Homochiral selection in the montmorillonite-catalyzed and uncatalyzed Prebiotic synthesis of RNA

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The reaction of D,L-5′-activated nucleotide of adenosine in the presence and absence of montmorillonite gave 60 : 40 and 96 : 4 ratios, respectively of the D,D- and L,L : D,L- and L,D-dimers.

In the RNA world paradigm for the origin of life, RNA is formed by polymerization of mononucleotides present on the primitive Earth.[1] The RNA provides both the required genetic information and the catalytic activity in the first life. RNA oligomers of A, C, I, G, and U are formed in the reaction of the corresponding activated mononucleotides on montmorillonite clay.[2–4] The information content of the heterogeneous mixture of oligomers formed in the montmorillonite-catalyzed reaction serves as a template for the formation of complementary RNAs.[3] Fifty mers of A form when the phosphoroimidazolide of adenosine (ImpA) is added daily to a decameric primer bound to montmorillonite.[5] RNAs containing about fifty monomer units are long enough to be effective templates and catalysts.[6]

There has been little or no success in the enantioselective formation of biomolecules in prebiotic experiments.[7] While chiral selection was observed in the self-directing oligomerization of activated tetranucleotides of pyranosyl-RNA[8] it has not been observed in the template-directed synthesis of RNA.[9]

It would have been a major impediment to the origins of the RNA world if RNAs composed of equal amounts of D- and L-nucleotides formed. These structures would have had less chance of forming the helices, stem loops, and pseudo knots required for the RNA to fold into the secondary and tertiary structures required for template-directed synthesis and catalysis.[6] In addition, polymers containing both D- and L-nucleotides would have increased the number of isomers formed with the resultant decrease in number of those longer RNAs required to initiate the origin of life.[6]

The potential for oligomer formation from racemic mixtures was explored in the reactions of D,L-ImpA and -ImpU on montmorillonite. The reactions of 15 mM of each of these racemic mixtures on montmorillonite resulted in the formation of oligomers comparable in length[10] to those formed from the corresponding D-enantiomers.[2,4]† This result stands in marked contrast to the RNA template-catalyzed reaction where the formation of oligo(G)s is strongly inhibited by the incorporation of a nucleotide of the opposite absolute configuration into the growing oligomer chain.[9]

The potential for homochiral selection in RNA synthesis was explored in the reaction of D,L-ImpA on montmorillonite to form pApA. Reaction mixtures were digested with alkaline phosphatase to dephosphorylate the pApA products to the corresponding ApAs. The products were separated into four peaks of increasing retention time which contained (1) D,D- and L,L-A$^{2'}$pA, (2) D,L- and L,D-A$^{2'}$pA, (3) D,D and L,L-A$^{3'}$pA and (4) D,L- and L,D-A$^{3'}$pA on two different reverse phase HPLC columns (Table 1).[11] The identity of the reaction products was established by coinjection with authentic samples. The average percentage of the homochiral products D,D- and L,L-ApA to the corresponding D,L- and L,D- diastereomers is 60 : 40 ± 3.8%, respectively.

Control reactions without montmorillonite resulted in the homochiral selection of 94 : 6 ± 3.2% for D,D- and L,L-ApA *vs.* D,L- and L,D-ApA using 0.15 and 0.60 mM D,L-ImpA (Table 2). The yields of dimeric products in the 0.15 and 0.60 mM montmorillonite-catalyzed reactions were 5.9 and 19 times greater, respectively, than those in the uncatalyzed reactions. The difference in the yields reflects the 500 times greater second order rate constant for the catalyzed *vs.* the uncatalyzed reactions of ImpA.[4,12]

The possibility that the homochiral selectivity observed in the montmorillonite-catalyzed reaction was due to an uncatalyzed solution phase process was evaluated. The percentages for the 0.15 and 0.60 mM catalyzed reactions in rows 7 and 9 of Table 1 were recalculated after subtracting the dimer yields in the control reactions. The selectivity would decrease from 65 : 35 to 59 : 41 for the 0.15 mM reaction and from 58 : 42 to 56 : 44 for the 0.6 mM reaction. These data show that the observed homochiral selection is observed in the reaction catalyzed by montmorillonite.

