

# OLIGOMERIZATION REACTIONS OF RIBONUCLEOTIDES: THE REACTION OF THE 5'-PHOSPHORIMIDAZOLIDE OF NUCLEOSIDES ON MONTMORILLONITE AND OTHER MINERALS

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(Received February 7, 1992)

**Abstract.** The reaction of ImpA in the presence of Na<sup>+</sup>-montmorillonite 22A or Na<sup>+</sup>-Volclay in aqueous, pH 8 solution gives a 50–60% yield of dimers and trimers (pA)<sub>2</sub> and (pA)<sub>3</sub>. The ratio of 3',5'-phosphodiester bond formation is twice as great as 2',5'-bond formation. The reaction requires the presence of Mg<sup>2+</sup> and is inhibited by 0.4 M imidazole. N-methylimidazole enhances the rate of the reaction but does not cause major changes in yield or product composition. Higher yields were obtained when Li<sup>+</sup>- or Ca<sup>2+</sup>-montmorillonites were used in place of Na<sup>+</sup>-montmorillonite. Little or no phosphodiester bond formation was observed with Mg<sup>2+</sup>- or Al<sup>3+</sup>-montmorillonite. Montmorillonites other than 22A and Volclay exhibited little or no catalysis. In addition, little or no catalysis was exhibited in ferruginous smectite, nontronite, allophane, imogolite or sepiolite. Oligomers were also formed by the reaction of ImpG, 2-methylImpG, ImpC and ImpU in the presence of Na<sup>+</sup>-montmorillonite. The pyrimidine nucleotides gave significantly lower yields of oligomers.

## 1. Introduction

The observation of the catalytic role of RNA in processing RNA transcripts (Been and Cech, 1988; Guerrier-Takoda *et al.*, 1983) suggests that RNA was the central biopolymer in the first life. This postulate has been challenged because of the difficulties encountered in discovering plausible prebiotic pathways to mononucleotides and oligonucleotides and the inhibition of template-directed syntheses of RNA oligomers by monomers of the opposite handedness (Joyce, 1989; Joyce *et al.*, 1984). We feel that one of the objections to the 'RNA first' hypothesis, the prebiotic formation of RNA oligomers, may be amenable to solution assuming the oligomerization reactions proceeded on mineral surfaces. The mineral would selectively adsorb the nucleotide monomers from aqueous solution on its surface and then catalyze their condensation to oligomers in the presence of a reagent which activates the phosphate groups.

Our research to date suggests that it may be possible to achieve a prebiotic synthesis of oligonucleotides on mineral surfaces in aqueous solution. We demonstrated that certain montmorillonite clays facilitate the formation of phosphodiester bonds between the monomers of ribonucleotides and deoxyribonucleotides when a water-soluble carbodiimide (EDAC)\* is used as the condensing agent. The 2',5'- and 3',5'-dimers of pA are formed from 5'-AMP with 3',5'-(pA)<sub>2</sub> constituting 37% of the dimeric mixture (Ferris *et al.*, 1989B). The dimer, trimer and tetramer were observed in the condensation reaction of 5'-dAMP (Ferris and Kamaluddin, 1989). The condensation reactions of deoxyribonucleotides were extended to 5'-dGMP, 5'-dCMP

and 5'-dTMP. The yield of dimeric products decreases in the order  $d(pA)_2 > d(pG)_2 \gg d(pC)_2, (pT)_2$  (Ferris *et al.*, 1990). Formation of the 3',5'-cyclic nucleotides and little oligomerization is observed if the 3'-phosphate derivatives of deoxynucleosides and the 5'-phosphates of cytidine and thymidine are the starting materials.

In a related study Sawai *et al.* (1989) observed that 1 mM  $UO_2^{2+}$  ion effected the conversion of 50 mM ImpA to a series of 2',5'-linked oligomers up to the hexadecamer of adenylic acid.

In the present study we report the results of an investigation of the condensation reaction of 5'-ImpA on minerals. 5'-Phosphorimidazolides may have formed on the primitive earth by the reaction of imidazole with the corresponding 3'-di- or tri-phosphate (Lohrmann, 1977). A brief description of the condensation reactions of the phosphorimidazolides of guanosine, cytidine and uridine is also given, as well as an investigation of the catalysis of ImpA condensation by  $UO_2^{2+}$ -montmorillonite. A preliminary report of some aspects of this research has been presented (Ferris *et al.*, 1989c).

