

SEQUENCE- AND REGIO-SELECTIVITY IN THE MONTMORILLONITE-CATALYZED SYNTHESIS OF RNA

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Abstract. The six binary montmorillonite clay-catalyzed reactions of the 5'-phosphorimidazolides of adenosine, cytidine, guanosine and uridine were performed and the eight dimers from each reaction were separated and analyzed by HPLC. A 16–51-fold higher yield of the 5'-purine-pyrimidine dimers over that of the 5'-pyrimidine-purines was observed. The total yield of the 5'-purine-pyrimidine dimers was in the 50–70% range while that of the 5'-pyrimidine-purine dimers was 1.3–7.0%. Less sequence selectivity was observed in the homodimers formed. Regioselectivity for the formation of 3', 5'-phosphodiester bonds over that found in the absence of clay was observed. The 5'-purine-pyrimidine, 5'-pyrimidine-pyrimidine and 5'-purine-purine dimers had 3', 5'-links in about half of their phosphodiester bonds. The percent phosphodiester links in the 5'-pyrimidine-pyrimidine dimers was 18%, a value close to that observed in the absence of the montmorillonite catalyst. The montmorillonite-catalyzed reaction of all four activated nucleotides was performed and the 24 products were separated and analyzed. The trends observed in the binary reactions were confirmed and the results also showed that the relative reactivity of the activated monomers was A>G>C>U in the ratio 8.2: 4.8: 1.3: 1 respectively. No 5'-pyrimidine-purines with a 5'-U and pG^{3'}pU, pC^{3'}pA and pC^{3'}pG were detected. These studies suggest that a limited population of RNAs would have formed in catalyzed prebiotic reactions.

1. Introduction

In the RNA world paradigm for the origin of life RNA is formed by polymerization of mononucleotides present on the primitive Earth (Crick, 1968; Gilbert, 1986; Orgel, 1968). We have shown that short homopolymers of A, C, G, U, and I are formed in the reaction of the corresponding 5'-phosphorimidazolides of mononucleotides which is catalyzed by montmorillonite clay (Ding *et al.*, 1966; Ertem and Ferris, 1996, 1992a; Kawamura and Ferris, 1999). Fifty mers of A form when the phosphorimidazolide of adenosine (ImpA) (Figure 1 R' = H, N = A) is added daily to a decameric primer bound to montmorillonite (Ferris *et al.*, 1996). RNAs containing about fifty monomer units are long enough to be effective templates (Joyce and Orgel, 1993) and catalysts (Joyce and Orgel, 1993) (Szostak and Orgel, 1993). In the RNA world the RNA provides both the genetic information

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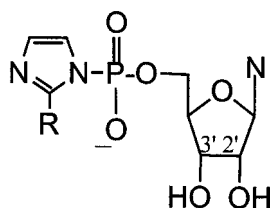


Figure 1.

and the catalytic activity required for its amplification. It should be possible to preserve the information content of the oligomers formed by clay mineral catalysis by template-directed synthesis. We found that the oligo(C)s formed in the presence of montmorillonite, which contain 2',5'- and 3',5'-linked phosphodiester bonds, pyrophosphate groups as well as linear and cyclic chains, serve as templates for the formation of the complementary oligo(G)s (Ertem and Ferris, 1996) (Ertem and Ferris, 1997). Thus it should be possible to maintain the information content of RNA formed by catalysis using template-directed synthesis.

The goal of the present research was to determine the effect of clay catalysis on the sequence- and regio-selectivity in the RNAs formed from a mixture of activated monomers. Here we report the formation of RNAs from binary, and quaternary mixtures of activated nucleotides in the presence of montmorillonite.

