Montmorillonite Catalysis of RNA Oligomer Formation in Aqueous Solution. A Model for the Prebiotic Formation of RNA

James P. Ferris* and Gözen Ertem

Contribution from the Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York 12180-3590

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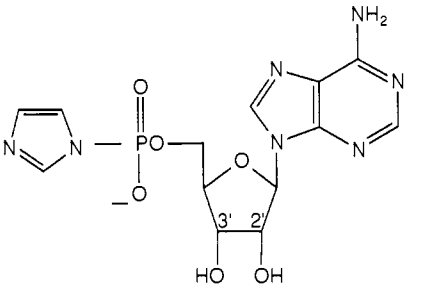
Abstract: Oligomers of adenylic acid of up to the 11-mer in length are formed by the reaction of the phosphorimidazolide of adenosine (ImpA) in pH 8 aqueous solution at room temperature in the presence of Na^+–montmorillonite. These oligomers are joined by phosphodiester bonds in which the 3′,5′-linkage predominates over the 2′,5′-linkage by a 2:1 ratio. Reaction of a 9:1 mixture of ImpA, A^5′ppA results in the formation of oligomers with a 3:1 ratio of 3′,5′- to 2′,5′-linked phosphodiester bonds. A high proportion of these oligomers contain the A^5′ppA grouping. A^5′ppA reacts much more rapidly with ImpA than does 5′-ADP (ppA) or 5′-ATP (pppA). The exchangeable cation associated with the montmorillonite effects the observed catalysis with Li^+, Na^+, NH_4^+, and Ca^{2+} being the more effective while Mg^{2+} and Al^{3+} are almost ineffective catalysts. 2′,5′-Linked oligomers, up to the tetramer in length, are formed using UO_2^{2+}–montmorillonite. The structure analysis of individual oligomer fractions was performed by selective enzymatic and KOH hydrolytic studies followed by HPLC analysis of the reaction products. It is concluded from the composition of the oligomers that the rate of addition ImpA to a 3′-terminus containing a 2′,5′-linkage is slower than the addition to a nucleoside joined by a 3′,5′-linked phosphodiester bond. The potential importance of mineral catalysis of the formation of RNA and other oligomers on primitive Earth is discussed.

Introduction

The regiospecific synthesis of oligonucleotides from unblocked monomers in aqueous solution has not been reported. Current synthetic methodology uses a stepwise solid-phase approach, blocked monomeric units, and anhydrous conditions for the formation of the phosphodiester bond.[1] In our search for the possible routes by which mononucleotides were condensed to oligonucleotides on primitive Earth, we concluded that the most likely starting materials were unblocked activated monomers and the most likely solvent was water, reagents and conditions which differ markedly from current synthetic methodology. The failure to detect the spontaneous condensation of activated monomers in solution[2] led to the conclusion that catalysts were essential for oligomer formation. The rate of oligomer formation must have been faster than the rate of hydrolysis of the activated monomers, another requirement which suggests catalysis was essential. Catalysts were also necessary to control the regiospecificity of the reaction in the absence of blocking groups.[3] If RNA or RNA analogs were the most important polymers in the first life on Earth,[4] then it is unlikely that other polymers such as polypeptides catalyzed their formation. It is proposed that minerals were the first catalysts,[3] and we report the oligomerization of aqueous solutions of the phosphorimidazolide of adenosine (ImpA) (1) on montmorillonite, a reaction system consistent with the above scenario for prebiotic formation of RNA oligomers.