## 2. Experimental

Many of the materials and methods used in this research were described previously (Ferris *et al.*, 1989b; Ferris and Kamaluddin, 1989; Ferris *et al.*, 1990). PIPES was obtained from Aldrich and alkaline phosphatase and ribonuclease T<sub>2</sub> were obtained from Sigma. Imidazole (Baker) was purified by recrystallization from benzene and 1-methylimidazole (Aldrich) was purified by vacuum distillation. HPLC analyses were performed on a Waters C18  $\mu$ -Bondapak reverse phase column (Ferris *et al.*, 1989b). The montmorillonite 22A used routinely in these studies was obtained from Wards Natural Science Establishment (American Petroleum Institute, 1951), Volclay SPV-200 was a gift from American Colloid Company, Arlington Heights, Illinois, Nontronite H-33a was provided by Prof D. Miller from the Rensselaer Geology Department (American Petroleum Institute, 1951), allophane (Ki-P) and imogolite (Ki-G) by Dr. Koji Wada of Kyushu University, Fukuoka, Japan and

\* Abbreviations: EDAC, 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide; 5'-AMP, 5'-adenosine monophosphate; 2',5'-(pA)<sub>2</sub>, adenylyl-2',5'-adenosine-5'-phosphate; 3',5'-(pA)<sub>2</sub>, adenylyl-3',5'-adenosine-5'-phosphate; AppA, P<sub>1</sub>,P<sub>2</sub>-diadenosine-5',5'-pyrophosphate; 2',5'-ApA, adenylyl-2',5'-adenosine; 3',5'-ApA, adenylyl-3',5'-adenosine; 5'-ImpA, adenosine 5'-phosphorimidazolide; ImpG, guanosine 5'-phosphorimidazolide; ImpC, cytidine 5'-phosphorimidazolide; ImpU, uridine 5'-phosphorimidazolide; 2-methylImpG, guanosine 5'-phosphor-2-methylimidazolide; GMP, 5'-guanosine monophosphate; 5'-d-AAMP, 2'-deoxy-5'-adenosine monophosphate; 3'-TMP, 3'-thymidine monophosphate; d(pA)<sub>2</sub>, deoxyadenyl-3',5'-deoxyadenosine-5'-phosphate; d(Ap)<sub>2</sub>, deoxyadenyl-3',5'-deoxyadenosine-3'-phosphate; (pT)<sub>2</sub>, thymidyl-3',5'-thymidine-5'-phosphate; dGMP, 2'-deoxy-5'-guanosine monophosphate; 5'-dCMP, 2'-deoxy-5'-cytidine monophosphate; d(pG)<sub>2</sub>, deoxyguanyl-3',5'-deoxyguanosine-5'-phosphate; d(pC)<sub>2</sub>, deoxycytidyl-3',5'-deoxycytidine-5'-phosphate; d(pN)<sub>2</sub>, deoxynucleoside-3',5'-deoxynucleoside-5'-phosphate; NppN, P<sub>1</sub>,P<sub>2</sub>-dinucleoside-5'-5'-pyrophosphate; HPLC, high performance liquid chromatography; PIPES, piperazine-N,N'-bis(2-ethanesulfonate); TRIS, Tris(hydroxymethyl)aminomethane; APH, alkaline phosphatase.

allophane by Dr. T. Itoh, Idemitsu Kasei Company, Ltd., Chiba, Japan. The other minerals used in this study were obtained from the Clay Minerals Society, Source Clays Repository, Department of Geology, University of Missouri, Columbia, Missouri 65211 (Van Olphen and Fripiat, 1979). The homoionic Volclays were prepared by the titration method (Banin *et al.*, 1985) while the homoionic smectites 22A, 28, H-33a, SWA-1 and Syn-1 were prepared by the saturation method (Brindley and Ertem, 1971).

ImpA was synthesized by using a modification of the procedure of Joyce *et al.* (1984). The literature procedure specifies the use of dry 5'-AMP as the starting material but does not describe how to remove this strongly bound water of solvation. Our studies (Kay, 1990) show that appreciable amounts of AppA and 5'-AMP are present in the ImpA preparation if this water is not removed. This was accomplished by dissolution of 5'-AMP (free acid) and imidazole in 20 mL of dimethylformamide and evaporation to near dryness on a rotary evaporator under vacuum at 40–45 °C. This was repeated two additional times. Using 5'-AMP dried in this way it was possible to prepare 5-ImpA which was greater than 90% pure by HPLC analysis. Comparable drying procedures were used by K. Kay in the synthesis of the phosphorimidazolides of guanosine, uridine, cytidine and the 2-methylimidazolide of guanosine, and his preparations were used in this study.

#### REACTION OF PHOSPHORIMIDAZOLIDES WITH MINERALS

A 14.5 mM solution of the 5'-phosphorimidazolide was prepared in a mixture of 0.2 M NaCl and 0.075 M MgCl<sub>2</sub> and the pH was adjusted to 8. To 1 mL of this solution was added 50 mg of montmorillonite 22A or another mineral and the pH was again adjusted to 8. The suspension was vortexed and allowed to stand at room temperature for 3–7 days. The pH at the end of the reaction is 8±0.2. Controls which did not contain mineral were run under the same conditions. The reactions containing mineral were centrifuged, the supernatant removed with a pipet and filtered through a 0.45 micrometer pore filter. To the mineral was added 1.0 mL of 0.1 M ammonium acetate, the mixture was vortexed and allowed to stand for 24 h at room temperature. The mixture was centrifuged, the supernatant removed, filtered and combined with the first supernatant for HPCL analysis on a Waters  $\mu$ Bondapak C-18 column. A precipitate formed in the 9 days reaction of UO<sub>2</sub><sup>2+</sup> with ImpA. This precipitate and the UO<sub>2</sub><sup>2+</sup>-montmorillonite 22A were treated with a mixture of 0.05 M ammonium acetate and 0.05 M ethylenediamine tetraacetate and these fractions were combined with the supernatants before HPLC analysis. Alkaline phosphatase hydrolysis was used to confirm the identity of the reaction products. The combined wash and supernatant was diluted 1:4 in 0.1 M TRIS (pH 8.2) and digested with 0.1 units of alkaline phosphatase for 12 h. The hydrolysate was analyzed by HPLC and the structures of the products with the terminal phosphates cleaved were determined by coinjection with standards of known structure. The yields were determined from the ratio of the peak area to that of the total area of the chromatogram. These yields were adjusted for the hyper-

chromicities of the dimeric and trimeric products of AMP and GMP (Ferris *et al.*, 1989b, 1990) and are given in mole percent.