2. Experimental Section

Dinucleotides (NpN) and ribonuclease T₂ were purchased from Sigma and alkaline phosphatase (APH) was from Worthington. Solvents and other chemicals were obtained from the same sources listed previously (Ferris and Ertem, 1993a). Homoionic Na⁺-montmorillonite was prepared from Volclay by titration method (Banin *et al.*, 1985). Volclay SPV-200 was a gift from the American Colloid Co., Arlington Heights, IL. The structure of Na⁺-montmorillonite was derived from its elemental analysis (Brindley and Ertem, 1971). Activated mononucleotides were prepared following the literature procedure (Joyce *et al.*, 1984) with slight modifications (Ferris and Ertem, 1992b). Anion exchange HPLC conditions were the same as described earlier (Ferris and Ertem, 1993a). The separation and analysis of the dinucleotides were accomplished using an Alltech C-18 Alltima reverse phase column (Kanavarioti, 1997). A Waters' μ Bondapak C-18 column (Ferris and Ertem, 1993b) was used to resolve the NpN isomers that had the same retention times on the Alltima column.

2.1. REACTIONS WITH BINARY AND QUATERNARY MIXTURES OF NUCLEOTIDES

The mixtures were prepared to contain each activated monomer at a final concentration of 14 mM in 0.1 M HEPES, 0.2 M NaCl and 0.075 M MgCl₂, pH 8, and Na⁺-montmorillonite (Ferris and Ertem, 1993a). Because of the insolubility of 2-MeImpG in the buffer-electrolyte system used, it was dissolved in sufficient water to prepare a 28 mM solution for the preparation of the binary mixtures. This solution was mixed with an equal volume of solution containing 28 mM of the accompanying monomer in 0.2 M HEPES, 0.4 M NaCl and 0.150 M MgCl₂, pH 8. 400 μL of the resulting solution was added to 20 mg of Na⁺-montmorillonite (Ferris and Ertem, 1993a) and reactions were run at 25 °C for 2 h and 3 d. In the reaction of four nucleotides the ImpA, ImpC and ImpU were each dissolved separately in 0.2 M HEPES, 0.4 M NaCl and 0.150 M MgCl₂, pH 8, to give a final concentration of 0.084 mM ImpN. The 2-MeImpG was dissolved in water to give 0.028 mM concentration and 68 μL of each of the ImpA, ImpC, and ImpU solutions was added to 204 μL of 2-MeImpG. This mixture was added to 20 mg of Na⁺-montmorillonite and allowed to react at 25 °C for 2 h. At the end of the reaction time the mixtures were centrifuged and the supernatants were removed. The clay was washed with 400 μL of 0.1 M ammonium acetate, the mixture centrifuged and the wash was combined with the supernatant. The pH of the combined supernatant and wash was adjusted to pH 4–5 with 10% HClO₄ (Schwartz and Orgel, 1985) and allowed to stand 25 °C for 4 h to hydrolyze the 5'-imidazole groups.

2.2. HPLC ANALYSES

The reaction products, 20–30 μL, were separated on anion exchange column and the oligomers with 3(-) charges ('dimers'), were collected and hydrolyzed with APH to convert the pNpN oligomers to NpN (Ferris and Ertem, 1993a). The APH hydrolysate was dialyzed, freeze-dried, dissolved in water and injected onto anion exchange column to separate the NpN dimers from NppNpN and cyclic trimers (Ertem and Ferris, 1997). The NpN fractions were collected, dialyzed and freeze dried, dissolved in water and analyzed on a C-18 Alltima column. The NpN dimers were identified by coinjections with authentic standards obtained from Sigma. C^{2'}pU was not commercially available but was identified by comparison of the relative retention times of U^{3'}pU, U^{3'}pC, pU, and C^{3'}pC with those of U^{2'}pU, U^{2'}pC and C^{2'}pC. HPLC peaks assigned to 3', 5'-NpNs were further identified by hydrolysis with ribonuclease T₂. Those NpNs that elute from Alltima column with the same retention times were collected and separated on a Waters' μ-Bondapak reverse phase column (Ferris and Ertem, 1992b), (Järvinen *et al.*, 1991). Yields, which are the average of two reactions, were calculated after correction for the dimers' absorption at 260 nm and for their hyperchromicities (Warshaw and Tinoco, 1966).