Low concentrations of D,L-ImpA were used to minimize trimer formation so that the product mixture would only be a measure of the chiral selection for dimer synthesis.[11] The amount of trimer formed was estimated from the relative total areas of the dimer and trimer peaks. The trimer fraction was

Table 1 Relative yields and ratios of D,D & L,L and D,L & L,D dimers from the montmorillonite-catalyzed reaction of D,L-ImpA

		μBondapak column				Alltima column			
D,L-ImpA (mM)		0.15	0.15	0.60	0.60	0.15	0.15	0.60	0.60
Reaction Time (h) D,D & L,L (%)		2	18	2	18	2	18	2	18
	A$^{2'}$pA	37	23	33	36	46	27	43	31
	A$^{3'}$pA	21	36	20	30	19	33	15	27
D,L & L,D (%)									
	A$^{2'}$pA	23	20	26	15	19	19	24	19
	A$^{3'}$pA	19	21	21	19	16	21	18	23
D,D & L,L : D,L & L,D[a]		58 : 42	59 : 41	53 : 47	66 : 34	65 : 35	60 : 40	58 : 42	58 : 42

[a] The average of all determinations of D,D & L,L : D,L & L,D is 60 : 40 ± 3.7%.

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Table 2 Relative yields and ratios of D,D & L,L and D,L & L,D dimers from the uncatalyzed reaction of D,L-ImpA (%)[a]

D,L-ImpA (mM)		0.15	0.15	0.60	0.60
D,D & L,L (%)					
	$A^{2'}$pA	49	57	60	58
	$A^{3'}$pA	42	39	31	39
D,L & L,D (%)					
	$A^{2'}$pA	2.5	trace	2.5	1
	$A^{3'}$pA	6.5	4	6.5	2
D,D & L,L : D,L & L,D[b]		91:9	96:4	91:9	97:3

[a] Procedures were the same as those given for Table 1. Reactions were carried out for 2 h and the products were analyzed on the Alltima column. [b] The average of all determinations of D,D & L,L : D,L & L,D is 94:6 ± 3.2%.

isolated from the reaction of 15 mM D,L-ImpA on montmorillonite and was dephosphorylated with alkaline phosphatase and coinjected with the products from the 0.15 and 0.6 mM D,L-ImpA reactions. The areas of the peaks with the same retention times as trimers in the 0.15 and 0.6 mM reactions of D,L-ImpA were summed and compared with the corresponding amount of dimers. In the reaction of 0.15 mM ImpA for 2 and 18 h the dimer yield was 12.5 fold higher than that of the total trimer yield. With 0.6 mM ImpA the combined dimer yield was 8.3 and 4.3 times greater than that of the trimer yield for the 2 h and 18 h reactions, respectively. These data show that little dimer is converted to trimer so that the yields in Table 1 mainly reflect the homochiral selectivity for dimer synthesis.

One of the five trimer peaks had the same retention time as the D,L- and L,D-$A^{3'}$pA (peak 4 above) in the dimer fraction. None of the compounds in the trimer fraction, that were not dephosphorylated with alkaline phosphatase, had a retention time that was identical with any of the dimer HPLC peaks. These findings establish that the chiral selectivity is not due to the enhancement of the peaks due to D,D- and L,L-ApA by trimers since the only enhancement is to the D,L- and L,D-$A^{3'}$ pA peak.

The product mixture was further characterized by ribonuclease T_2 digestion where the two HPLC peaks with the longest retention times, peaks 3 and 4 above, were decreased by about 50%. This is consistent with the known cleavage of D,D-$A^{3'}$ pA and, as discovered in this study, the cleavage of D,L-$A^{3'}$ pA with ribonuclease T_2 and the resistance of L,L-$Ap^{3'}A$, L,D-$A^{3'}$ pA, and the 2′, 5′-linked ApAs to cleavage with ribonuclease T_2.