Products whose structures could not be established by alkaline phosphatase hydrolysis were separated by preparative HPLC on the analytical column. The eluates were freeze-dried, dissolved in 10% methanol-water and separated from inorganic phosphate by isocratic elution from the HPLC column with 10% methanol. The purified substance was obtained after freeze drying.

Structure analysis of  $p^{5'}A^{3'}p^{5'}A^{2'}p^{5'}A$  – This substance was purified by HPLC, dissolved in 15 mM ammonium acetate (pH 4.5) and digested with 0.5 units of ribonuclease  $T_2$  at 37 °C for 24 h and analyzed by HPLC. Two peaks with retention times 4 and 26 min were observed. The peak at 26 min was established as  $A^2p^5A$  by coinjection with an authentic standard while the rapidly eluting peak is the more polar pAp. Digestion of  $p^{5'}A^{3'}p^{5'}A^{2'}p^{5'}A$  at pH 8.2 with 0.05 units of alkaline phosphatase gave a HPLC peak at 31 min which is between that of 2',5'-ApApA at 29 min and 3'5'-ApApA at 35 min. The retention time of 31 min is consistent with the presence of both 2',5'- and 3',5'-links in this compound as established by the ribonuclease  $T_2$  study.

### 3. Results and Discussion

The yields and structures of the products formed by the condensation of the 5'-phosphorimidazolide of adenosine (5'-ImpA) on  $Na^+$ -montmorillonite 22A differ from those observed in the reaction of EDAC with 5'-AMP (Ferris *et al.*, 1989b). Appreciable yields of trimeric products are obtained (Table I) where none were detected when EDAC was used. About 65% of the phosphodiester bonds formed are 3'5'-linked while only 35% 3'5'-linkage were observed using EDAC. Virtually none of the pyrophosphate derivative AppA was observed starting from ImpA while a 4.8% yield of AppA is observed using EDAC. AppA is the principal reaction product when 5'-ImpA reacts in aqueous solution at pH 8 in the absence of  $Na^+$ -montmorillonite 22A (Table I).

The yields of dimeric and trimeric products provide insight into the process by which oligomers are formed. A 2:1 ratio of 3'5'-(pA)<sub>2</sub>:2',5'-(pA)<sub>2</sub> is observed but a 2:1 ratio of  $p^{3'}A^{5'}pA^{2'}p^{5'}A$ :3'5'-(pA)<sub>3</sub> is formed. The absence of 2',5'-(pA)<sub>3</sub> and  $pA^{3'}p^{5'}A^{2'}p^{5'}A^{2'}p^{5'}A$  suggests that the elongation of 2',5'-linked oligomers is an inefficient process on montmorillonite. The elongation of 3'5'-(pA)<sub>2</sub> proceeds efficiently but with a 2:1 preference for the addition of the next nucleotide by a 2',5'-linkage over a 3'5'-linkage.

Phosphodiester product formation is observed in the reaction of 5'-ImpA with 10, 50 or 250 mg of montmorillonite mL<sup>-1</sup>. The yield of products containing the phosphodiester bond is less when 10 mg of  $Na^+$ -montmorillonite 22A is used. The product ratios are not appreciably changed by sonicating the reaction mixture to keep the clay suspended or by using a fraction of the clay that had a particle size less than 2 microns although somewhat higher yields were obtained using the

TABLE I  
Reaction of ImpA on Na<sup>+</sup>-Montmorillonite  
Yield (%)<sup>a</sup>

	pA	2',5'-(pA) <sub>2</sub>	AppA	3',5'-(pA) <sub>2</sub>	3',5'-(pA) <sub>3</sub>	pA3'p5'A2'p5'-A	Phospho- diester Product	3',5'-links
Na-22A-ImpA	82	5.2	0	8.3	1.5	3.0	18	64
Na-Yol-ImpA	62	8.0	0.3	15	5.2	9.5	38	67
Na-22A(10 mg)-ImpA	85	4.5	3.4	5.0	0.6	1.1	11	57
Na-22A(250 mg)-ImpA	88	3.2	0	6.6	0.4	1.4	12	66
Na-22A-ImpA <sup>b</sup>	72	6.4	0.6	8.7	1.5	2.2	19	66
Na-22A(<2 μ-ImpA	77	6.0	0	11	2.1	3.9	23	66
ImpA (no clay)	74	1.5	10	0.4	0	0	2.9	21

<sup>a</sup> 0 signifies less than 0.1% yield; initial pH=8.