3. Results and Discussion

3.1. BINARY REACTIONS

The reactions of mixtures of the 5'-phosphorimidazolides of two nucleosides on montmorillonite were carried out as described previously (Ferris and Ertem, 1993a) for the formation of homopolymers. The dinucleotides formed were investigated because authentic samples of both the 2', 5- and 3', 5'-isomers were available and separable by reverse phase HPLC (Ferris and Ertem, 1992b), (Kanavarioti, 1997). RNA oligomers were formed in the clay-catalyzed reaction of binary mixtures of the 5'-phosphorimidazolides of the nucleosides A + U, A + C, A + G, G + C, G + U, and C + U. The dimers were separated from the other oligomers by anion-exchange HPLC (Ferris and Ertem, 1992a) and the 5'-phosphate groups were cleaved using alkaline phosphatase to give the 2', 5'- and 3', 5'-linked NpN derivatives (N = A, G, C, U). The eight dimeric products formed from each binary mixture of activated nucleotides were separated by reverse phase HPLC and identified by coinjection with authentic standards and by enzymatic hydrolyses of the 3', 5'-linked isomers (Table I).

Since the dimer yields depend both on their relative rates of formation and elongation yields were measured after 2 h and 3 d for four of the binary mixtures. Major variations in the yields of dimers with time (60%) were observed in some of the products of the A + C and A + U reactions. In each case there was a decrease in the yields of pA^{3'}pA and pA^{2'}pA with time (Table I). This finding indicates that pApA undergoes rapid elongation relative to the other dimers present and it is a likely grouping at the 5'-end of the RNAs formed. There were major increases in the yields of pC^{3'}pC, pC^{2'}pC and pU^{3'}pU with time in the A + C and A + U reactions suggestive of a slower rate of elongation of these dimers compared with the other dimeric products. These trends in the variation of the yields with reaction times were not observed in the G + C and C + U reactions.

Sequence selectivity was observed in the clay-catalyzed reactions of binary mixtures of activated purine and pyrimidine nucleotides (Table II). There was a 16–51 fold higher yield of the dimers with the purine nucleotide in the 5'-position over that of the dimers with the pyrimidine in the 5'-position in the three day reactions. In all these reactions the total yields of the purine-pyrimidine dimers is in the 50–70% range while the corresponding pyrimidine-purine dimer yields are in the 1.3–7.0% range. The high yields of the purine-pyrimidine dimers suggest that these sequences will, along with pApA, predominate at the 5'-ends of the RNA formed by clay-catalysis.

Some sequence selectivity was observed in the homodimers but it was significantly less than that observed in the reactions between purine and pyrimidine nucleotides (Table II).

The sequence selectivity observed in the clay-catalyzed reactions, using 0.03 M total nucleotide concentrations, differs significantly from that of the uncatalyzed

TABLE I
Dimer yields in binary reactions (%)^a

A + C			A + U			G + C		
	2 h	3 d		2 h	3 d		2 h	2 d
pC ^{2'} pC	6.3	17	pU ^{2'} pU	7.0	9.6	pC ^{2'} pC	20	7.3
pC ^{2'} pA	2.6	1.3	pU ^{2'} pA	1.1	2.0	pC ^{2'} pG	1.1	2.9
pA ^{2'} pC	24	30	pA ^{2'} pU	17	26	pG ^{2'} pC	30	42
pA ^{2'} pA	13	4.2	pA ^{2'} pA	17	12	pG ^{2'} pG	18	8.6
pC ^{3'} pC	2.7	7.4	pU ^{3'} pU	1.0	3.1	pC ^{3'} pC	5.9	2.4
pC ^{3'} pA	4.4	0.51	pU ^{3'} pA	1.0	2.0	pC ^{3'} pG	0.38	0.96
pA ^{3'} pC	35	36	pA ^{3'} pU	38	43	pG ^{3'} pC	20	19
pA ^{3'} pA	12	4.7	pA ^{3'} pA	18	2.6	pG ^{3'} pG	5.4	16