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Experimental Section

General Details. The chemicals were obtained from the following sources: Imidazole from Baker was recrystallized from benzene, 1-methylimidazole from Baker was vacuum-distilled, A^5′ppA, Ap_3A, Ap_4A, $Ap^{2'}A$, $Ap^{3'}A$, $Ap^{2'}A^{2'}A$, 5′-AMP, ATP, ADP, alkaline phosphatase (APH), and ribonuclease T_2 ($RNase\ T_2$) were obtained from Sigma, PIPES buffer was obtained from Aldrich, montmorillonite 22A was from Wards Natural Science Establishment,[5] and Volclay SPV-200 was a gift from the American Colloid Co., Arlington Heights, IL. The homoionic Volclay was prepared by the titration method.[6] This material contains quartz and other particulates which form a discernible bottom layer in the final centrifugation step. Careful removal of the top layer yields 2.5–3.0 g of Na^+–Volclay from an initial 6 g. The homoionic montmorillonite 22A was prepared by the saturation method.[7] ImpA was synthesized using a modification of the procedure of Joyce et al.,[8,9] and pA^5′ppA and pA^5′ppAp were synthesized as described previously.[10]

HPLC analyses were performed on a Waters μBondapak C-18 column using a gradient of 0.005 M NaH_2PO_4 in 5% CH_3OH at pH 3.5 mixed with 0.01 M NaH_2PO_4 in 40% CH_3OH at pH 4.0 and on a HEMA-IEC BIO Q anion-exchange column from Alltech using a gradient of 0–0.4 M $NaClO_4$ at pH 8 with 2 mM Tris buffer. No Tris was used when samples were collected for further analysis.

Reaction of ImpA or Mixtures of A^5′ppA, 5′-ADP, or 5′-ATP with ImpA on Montmorillonite. A solution of ImpA alone or a mixture with A^5′ppA or the di- or triphosphate of adenosine with a combined concentration of 14.5 mM was prepared in 0.2 M NaCl and 0.075 M

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$MgCl_2$, unless noted otherwise, and the pH was adjusted to 8. To 1 mL of this solution was added 50 mg of homoionic montmorillonite, and the pH was again adjusted to 8. The suspension was vortexed and allowed to stand at room temperature for 3–7 days. Controls which did not contain montmorillonite were reacted under the same conditions. The pH at the end of the reaction was 8 ± 0.2. The reaction mixtures with montmorillonite were centrifuged, and the supernatant was removed with a pipet and filtered through a 0.45 μm pore filter. To the montmorillonite was added 1.0 mL of 0.1 M ammonium acetate, and the mixture was vortexed and allowed to stand for 24 h at room temperature. The mixture was then centrifuged, and the supernatant was removed, filtered, and combined with the first supernatant for HPLC analysis using the anion-exchange column.

HPLC and Enzymatic Analysis of Oligomers. The trimer, tetramer, and pentamer peaks were collected from the anion-exchange HPLC column and stored in LN_2. The homogeneity of the collected fraction was determined by reinjection of an aliquot of the collected fraction. A 0.3-mL aliquot of the fraction was treated with 0.1 unit of APH at 37 °C for 16 h. The pH increased from 5 to 7.5 during the course of the hydrolysis. Alternatively, the pH was adjusted to 8 initially. The ratio of the percentage of the peak(s) with the same retention time as starting material to that of the percentage before hydrolysis was used to calculate the percentage of $A^{5'}$ppA-containing oligomers in the fraction.

RNase T_2 hydrolyses were performed on 0.3 mL for 2 h at 37 °C and pH 4.5 with 0.3 unit of enzyme. Subsequent hydrolysis of the terminal phosphate groups of the RNase T_2 hydrolysis products was performed by the addition of 0.18 unit of enzyme and incubation for 8 h at 37 °C. The hydrolysis products were analyzed by anion-exchange HPLC, and the products were identified by coinjection with authentic samples. Control experiments showed that $pA^{5'}$ppAp and $pA^{5'}$ppA undergo slow hydrolysis with RNase T_2, so the ratio of these products was determined by hydrolysis of the collected fractions with 1 M KOH for 50 h at room temperature.[11] Because KOH cleaves 2′,5′-linked phosphodiester bonds, this method ensures that all the incorporated $A^{5'}$ppA is converted to its mono- or diphosphorylated derivatives. Since pAp and $pA^{5'}$ppA have the same retention times, their amounts were determined from the ratio of pA-containing oligomers to those containing $A^{5'}$ppA after APH hydrolysis and the yield of $pA^{5'}$ppAp and the combined yield of pAp and $pA^{5'}$ppA after KOH hydrolysis.