<sup>b</sup> Reaction sonicated.

TABLE II  
Effect of Buffer and Salts on Yields  
Yield (%)

	pA	2',5'-(pA) <sub>2</sub>	AppA	3',5'-(pA) <sub>2</sub>	AppA	3',5'-(pA) <sub>2</sub>	3',5'-(pA) <sub>3</sub>	Ap(pA) <sub>2</sub>	Ap(pA) <sub>3</sub>	pA3'p5'A2'p5'-A	2',5'-(pA) <sub>3</sub>	Phospho- diester Product	3',5'-links
1. Na <sup>+</sup> -22A-ImpA in H <sub>2</sub> O	78	2.8	0.3	1.4	0	0	0	0	0	0	0	4.2	33
2. Na <sup>+</sup> -22A-ImpA in 0.2 M PIPES	76	1.8	0.4	4.3	0.4	0	0	0	0	1.0	0	7.0	74
3. Na <sup>+</sup> -Vol-ImpA in 0.075 M MgCl <sub>2</sub>	56	12	0.3	14	5.1	0	0	0	0	8.8	0	40	59
4. Na <sup>+</sup> -Vol-ImpA, 25 °C <sup>a</sup>	36	9.8	0	12	3.0	0	0	0	0	7.0	1.8	33	61
5. ImpA (no clay), 25 °C <sup>a</sup>	80	5.0	63	1.0	0	0	0	0	0	0.1	0.3	6.0	17
6. Na <sup>+</sup> -Vol-ImpA, 2 °C <sup>a</sup>	30	9.1	0	18	5.9	0	0	0	0	14	2.0	49	62
7. ImpA (no clay), 2 °C <sup>a</sup>	78	9.0	5.0	1.9	0.1	0	0	0	0	0.6	1.3	13	18
8. Na <sup>+</sup> -Vol-ImpA pH 6.5 <sup>b</sup>	67	5.9	4.3	4.3	0.7	1.0	0	0	0	2.4	0	14	52
9. ImpA (no clay) <sup>b</sup>	79	1.6	16	0.4	0	0	0	0	0	0	0	2.0	20
10. Na <sup>+</sup> -Vol-ImpA <sup>c</sup>	76	8.1	2.5	3.6	1.0	1.1	0.4	0	0	1.9	0	16	43
11. ImpA (no clay) <sup>c</sup>	87	0.7	7.0	0.3	0	0	0	0	0	0	0	1.0	30
12. Na <sup>+</sup> -Vol-ImpA <sup>d</sup>	71	5.3	0.8	12	3.4	0.8	0.7	0	0	3.6	0	26	73
13. ImpA (no clay) <sup>d</sup>	75	1.0	19	0.2	0	0	0	0	0	0	0	1.2	17

<sup>a</sup> Medium: 0.2 M NaCl, 0.075 M MgCl<sub>2</sub>, 0.1 M 1-methylimidazole pH 8.0.

<sup>b</sup> Medium: 0.1 M NaCl, 0.4 M MgCl<sub>2</sub>, 0.4 M Imidazole pH 6.5.

<sup>c</sup> Medium: 0.1 M NaCl, 0.075 M MgCl<sub>2</sub>, 0.4 M Imidazole pH 6.5.

<sup>d</sup> Medium: 0.1 M NaCl, 0.4 M MgCl<sub>2</sub>, pH 6.5.

smaller particle size montmorillonite (Table I). Oligomer formation proceeds somewhat more efficiently at 2 °C than at 25 °C (Table II).

A greater efficiency of phosphodiester bond formation was observed when Na<sup>+</sup>-Volclay was used instead of Na<sup>+</sup>-montmorillonite 22A (Table I). This was not expected since low yields of phosphodiester linked dimers were observed when 5'-dAMP is reacted with EDAC in the presence of Na<sup>+</sup>-Volclay (Ferris *et al.*, 1990). In subsequent studies Na<sup>+</sup>-Volclay and Na<sup>+</sup>-montmorillonite 22A were used interchangeably since comparable reaction products are obtained from each.

The yield of phosphodiester products is strongly dependent on the concentration of Mg<sup>2+</sup> in the reaction solution (Table II). Very low yields are obtained when no salts were used (Table I-1). A small improvement in yield was observed when the ionic strength was increased by use of 0.2 M PIPES (Table II-2). There is a dramatic increase in the yield when 0.075 M MgCl<sub>2</sub> is added to the reaction mixture with an overall 40% yield of products which contain the phosphodiester bonds (Table II-3). Addition of 0.4 M imidazole to these reaction solutions containing 0.075 or 0.4 M Mg<sup>2+</sup> depresses the total yield of phosphodiester products by almost one half (Table II-8, 10). A good yield of phosphodiester products is observed with 0.4 M MgCl<sub>2</sub> and 0.1 M NaCl (Table II-12). The addition of 0.1 M N-methylimidazole results in a 49% yield of products which contain the phosphodiester bond (Table II-4, 6) but the yield of phosphodiester products in the absence of clay is also enhanced (Table II-5, 7) so the net yield is comparable to that obtained with 0.075 M MgCl<sub>2</sub> alone (Table II-3). The extent of 3'5'-bond formation was in the 61–73% range for all the reactions in Table II except when no salts were added (1) and those media containing 0.4 M imidazole (8, 10); reactions which proceed in low yield.