C + U			G + A		G + U	
	2h	3 d		3 d		3 d
pC ^{2'} pC	27	24	pA ^{2'} pA	12	pU ^{2'} pU	9.5
pC ^{2'} pU	18	28	pA ^{2'} pG	12	pU ^{2'} pG	1.9
pU ^{2'} pC	20	20	pG ^{2'} pA	23	pU ^{2'} pU	38
pU ^{2'} pU	17	15	pG ^{2'} pG	17	pG ^{2'} pG	14
pC ^{3'} pC	5.9	6.9	pA ^{3'} pA	9.3	pU ^{3'} pU	1.6
pC ^{3'} pU	5.5	2.6	pA ^{3'} pG	5.6	pU ^{3'} pG	0.65
pU ^{3'} pC	~0	~0	pG ^{3'} pA	13	pG ^{3'} pU	32
pU ^{3'} pU	6.8	4.2	pG ^{3'} pG	9.6	pG ^{3'} pG	3.6

^a 5' – Terminal imidazolides present in the reactions were cleaved by adjusting the pH to 4–5 with 10% HClO₄ and incubation at 25 °C for 4 h. The dimer fraction was separated by anion-exchange chromatography on a HEMA IEC BIO Q column (Ferris and Ertem, 1993). This fraction was hydrolyzed by alkaline phosphatase and the NpN fraction was separated from the NppNpN and cyclic trimer oligomers by anion-exchange HPLC (Ertem and Ferris, 1997). The NpN isomers were analyzed by HPLC on Alltima C-18 (Kanavarioti, 1997) and Waters μ Bondapak (Ferris and Ertem, 1993) (Järvinen *et al.*, 1991) columns and detected at 260 nm. Dimers were identified by coinjection with authentic samples obtained from Sigma. C^{2'}pU was not commercially available but was identified from the relative retention times of CpU, UpC, UpU and CpC isomers. The structural assignments were supported by the lability of the 3', 5'-linked dimers and the stability of the 2', 5'-linked dimers on treatment with ribonuclease T₂. Yields, which are the average of two reactions, were calculated after correction for the dimers' adsorption at 260 nm and for their hyperchromicities (Warshaw and Tinoco, 1966).

reactions. Little sequence selectivity was observed in the dimers formed from the reactions of mixtures of 0.5–1 M solutions of the activated nucleotides of G, C, and U (Kanavarioti, 1998). Clearly the selectivity observed in the clay-catalyzed

TABLE II
Yield ratio for each sequence in the binary reactions

Reaction	Dimers	Ratio	
		2 hr reaction	3 day reaction
Heterodimers			
A + C	pApC/pCpA	8.4	51
A + U	pApU/pUpA	26	17
G + C	pGpC/pCpG	33	16
G + U	pGpU/pUpG	–	27
G + A	pGpA/pApG	–	2.0
C + U	pCpU/pUpC	1.2	1.5
Homodimers			
A + C	pApA/pCpC	2.8	0.36
A + U	pApA/pUpU	4.4	1.1
G + C	pGpG/pCpC	0.90	2.5
G + U	pGpG/pUpU	–	1.6
G + A	pGpG/pApA	–	1.2
C + U	pCpC/pUpU	1.4	1.6

^a Sum of the yields for the 2',5'- and 3',5'-linked isomer of each dimer was used in determining the ratios.

reactions is controlled by surface processes on the mineral and is due to differences between the reactivity of purine and pyrimidine nucleotides on the clay.

Differing regioselectivities for the formation of 3',5'- or 2',5'-phosphodiester bonds were also observed (Table III). The 5'-purine-pyrimidine, 5'-pyrimidine-purine and 5'-purine-purine dimers have an average of 57, 42 and 55% 3',5'-linked phosphodiester bonds, respectively in the 2 h reaction. The average percentage of 3',5'-phosphodiester bonds in the 5'-pyrimidine-pyrimidine dimers is 18% (Table III).

The percent 3',5'-phosphodiester bond formation in clay-catalyzed reactions differs markedly from that observed in uncatalyzed reactions. The proportions of 3',5'-linked dimers formed is 10–17% in the uncatalyzed reactions of 0.1 M concentrations of A, G, U and C nucleotides (Lohrmann and Orgel, 1978). The proportion of the 3',5'-linked isomer increases to about 25% when the concentrations of reacting activated nucleotides is increased from 0.1 to the 1 M range (Kanavarioti, 1997).

