.1M HEPES + Volclay.
[a]Yields were determined from the peak areas of the HPLC chromatograms.

may be due to the increased rate of hydrolysis of ImpA at higher temperatures (Table III). The yield of 5′-AMP increases at higher temperatures, a finding consistent with the decrease in the chain lengths of the oligomers.

3.3. Effect of pH

The effect of changes in pH on oligomer formation was investigated (Figure 3). Dimeric products containing phosphodiester bonds were formed at pH 5–10 with the optimal yield at pH 7–8. The longest oligomers were observed in this pH range (Figure 4). The formation of $A^{5'}$ ppA occurred in the pH 1-6 range with the optimal yield at pH 2–3. The reactions were monitored at different times to determine if

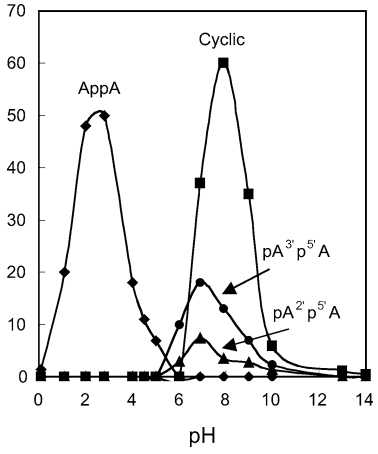

Figure 3. pH profile of the dimer yields. Reaction of 0.015M ImpA in 0.35 M NaCl, 0.1 M buffer, and Na^{+}-montmorillonite. The yields were determined from the uncorrected peak areas of the reverse phase HPLC chromatograms.

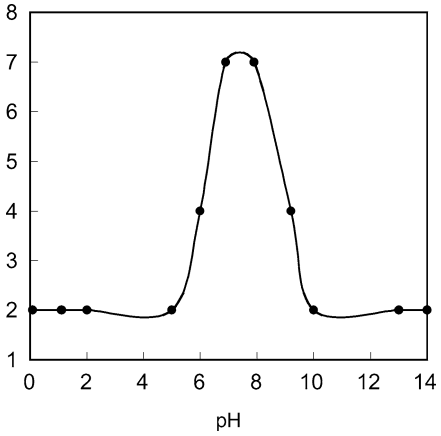

Figure 4. Variation in the length of the longest oligomers formed with pH. See Figure 3 caption for reaction conditions. The lengths of the oligomers were determined from the anion-exchange HPLC chromatograms.

hydrolysis resulted in a decrease in the products. The yields of products containing phosphodiester bonds were constant over a 7-day reaction time at pH 7–8.

It was expected that RNA oligomers might have formed more efficiently under acidic conditions, because the protonated ImpA would have bound directly to negative sites of interlayers of the clay (Dawson *et al*., 1969; Banin *et al*., 1985; Ertem and Ferris, 1998). Instead large amounts of $A^{5'}$ppA formed around pH 2. This is probably because protonated imidazole groups are displaced by the negatively charged phosphate groups formed by the hydrolysis of the activated monomers (Kanavarioti *et al*., 1989; Ruzicka and Frey, 1993).

It was also expected that RNA oligomers would form more efficiently around pH 10, because the hydrolytic rate of ImpG and ImpU are lowest at that pH (Kanavarioti *et al*., 1989; Ruzicka and Frey, 1993). However, most of ImpA did not react at pH 10.

3.4. Catalysis of oligomer formation

3.4.1. *Reaction of ImpA*

Three meteorites and twelve minerals were investigated as potential catalysts for the formation of RNA oligomers from ImpA. Meteorites were investigated because they contain mineral assemblages with associated organic compounds (Cronin and Chang, 1993). The meteorites were powdered samples of the Murchison meteorite and two Antarctic meteorites. Minerals were selected on the basis of their potential for binding to the phosphate group of the activated mononucleotides.