It has been demonstrated that Mg<sup>2+</sup> enhances the rate of hydrolysis of ImpG by coordination of the oxygen of the phosphoroimidazolide grouping (Kanavarioti *et al.*, 1989). The central role of Mg<sup>2+</sup> in the oligomerization reaction probably reflects a comparable activation by coordination.

The exchangeable cation associated with montmorillonite 22A influences the extent of conversion of ImpA to products containing phosphodiester bonds (Table III). The Li<sup>+</sup>-derivative gives the best conversion (28%) to dimers and trimers. The NH<sub>4</sub><sup>+</sup>-, Na<sup>+</sup>- and H<sup>+</sup>-montmorillonites 22A give about the same yield (35 ± 5%) of oligomeric products. The H<sup>+</sup>-montmorillonite must contain exchangeable Na<sup>+</sup> because considerable NaOH had to be added to the reaction solution to adjust the pH to 8.

More pronounced differences in the extent of phosphodiester bond formation was observed with Mg<sup>2+</sup>-, Ca<sup>2+</sup>- and Al<sup>3+</sup>-montmorillonite 22A (Table III). A 29% yield of phosphodiester products was obtained using Ca<sup>2+</sup>-montmorillonite while only a 9% yield was obtained using Mg<sup>2+</sup>- as the exchangeable cation. The phosphodiester product yield obtained with Al<sup>3+</sup>-montmorillonite 22A was comparable to that of control reactions where no montmorillonite was used. There must be very little exchange of Mg<sup>2+</sup> for Na<sup>+</sup> in Na<sup>+</sup>-montmorillonite because the 0.075 M

TABLE III  
Effect of Interlayer Cation on ImpA Reaction  
Yield (%)<sup>a</sup>

	pA	2',5'-(pA) <sub>2</sub>	AppA	3',5'-(pA) <sub>2</sub>	3',5'-(pA) <sub>3</sub>	pA3'p5'A2'p5'-A
<i>Monovalent</i>						
Li-22A-ImpA	72	7.2	0	12	2.7	6.1
NH <sub>4</sub> -22A-ImpA	80	7.4	0	6.7	0.6	3.2
H-22A-ImpA	84	5.7	0.3	7.8	1.0	2.5
<i>Di- and Tri-valent</i>						
Ca-22A-ImpA	67	6.5	0	13	3.4	6.1
Mg-22A-ImpA	85	2.8	3.2	2.5	0.1	0.3
Al-22A-ImpA	93	1.1	2.5	0.2	0	0

<sup>a</sup> 0% signifies less than 0.1% yield; initial pH = 8.

MgCl<sub>2</sub> added to Na<sup>+</sup>-montmorillonite facilitates phosphodiester bond formation. Much less phosphodiester bond is observed when the Mg<sup>2+</sup> is the exchangeable cation on the montmorillonite.

The difference in reaction products between Ca<sup>2+</sup>- and Mg<sup>2+</sup>-montmorillonite may be due to presence of an interlayer magnesium hydroxide complex in the montmorillonite (Ohtsuka *et al.*, 1990). This complex fills the interlayer space and prevents the binding of ImpA. The interlayer calcium hydroxide complex is water soluble so can be displaced from the interlayer by water or ImpA.

The explanation for the absence of catalysis by Al<sup>3+</sup>-montmorillonite is similar to that given for Mg<sup>2+</sup>. Aluminum ion is known to hydrolyze in water to form polymeric cations which bind at the negative sites on the basal surfaces of the clay (Cotton and Wilkinson, 1980; Pinnavaia, 1983). These pillars of aluminum hydroxide may impede binding by filling the space between the sheets so that there is little room for binding to occur (Newman, 1987).

The efficient UO<sub>2</sub><sup>2+</sup>-catalyzed condensation of ImpA to 2',5'-linked oligomers (Sawai *et al.*, 1989) prompted investigation of the reaction of ImpA on UO<sub>2</sub><sup>2+</sup>-montmorillonite. A control reaction was performed concurrently in which the amount of UO<sub>2</sub><sup>2+</sup> present was the same as that bound to the montmorillonite 22A. A higher yield of 2',5'-pApA (11 versus 4.6%), and comparable yield of 2',5'-(pA)<sub>3</sub> (8.0 versus 7.8%) was obtained with UO<sub>2</sub><sup>2+</sup>-montmorillonite 22A. But the yields of longer oligomers dropped off markedly after the trimer and essentially went to zero after pentamer with UO<sub>2</sub><sup>2+</sup>-montmorillonite 22A while the yield of oligomers from the UO<sub>2</sub><sup>2+</sup>-catalyzed reaction remained constant at 1–2% up to the octamer. The structures of the longer oligomers were not determined but are reported to be 2',5'-linked (Sawai *et al.*, 1989).