The regioselectivities observed in phosphodiester bonds of the homodimers formed in this study follow the trend observed in the dimers formed in the reaction of individual activated nucleotides of A, U and C on montmorillonite. The percentages of 3',5'-links in pA^{3'}pA (Ferris and Ertem, 1992a), pU^{3'}pU (Ding *et*

TABLE III
3',5'-Linked dimers in the binary reactions (%)

Reaction	Dimer	2 hr reaction	3 day reaction
1. A + C	pApC	59	55
	pCpA	63	28
	pApA	48	52
	pCpC	30	30
2. A + U	pApU	69	62
	pUpA	48	50
	pApA	51	18
	pUpU	13	24
3. G + C	pGpC	40	31
	pCpG	27	25
	pGpG	23	65
	pCpC	23	25
4. G + U	pGpU	–	46
	pUpG	–	25
	pGpG	–	20
	pUpU	–	14
5. G + A	pGpA	–	36
	pApG	–	32
	pGpG	–	36
	pApA	–	44
6. C + U	pCpU	23	8.5
	pUpC	~0	~0
	pCpC	18	22
	pUpU	22	22

al., 1996) and pC^{3'}pC (Ertem and Ferris, 1996) are 61, 19 and 26% respectively, as compared to 49, 16 and 24% in the present work.

3.2. QUATERNARY REACTION

The investigation of the structures of the dimeric products from the 2 h reaction of a mixture of the four activated nucleotides of A, C, G and U was undertaken

TABLE IV
Dimer yields in the reaction of the activated nucleotides of A, C, G and U (%)

Dimer	Yield	Dimer	Yield	Dimer	Yield	Dimer	Yield
pA ^{2'} pA	5.1	pC ^{2'} pA	0.92	pG ^{2'} pA	8.6	pU ^{2'} pA	0
pA ^{3'} pA	8.2	pC ^{3'} pA	0	pG ^{3'} pA	0.04	pU ^{3'} pA	0
pA ^{2'} pC	7.9	pC ^{2'} pC	1.8	pG ^{2'} pC	7.4	pU ^{2'} pC	2.3
pA ^{2'} pC	15	pC ^{3'} pC	1.8	pG ^{3'} pC	6.1	pU ^{3'} pC	0
pA ^{2'} pG	3.6	pC ^{2'} pG	2.3	pG ^{2'} pG	4.1	pU ^{2'} pG	0
pA ^{3'} pG	0.11	pC ^{3'} pG	0.	pG ^{3'} pG	0.06	pU ^{3'} pG	0
pA ^{2'} pU	0.51	pC ^{2'} pU	0.92	pG ^{2'} pU	4.6	pU ^{2'} pU	2.7
pA ^{3'} pU	13	pC ^{3'} pU	0.46	pG ^{3'} pU	0	pU ^{3'} pU	1.5

to determine if the trends in the yields observed in the binary systems is the same in a more complex reaction mixture (Figure 2) (Table IV)). The 5'-purine dimers, pApC, pGpC, pApU, pApA, pGpA, pGpU, pGpG and pApG constitute 84% of the reaction products while the 5'-pyrimidine-purines pUpA, pUpG, pCpA and pCpG are 3.2%. The ratio of the 5'-purine-pyrimidine to the 5'-pyrimidine-purine dimers was 17:1 and there is a 5.7:1 ratio of the dimers with 5'-purines versus those with 5'-pyrimidines. The yield data show that the relative rates of the initiation of dimer formation decreased in the order A > G > C > U by 8.4:4.8:1.3:1, respectively. These findings confirm the trends observed in the binary mixtures discussed above where 5'-purine-pyrimidine and 5'-purine-purine dimers are formed in significantly higher yields than 5'-pyrimidine-purine dimers.

The relative reactivities of the four nucleotides on one reaction mixture could not be predicted from the binary reaction data. The absence of 5'-pyrimidine-purine dimers with a U in the 5'-position, the absence of pG^{3'}pU, pC^{3'}pA, pC^{3'}pG and the low yields of pA^{2'}pU, pA^{3'}pG, pG^{3'}pA and pG^{3'}pG were not expected. These low yields are mainly a function of the lower relative reactivity of activated G and U nucleotides in the clay-catalyzed reactions.