The high homochiral selectivity observed in the uncatalyzed reaction may reflect chiral selection in the stacking of the activated nucleotides[13] coupled with intrastack reactions to form dimers. The lower selectivity in the uncatalyzed reaction may reflect formation of a monolayer of activated purine nucleotides on the montmorillonite platelets.[14]

The observance of both oligomer formation and chiral selection in the montmorillonite-catalyzed reaction of activated D,L-nucleotides on montmorillonite catalysis is consistent with previous findings in montmorillonite-catalyzed reactions. For example, regioselectivity was observed in the phosphodiester bonds formed between purine nucleotides[2] and sequence selectivity was observed in the reaction of mixtures of activated purine and pyrimidine nucleotides.[11] In most of these previous studies selectivity, but not specificity, was observed. This indicates that montmorillonite exhibits partial control on the course of the condensation reactions. This limited control explains why the oligomerization of D,L-mixtures proceeds on montmorillonite. The same limited control resulted here in the preferential formation of homochiral dimers.

It is expected that the bias towards the formation of homochiral oligomers will increase as the chain length increases and the homochiral strands fold into secondary and tertiary structures. This process, together with the potential for the more rapid hydrolysis of those RNAs that contain both enantiomers because they do not form secondary and tertiary structures, could enhance the proportion of homochiral RNAs.[15,16] The scenario of chiral selection and more rapid hydrolysis of heterochiral oligomers could have resulted in an even greater preponderance of homochiral RNA. This may have led to two RNA worlds, one with D- and the other with L-RNA.[17] A chance event may have favored the survival of the D-RNA world which was the precursor to the D-RNA (and D-DNA) present in life on Earth today.

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Notes and references

† The L-enantiomers of adenosine and uridine[18] were phosphorylated at the 5′-position[19] and the phosphate was converted to the L-phosphoroimidazolides of adenosine (ImpA) and uridine (ImpU).[20] Solutions of 0.15 M each of D- and L-ImpA were prepared in 0.2 M NaCl, 0.075 M $MgCl_2$ and 0.1 M HEPES at pH 8. The ImpA concentrations were determined by quantitative HPLC analysis. At the end of the reaction time the mixture was centrifuged and the supernatant was removed. The montmorillonite was washed with two 40 μL portions of ammonium acetate. The combined supernatant and extracts was adjusted to pH 4 with 1 M $HClO_4$ and incubated at 37 °C to cleave the imidazole groups from the activated nucleotides. The pH of 200 μL of the reaction mixture was then adjusted to pH 8 and 0.25 units of alkaline phosphatase (bacterial, Sigma) was added and the mixture was incubated at 37 °C for 1 h. The analysis of the ApA isomers was performed on a Waters μBondapak C-18 column[2] and on an Alltima C-18 column.[11,21] Authentic samples of the 2′,5′- and 3′,5′-isomers of L,L-ApA, D,L-ApA and L,D-ApA were prepared by appropriate reactions of the activated monomers with an excess of the corresponding adenosine derivatives. For example, L,D-ApA was prepared in the reaction of excess L-adenosine with D-ImpA. The D,D- and L,L-enantiomers and the D,L- and L,D-diastereomers had the same retention times on the two different reverse phase HPLC columns used. The D,D-enantiomers were characterized by coinjection with authentic standards.[2] Further characterization was performed on some samples by adjusting the pH of 100 μL of the reaction products to 4, adding 2 units of ribonuclease T_2 (Sigma) and incubating the mixture for 1 h at 37 °C. The ribonuclease T_2 cleaves D,D-$A^{3'}$ pA and D,L-$A^{3'}$ pA. It does not cleave any of the other reaction products. The combined peaks due to D,D-and L,L-$A^{3'}$A and D,L- and L,D-$A^{3'}$pA were diminished by about 50% by treatment with ribonuclease T_2.

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