One explanation for the shorter oligomers obtained using UO<sub>2</sub><sup>2+</sup>-montmorillonite 22A is that there is hindrance to formation of longer 2',5'-linked oligomers when the uranyl ion is bound to smectite clay. We have observed that 3',5'-linked oligomers



TABLE IV  
Effect of Mineral on Phosphodiester Bond Formation  
Yield (%)<sup>a</sup>

	pA	2',5'-(pA) <sub>2</sub>	AppA	3',5'-(pA) <sub>2</sub>	Unreacted ImpA
Na-Montmorillonite, Little Rock, Ark. (28)	87	4.6	3.2	0.8	4.2
Na-Ferruginous Smectite, Grant County, WA (SWA-1)	87	3.4	3.1	0.4	1.7
Na-Nontronite, Garfield, WA (H-33a)	65	5.7	19	1.1	2.8
Na-Barasym, SSM-100 (Syn-1)	87	1.9	3.9	0.3	4.8
Sepiolite, Two Crows, Nev. (Sep. Nev-1)	60	1.7	8.0	1.6	17
Allophane, Ki-P	88	0.9	7.0	0.1	3.2
Allophane, (Itoh)	93	1.1	3.2	0	2.0
Imogolite, Ki-G	82	0.5	2.7	0.2	12

<sup>a</sup> 0% signifies less than 0.1% yield; initial pH = 8.

are the predominant reaction products on Na<sup>+</sup>-montmorillonite so there may be geometric constraints on the montmorillonite surface to the formation of 2',5'-linked oligomers. The inhibitory effect must start at the trimer level because a much higher yield of 2',5'-(pA)<sub>4</sub> formed in the solution phase than in the presence of UO<sub>2</sub><sup>2+</sup>-montmorillonite 22A.

The effect of mineral source and structure on oligonucleotide formation was investigated briefly (Table IV). The sodium form of a montmorillonite from Little Rock, Arkansas (API 28) was studied because its iron content (4.0% Fe<sub>2</sub>O<sub>3</sub>) (American Petroleum Institute, 1951) is similar to that of Na<sup>+</sup>-22A (5.65% as Fe<sub>2</sub>O<sub>3</sub>) (American Petroleum Institute, 1951). However, its effect on the formation of 3'5'-(pA)<sub>2</sub> from 5'-ImpA is close to 10 fold less (0.8 vs. 8.3% yield) (Table I). This suggests that factors other than iron content are responsible for the observed catalysis (Ferris *et al.*, 1990). The effective catalysis of 3'5'-(pA)<sub>2</sub> formation by Volclay (Table I) which contains 3.25% iron as Fe<sub>2</sub>O<sub>3</sub> (Brindley and Ertem, 1971) is also not consistent with a proposed correlation between iron content and oligonucleotide formation (Ferris *et al.*, 1990). Two other montmorillonites were also tested for their ability to convert 5'-ImpA to 3'5'-(pA)<sub>2</sub>. Neither Barasym SS-100 (Syn-1), a synthetic mica montmorillonite which has very low levels of iron (0.02% Fe<sub>2</sub>O<sub>3</sub>) (Van Olphen and Fripiat, 1979) nor a ferruginous smectite (SWa-1) which has very high levels of iron (28.7 Fe<sub>2</sub>O<sub>3</sub>) (Goodman *et al.*, 1976; Lear and Stucki, 1987) caused the formation of higher levels of 3'5'-(pA)<sub>2</sub> than are formed in the absence of mineral (Table III). Allophane, a glassy aluminum silicate formed by the weathering of volcanic ash (Newman, 1987), and imogolite, a tubular allophane (Newman, 1987) did not induce the formation of 3'5'-(pA)<sub>2</sub> (Table III). Sepiolite (SepNev-1), a magnesium silicate with a ribbon-like structure (Post, 1978; Newman, 1987), exhibits modest activity for 3'5'-(pA)<sub>2</sub> formation (Table IV).

TABLE V  
Effect of Base on Phosphodiester Bond Formation  
Yield (%)<sup>a</sup>

	pN	2',5'-(pN) <sub>2</sub>	NppN	3',5'-(pN) <sub>2</sub>
Na <sup>+</sup> -22A-ImpG	80	3.3	0	3.9
ImpG, no clay	87	1.4	8.9	0.4
Na <sup>+</sup> -22A-2-MethylImpG	88	4.6	0	2.6
2-MethylImpG, no clay	75	2.6	2.7	0.8
Na <sup>+</sup> -22A-ImpC	88	4.2	0	1.3
ImpC, no clay	92	0.6	6.4	0
Na <sup>+</sup> -22A-ImpU <sup>b</sup>	80	5.4	0	1.3
ImpU, no clay	95	0.7	0	0.2

<sup>a</sup> 0% signifies less than 0.1% yield; initial pH = 8.

<sup>b</sup> Three more peaks appeared after APH hydrolysis; the compounds formed by APH hydrolysis were cleaved by ribonuclease A indicating there were 3',5'-linked.