The data for the regioselectivity of the phosphodiester bond formation are less clear than the findings in the binary reactions because of the low yields of many of the dimeric products. In the instances where there were significant yields of both the 3',5'- and 2',5'-isomers, the percent yield of the 3',5'-isomers of pApC, pApA and pGpC were 66, 62 and 45%, respectively. These yields are close to the 50% range observed for the dimers in the binary reactions.

The observed regioselectivities may reflect differences in the binding of purine and pyrimidine nucleotides to montmorillonite with purines binding more strongly than pyrimidines (Ferris *et al.*, 1989). The phosphorimidazolides (Figure 1) (13 mM) bind in the following increasing order to fifty mg of montmorillonite in the

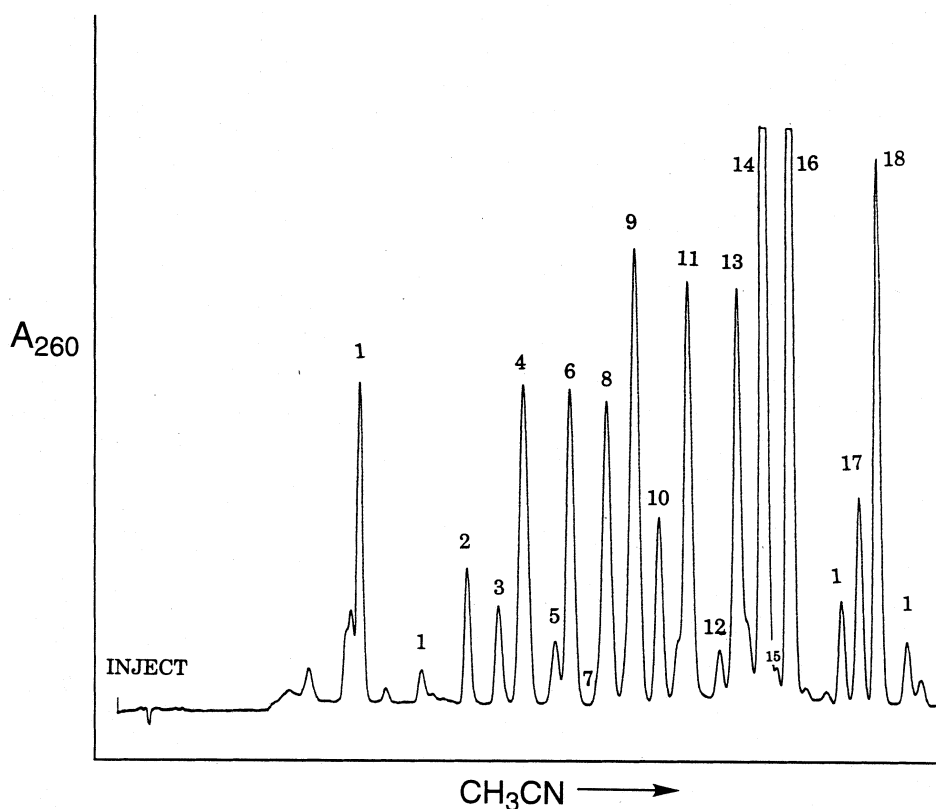


Figure 2. The HPLC elution profile, on C-18 Alltima reverse phase column (Kanavarioti, 1997), of dimers formed by the 2 h reaction of a mixture of the phosphorimidazolides of adenosine, cytosine, uridine and the phosphor-2-methylimidazolide of guanosine on montmorillonite and their hydrolysis with alkaline phosphatase (see footnote to Table I for experimental details). Of all the possible dimers $U^{2'}pU$ has the shortest retention time and $A^{3'}pA$ the longest, so that peaks outside the limits of these retention times not due to dimers. The reaction products were identified by coinjection with authentic standards. Overlapping peaks were resolved on a Waters μ Bondpak column. The products are 1. unknown 2. $U^{2'}pU$, 3. $C^{2'}pU$ 4. $U^{2'}pC$ and $G^{2'}pU$ 5. $C^{2'}pC$, 6. $A^{2'}pU$, $U^{3'}pU$ and $G^{2'}pG$ 7. $C^{3'}pU$ 8. $G^{2'}pC$ 9. $G^{2'}pA$ 10. $A^{2'}pG$ 11. $C^{3'}pC$ and $A^{2'}pC$ 12. $C^{2'}pA$ and $A^{3'}pG$ 13. $A^{2'}pA$ 14. $A^{3'}pU$ and $G^{3'}pC$ 15. $C^{2'}pG$ 16. $A^{3'}pC$ 17. $G^{3'}pA$ and $G^{3'}pG$ 18. $A^{3'}pA$.