None of the meteorites and only one of the minerals catalyzed the formation of RNA oligomers from ImpA. Galena (PbS) catalyzed the formation of $pA^{2'}pA$ and $pA^{3'}pA$, a finding that was reported previously (Sleeper and Orgel, 1979). The

TABLE IV
Reaction of ADP in the presence and absence of minerals for 4 days

		Yield of reaction products (%)[a]			
Reaction mixture	Temperature	AMP	ADP	ATP	Unknown
ADP + Brucite	37 °C	15.4	79.4	3.6	1.0
ADP + Talc	37 °C	14.5	80.0	3.8	1.0
ADP + Siderite	37 °C	14.8	80.1	3.8	0.9
ADP + Magnetite	37 °C	15.5	79.6	3.7	0.7
ADP + Galena	37 °C	14.9	79.0	4.0	1.2
ADP	37 °C	15.9	78.8	3.9	0.8
ADP + Olivine	37 °C	15.1	79.6	3.9	0.9
ADP + Olivine	21 °C	14.9	79.3	4.2	1.2
ADP	21 °C	14.9	79.9	4.2	0.9

Reaction: 0.015M ADP + 0.2M NaCl + 0.075M $MgCl_2$ + 0.1 M HEPES, pH 8 + mineral (50 mg/mL)
[a]Yields were determined from the peak areas of the HPLC chromatograms.

insoluble mineral and not the soluble Pb^{2+} was shown to be the catalyst because a solution in contact with the powdered mineral, when filtered to remove mineral particles, did not catalyze oligomer formation. Galena was less effective than montmorillonite as a catalyst as shown by the observation of only dimers as products from the reaction of ImpA.

3.4.2. *Mineral Catalysis of the Reactions of ATP, ADP, 3′,5′-Cyclic AMP and 2′,3′-Cyclic AMP*

The minerals magnesite, brucite, talc, olivine, dolomite, calcite, hematite, goethite, siderite, magnetite, galena, sphalerite and chalcosite were investigated as possible catalysts for the oligomerization of the adenine nucleotides at pH 8.0 and 5.8 in the presence of buffers alone and in the presence of buffers together with 0.075 M $MgCl_2$ and 0.2 M NaCl. The reactions were carried out for 4 days at 37 °C and the products were analyzed by anion exchange HPLC (Table IV).

Reactions performed only in the presence of buffer generally resulted in the hydrolysis of ATP and ADP to AMP and adenosine (Figure 5a). While those where the additional salts were added underwent little or no reaction. In the cases where the HPLC retention times of some of the products were comparable to those expected for $pA^{3'}pA$ or $pA^{2'}pA$ that have comparable retentions to ADP on a Biosphere anion exchange column, the reaction mixtures were hydrolyzed with alkaline phosphatase and analyzed for the presence of $A^{3'}pA$ or $A^{2'}pA$ by HPLC on a reverse phase column. In every instance adenosine was the major product confirming that these reaction products were ADP and not the isomers of pApA.

2′,3′-Cyclic AMP (Figure 5b) underwent hydrolysis to AMP in the presence and absence of added minerals. 3′,5′-Cyclic AMP (Figure 5b) was more resistant to