The oligomers formed in the reaction of the 5'-phosphorimidazolides of guanosine, cytidine and uridine on Na<sup>+</sup>-22A were investigated briefly (Table V). The 2',5'- and 3'5'-linked dimeric products were characterized by hydrolysis with alkaline phosphatase and coinjection with authentic samples of dinucleoside monophosphates. The formation of 5,5'-dinucleoside pyrophosphate (NppN) was inferred from the principal HPLC peak in the absence of montmorillonite and its resistance to hydrolysis by alkaline phosphatase. AppA is the principal product of the reaction of ImpA at pH 8 (Table I). The combined yield of dimers from ImpG was about 15%, a yield that is less than that obtained with ImpA. Also with ImpG the 2',5'-isomer ratio of 2',5'- to 3'5'-isomer is approximately the same while with ImpA the 3'5'-isomer is favored. The absence of authentic samples precluded the structure determination of other reaction products. The yield and distribution of dimeric products from 2-methylImpG was comparable to that obtained from ImpG. Thus it appears that the substitution on the imidazole ring has little effect on the course of the reaction. This finding is in marked contrast to the difference in regioselectivity observed in template directed synthesis where the 2',5'-linked oligomers are the predominant product with the imidazolides and the 3'5'-products form from the 2-methylphosphorimidazolides.

Lower yields of dimeric products (6–7%) were obtained when the pyrimidine nucleotides ImpU and ImpC were used. The 2',5'-isomer predominates in each reaction. There were three additional products formed from ImpU which, after hydrolysis of the terminal phosphate groups with alkaline phosphatase, had longer retention times than 3'5'-UpU. Two of these were further digested with ribonuclease A suggesting that they were 3'5'-linked oligomers. The longer retention times and the hydrolysis by alkaline phosphatase demonstrates that these are 3'5'-linked trimeric and/or higher oligomeric species. The structures of these products will be the subject of a future investigation.

#### 4. Conclusions

The formation of the phosphodiester bond proceeds in aqueous solution and moderate pH in the presence of some montmorillonite clays. The reaction proceeds under a variety of reaction conditions and with varying amounts and particle sizes of the montmorillonite.  $\text{Li}^+$ ,  $\text{Na}^+$ ,  $\text{Ca}^{2+}$  and  $\text{NH}_4^+$  are among the more effective exchangeable cations associated with the montmorillonite for the formation of the phosphodiester bond. The cations of sodium and calcium are prevalent in sea water and are associated with contemporary clays (Grim and Güven, 1978). They have also been postulated to be present in the oceans of the primitive Earth (McClendon, 1976; Egami, 1974).

The use of the imidazolid group in place of EDAC results in higher yields of products which contain phosphodiester bonds and a greater proportion of 3'5'-linkages in these products. A ten-fold excess of EDAC was required for the formation of a 30% yield of products with phosphodiester bonds in a two-step process (activation and condensation). Comparable yields are obtained in the condensation of only one equivalent of ImpA to phosphodiester products (Ferris *et al.*, 1989b). Appreciable yields of trimeric products (5–15%) are observed in the present study while 2.1% was the highest yield of trimeric product detected in previous studies where EDAC was the condensing agent (Ferris and Kamaluddin, 1989).

The 2:1 preponderance of 3'5'- over 2',5'-linkages is the most significant advantage of the imidazolid over EDAC as a phosphate activating group. The observation that the *Tetrahymena* ribozyme does not elongate 2',5'-linked substrates (Young and Cech, 1989) indicates that 3'5'-phosphodiester bonds were important if life was based on RNA on the primitive Earth. Previous potentially prebiotic syntheses starting from 3'-phosphorimidazolides yield a preponderance of the 2',5'-linked isomers (Sawai and Orgel, 1975; Sleeper and Orgel, 1979; Lohrmann and Orgel, 1978; Sawai *et al.*, 1989). The solid phase reaction of the 2',3'-cyclic phosphate of adenosine in the presence of diamine catalysts gives a 1.65–1.85 ratio of 3'5'- to 2',5'-linkages (Verlander *et al.*, 1973).

The acidity of montmorillonites is believed to have a central role in the formation of oligomers from the phosphorimidazolides of purine nucleotides (Loeppert *et al.*, 1986). The higher yields obtained from the phosphorimidazolides of purine nucleosides may reflect their greater binding to montmorillonite (Ferris *et al.*, 1989a; Ferris and Kamaluddin, 1989; Ferris *et al.*, 1990). The principal interaction between purine nucleotides and montmorillonite is postulated to be an ionic one between the protonated heterocyclic ring and negative centers on the montmorillonite. It is proposed that the acidic sites on the montmorillonite serve as acid catalysts for the reaction. The montmorillonite may protonate the imidazole group of the phosphorimidazolid and/or the oxygen of the phosphate and thus accelerate the reaction with the alcohol groups of other nucleotides to form the phosphodiester bond. The addition of montmorillonite makes it possible to perform an acid catalyzed

reaction in an aqueous solution where the bulk pH is 8.

The association of the protonated heterocyclic ring with the montmorillonite orients the hydroxyl and phosphorimidazolide groups of the nucleotides away from the surface of the clay where they will be accessible for phosphodiester bond formation (Ferris *et al.*, 1989a; 1990). The difference in the regiospecificity between the reactions using EDAC and those with the phosphorimidazolide group may reflect changes in orientation of the reacting nucleotides on the montmorillonite. In addition, EDAC which contains a protonated amine group, may compete with the 5'-AMP for binding sites on the montmorillonite. Alternatively, the difference in regiospecificity may be because the two reactions proceed by very different pathways.