presence of 0.075 M Mg^{2+} : ImpU (7.6%), ImpC (9%), ImpG (45%), ImpI (52%) and ImpA (64%) (Kawamura and Ferris, 1999). The phosphorimidazolides of the purine nucleotides exhibit significant yet diminished bindings to montmorillonite in the absence of Mg^{2+} while ImpU does not bind at all. These data suggest that both the purine and pyrimidine nucleotides bind to montmorillonite via a Mg^{2+} salt bridge between the negative phosphate group and the negative charges on the montmorillonite surface. There is an additional Van der Waals interaction associated with the purine nucleotides, which enhances their interaction with the montmorillonite (Kawamura and Ferris, 1999) (Lailach *et al.*, 1968).

There are at least two possibilities for the Van der Waals interaction. One is between the purine bases and the clay surface (Lailach *et al.*, 1968) and the second is between the purine bases of the nucleotides bound to the clay surface. The latter explanation would explain the predominance of 5'-purine-pyrimidine sequences. If the nucleotides stack on the clay surface while bound to the clay via salt bridges it would be expected that the pyrimidines would stack between two purines and be in the 3'-position in the dimers. There would be little binding of pyrimidines on the end of such an assembly because the stacking energy would be lower. This explanation is also consistent with the appreciable yield of dimers which contain two purine rings since there would also be assemblages of stacked purines as well. The differences observed in the regioselectivity of phosphodiester bond formation might also reflect the difference between the binding of nucleotides in which either purine nucleotides or a purine and pyrimidine nucleotides bind as stacked aggregates.

4. Conclusions

Purine nucleotides initiate the formation of over 80% of the oligomers formed in the reaction of mixtures of the activated nucleotides of A, G, C and U. About half of the phosphodiester bonds will be 3', 5'-linked in these oligomers. For those chains initiated by pyrimidines, C will initiate most and the second nucleotide will be a pyrimidine nucleotide linked by a 2',5'-phosphodiester bond. The low levels of inclusion of 5'-pyrimidine-pyrimidine units, which are mainly 2',5'-linked, indicates that about half of the phosphodiester bonds in these oligomers will be 3',5'-linked. The presence of 2',5'-linkages in these initial oligomers would not have deterred the origin of the RNA world since oligo(C) oligomers containing less than 50% 3',5'-linkages are effective templates for the formation of the complementary RNAs (Ertem and Ferris, 1996).

These studies establish the central role that catalysis may have had in the chemical processes leading the origin of life. Montmorillonite catalysis not only made it possible to form biopolymers in aqueous solution, where hydrolysis is an important competing reaction (Ferris and Ertem, 1993b; Ferris *et al.*, 1996), but it also could have limited the structural diversity of the polymers formed. The synthesis of all possible isomers in the prebiotic formation of the polymers of life is a problem that has not been addressed experimentally (Haldane, 1965; Miller and Orgel, 1974; Shapiro, 1986). The problem is that in the absence of constraints on the chemistry all possible isomers of a protein or nucleic acid are formed. The amounts of each biopolymer essential for the first life would have been severely limited because of the large amounts of monomers required to form all possible isomers. In addition, it would have been difficult to select this particular isomer from all the other ones formed. In one example it has been calculated that there are 10^{30} isomers of all possible fifty mers of RNA and that mixture containing one copy of each

of these would weight 10^{10} g or about 10^{-9} of the present biosphere (Joyce and Orgel, 1993). Clearly a more specific synthesis of RNAs is required and it has been demonstrated here that catalysis could have provided some of the required specificity.

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