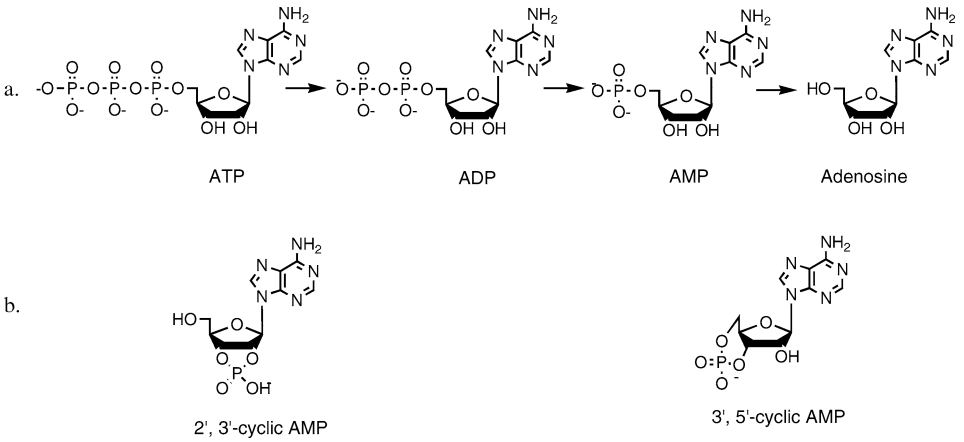

Figure 5. (a) Steps in the hydrolysis of ATP to adenosine. (b) Structures of cyclic phosphates.

hydrolysis and in most reactions yielded only about 5% AMP as a reaction product. ATP did not oligomerize in the presence of meteorites Yamato-791717 (CO3) and Yamato-86751 (CV3).

The reaction of ADP in the 0.1 M HEPES, 0.2 M NaCl and 0.075 M $MgCl_2$ in the presence and absence of minerals yielded AMP and ATP (Table IV). The products were identified by coinjection with authentic samples using ion exchange and reverse phase HPLC. The structural assignments were confirmed by electrospray mass spectrometry using an ion exchange column.

It was established that minerals did not have a role in the formation of ATP since it was formed in their absence. It was established that the formation of ATP was due to the presence of 0.1M HEPES in the reaction mixture. It does not appear to be an ionic strength effect since ATP was not formed when MOBS replaced HEPES (Figure 2) in the reaction (Table V). The functional groups of HEPES and MOBS differ by the presence of a hydroxyl group in HEPES (Figure 2). This suggests that the hydroxyl group in HEPES has a role in the reaction.

There are reports of the conversion of ADP to ATP in the presence of cyclodextrin, creatine and oxygen (Hattori *et al.* 1984) and with NADH and riboflavin (Lozinova and Arutyunyan, 1990) but there are no reports of ATP formation in the presence of a buffer-salt mixture. The yield of ATP decreased in the absence of oxygen (Hattori *et al.* 1984). The formation of ATP from ADP by Lozinova and Arutyunyan (Lozinova and Arutyunyan 1990) still occurred in the absence of oxygen. Both reports propose that ATP formation proceeds via a free radical pathway.

3.4.3. *Hydrolysis of ATP by Meteorites*

The meteorites did not initiate the condensation of ATP to oligomers but rather catalyzed the hydrolysis of ATP to ADP and AMP. The pseudo first order rate

TABLE V
Formation of ATP from ADP

Reaction Mixtures[a]	Time (days)	Yields of Products[b] AMP	ADP	ATP	Unknown
ADP,NaCl,$MgCl_2$,HEPES	30	21.8	48.3	29.2	0.7
ADP, NaCl, $MgCl_2$	30	12.8	86.0	0.7	0.3
ADP, HEPES	30	30.7	65.3	3.3	0.3
ADP, $MgCl_2$	30	13.5	85.7	0.4	0.2
ADP, NaCl	30	3.9	94.5	1.1	0.3
ADP, H_2O	30	3.9	94.4	1.1	0.3
ADP, NaCl $MgCl_2$, MOBS	38	6.4	90.6	1.7	0.8
ADP, NaCl $MgCl_2$, MOBS	63	10.9	86.1	1.6	0.5

[a]0.015 M ADP, 0.2 M NaCl, 0.075 M $MgCl_2$, 0.1 M HEPES or 0.1 M MOBS, pH 8, 25 °C. Where no buffer was used the pH was adjusted daily using 0.1 M NaOH.
[b]Yields were determined from the peak areas of the HPLC traces.