### Acknowledgements

We thank Dr. Koji Wada, Dr. T. Itoh and the American Colloid Company for providing some of the minerals used in this study. Mr. K. Kay synthesized some of the 5'-phosphorimidazolides. Professor L. Interrante informed us of the research paper of Ohtsuka *et al.* (1990). This research was supported by Grant CHE-85-06377 from the National Science Foundation. The HPLC was provided by NASA Grant NGR 3-018-148.

### References

- American Petroleum Institute: 1951, Clay Mineral Standards, American Petroleum Institute, Project 49, Preliminary Report 7B, Chemical Analysis, Columbia University, NY.
- Banin, A., Lawless, J.G., Mazzurco, J.; Church, F. M., Margulies, L. and Orenberg, J.: 1985, *Origins of Life* **15**, 89.
- Been, M. D. and Cech, T. R.: 1988, *Science* **239**, 1412.
- Brindley, G. W. and Ertem, G.: 1971, *Clays Clay Min.* **19**, 399.
- Cotton, F. A. and Wilkinson, G.: 1980, 'Advanced Inorganic Chemistry', 4th Edition, John Wiley, N.Y., p. 33.
- Egami, F.: 1974, *J. Mol. Evol.* **4**, 115.
- Ferris, J. P., Edelson, E. H., Mount, N. M., and Sullivan, A. E.: 1979, *J. Mol. Evol.* **13**, 317.
- Ferris, J. P., Ertem, G. and Agarwal, V.: 1989a, *Origins Life Evol. Biosphere* **19**, 153.
- Ferris, J. P., Ertem, G., and Agarwal, V.: 1989b, *Origins Life Evol. Biosphere* **19**, 165.
- Ferris, J. P. and Kamaluddin: 1989, *Origins Life Evol. Biosphere* **19**, 609.
- Ferris, J. P., Kamaluddin, and Ertem, G.: 1990, *Origins Life Evol. Biosphere* **20**, 279.
- Ferris, J. P., Kamaluddin, Kezbekus, P., Ertem, G., and Hagan, W. J., Jr.: 1989c, *Origins Life Evol. Biosphere* **19**, 325.
- Goodman, B. A., Russell, J. D., Fraser, A. R., and Woodhams, F. W. D.: 1976, *Clays Clay Miner.* **24**, 53.
- Grim, R. E. and Güven, N.: 1978, 'Developments in Sedimentology 24. Bentonites, Geology, Mineralogy, Properties and Uses', Elsevier, Amsterdam, pp. 16, 17.
- Guerrier-Takoda, C., Gardiner, K., Marsh, T., Pace, N., and Altman, S.: 1983, *Cell* **35**, 849.
- Joyce, G. F.: 1989, *Nature* **338**, 217.
- Joyce, G. F., Inoue, T., and Orgel, L. E.: 1984, *J. Mol. Biol.* **176**, 279.
- Joyce, G. F., Visser, G. M., van Boeckel, C. A. A., van Boom, J. H., Orgel, L. E., and van Westrenen, J.: 1984, *Nature* **310**, 602.
- Kanavarioti, A., Bernasconi, C. F., Doodokyan, D. L., and Aberas, D. J.: 1989, *J. Am. Chem. Soc.* **111**, 7247.

- Kay, K., B. S. Thesis, Rensselaer Polytechnic Institute, 1990.
- Lear, P. R. and Stucki, J. W.: 1987, *Clays Clay Miner.* **35**, 373.
- Loeppert, R. H., Zelanzy, L. W., and Volk, B. G.: 1986, *Clays Clay Miner.* **34**, 87.
- Lohrmann, R.: 1977, *J. Mol. Evol.* **10**, 137.
- Lohrmann, R. and Orgel, L. E.: 1978, *Tetrahedron* **34**, 853.
- McClendon, J. H.: 1976, *J. Mol. Evol.* **8**, 175.
- Newman, A. C. D.: 1987, 'Chemistry of Clays and Clay Minerals', ed., A. C. D. Newman, John Wiley, New York, pp. 258–260.
- Ohtsuka, K., Suda, M., Tsunoda, M., and Ono, M.: 1990, *Chem. Mater.* **2**, 511.
- Pinnavaia, T. J.: 1983, *Science* **220**, 365.
- Post, J. L.: 1978, *Clays Clay Miner.* **26**, 58.
- Sawai, H., Kuroda, K., and Hojo, T.: 1989, *Bull. Chem. Soc. Jpn.* **62**, 2018.
- Sawai, H. and Orgel, L. E.: 1975, *J. Am. Chem. Soc.* **97**, 3532.
- Sleeper, H. L. and Orgel, L. E.: 1979, *J. Mol. Evol.* **12**, 357.
- Van Olphen, H. and Fripiat, J. J. (eds.): 1979, 'Data Handbook for Clay Minerals and Other Non-Metallic Minerals', Pergamon, Oxford.
- Young, B. and Cech, T. R.: 1989, *J. Mol. Evol.* **29**, 480.