TABLE VI
Hydrolysis rates and half-life of ATP in the presence of meteorites

Meteorite	K_1/day	$T_{1/2}$
Murchison	3.2	5 h
Y7917127 (CO3)	0.17	4 day
Y86751 (CV3)	0.11	6 day
Blank	0.01	2 month

Hydrolytic conditions: 1 mM ATP, 10 mM MOBS, pH 7, 37 °C.

constants and half-lives were calculated from the slopes of the plots of the log of the change in concentration of ATP with time (Table V). The meteorites were effective catalysts as shown by 320-fold increase in the rate constant for the hydrolysis of ATP by the Murchison meteorite over that observed in its absence.

4. Conclusions

Presence of salts in the reaction medium enhances the formation of RNA oligomers in the montmorillonite-catalyzed reaction of nucleoside phosphorimidazolides. Previously it was believed that the reaction required the presence of Mg^{2+} to coordinate with the activated phosphate group but the present studies indicate that the length of the oligomers formed is mainly a function of the ionic strength of the reaction solution. For example, it was demonstrated that the same length oligomers were obtained using 1 M NaCl as was observed with a mixture of 0.075 $MgCl_2$

and 0.2 M NaCl. The chain lengths decreased as the concentration of NaCl was decreased (Table I).

A variety of salts that may have been present in an evaporating body of water, similar to the salt deposits discovered by the Opportunity Rover on Mars, were investigated. Sodium sulfate (ionic strength 0.60) does not enhance the formation of longer oligomers as much as does NaCl (ionic strength 0.42). Salts that inhibit the reaction are those that displace the imidazole group from the activated nucleotide or block binding of activated nucleotides to clays. These include NH_4^+ (probably as NH_3), HCO_3^- and $H_2PO_4^-$. The concentrations of NH_4^+ and $H_2PO_4^-$ on the primitive Earth were probably lower than that was required for inhibition. The concentration of HCO_3^- would have been high enough to inhibit the reaction only if the early Earth had a CO_2 atmosphere of 10 bars or higher. Higher salt concentrations would have been present in evaporating bodies of water.

A selection of minerals and meteorites were investigated as possible catalysts for the formation of oligomers from ImpA. Only galena (PbS) catalyzed the formation of the dimers $pA^{2'}pA$ and $pA^{3'}pA$, a finding that was reported previously (Sleeper and Orgel 1979). Our studies established that the reaction was catalyzed by the mineral surface and not by soluble PbS. The dimer yields formed by PbS catalysis were much lower than those formed by montmorillonite catalysis.

The pH of the reaction solution has a marked effect on the products formed from the reaction of ImpA. Reactions carried out in the pH 6–10 range yielded oligomers as reaction products with the optimal pH 7–8. Reactions performed at pHs less than 6 yielded $A^{5'}$ ppA, with an optimal pH of 2–3. The requirement for a basic pH for oligomer formation differs from the highly acidic pH predicted for the evaporated body of water found by the Opportunity Rover on Mars. It is proposed that the Martian conditions were very acidic because crystals of the mineral Jarosite [$(KFe_3(SO_4)_2(OH)_6$] were detected (Madden, *et al*., 2004; Squires *et al*., 2004). It is possible that an environment similar to that observed by the Opportunity Rover was also present on the early Earth. This highly acidic environment would have generated an acidic montmorillonite that was gradually converted to a neutral catalytic montmorillonite as the acidity on the Earth decreased.

The lengths of the oligomers formed by the montmorillonite catalysis of the reaction of ImpA decrease as the temperature is raised to 50 °C. The yield of 5′-AMP formed by hydrolysis of ImpA increases at higher temperature. These observations suggest that the increase in rate of hydrolysis results in a decrease in the relative rate of oligomer formation and hence the lower yields of the longer oligomers.

The search for mineral or meteorite catalysis of the oligomerization of other activated phosphate derivatives was not successful. Where a reaction was observed the predominant product formed was due to the hydrolysis of the activating group on the monomers. These studies did lead to the discovery that ADP was converted to ATP and AMP at room temperature in the presence of HEPES buffer. It has been proposed that similar transformation proceed by free radical processes but it seems

unlikely that free radicals would be generated under the reaction conditions used in the present study.

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