Results and Discussion

The formation of oligomers containing up to 11 monomer units was demonstrated by anion-exchange HPLC analysis[12] of the products of the reaction of aqueous pH 8 solutions of ImpA and ImpA, $A^{5'}$ppA mixtures in the presence of some montmorillonite clays (Figures 1 and 2). Oligomers of chain length greater than the trimer were not detected in previous studies because they did not elute from a C-18 reverse-phase column.[9,10] The separations on anion-exchange HPLC columns are based principally on the number of negative charges on each molecule, so the oligomer chain lengths are determined by simply counting the peaks starting from the monomer.[11,12] Diadenosine pyrophosphate ($A^{5'}$ppA), which is incorporated into some of the oligomers, has two negative charges and thus has the same charge as a terminal 5′-AMP (pA). The retention times of oligomers which contain $A^{5'}$ppA groupings are essentially the same as those with of oligomers a 5′-terminal phosphate group. Consequently, $A^{5'}$ppA is considered to be a monomer unit because it behaves like a terminal monomer unit on HPLC columns even though it contains two nucleotide groupings.

Oligomer Chain Lengths. The reaction of 0.015 M ImpA in pH 8 aqueous solution in the presence of the Wyoming montmorillonite Na^+–Volclay (Na^+–Vol) yielded oligoadenyaltes up to the undecamer in length (Table I, Figure 1). Most condensation reactions of 5′-AMP yield $A^{5'}$ppA as a product,[14] so the absence of $A^{5'}$ppA suggested that it was incorporated into the oligoadenylate products. Reaction of a mixture of 9:1 ImpA, $A^{5'}$ppA (Figure 2) resulted in higher yields of trimers than a reaction with ImpA alone[10] but somewhat lower yields of higher molecular weight oligomers (Table I). The yields of dimers and trimers increase and those of higher oligomers decrease as the ImpA:$A^{5'}$ppA ratio is changed from 9:1 to 4:1 to 1:1 (Table I). $A^{5'}$ppA$^{3'}$pA is the predominant product when a 1:1 ratio is used while AppA$^{3'}$pA$^{3'}$-pA predominants with a 9:1 or a 4:1 ImpA:$A^{5'}$ppA ratio.

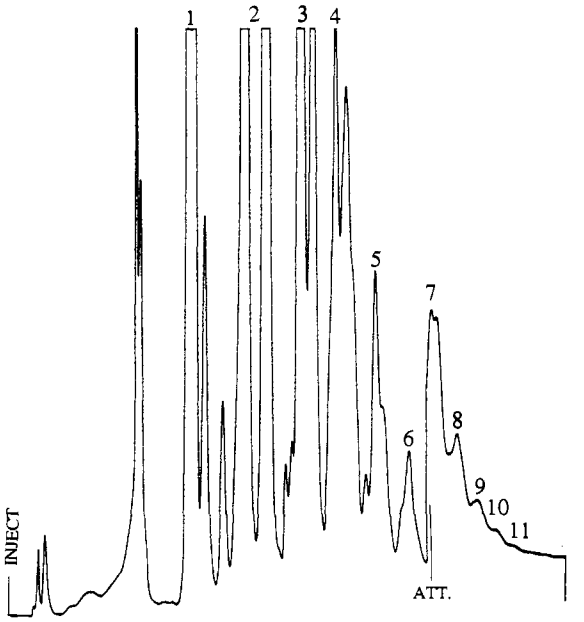

Figure 1. Anion-exchange HPLC of the reaction of ImpA on Na^+–Vol.

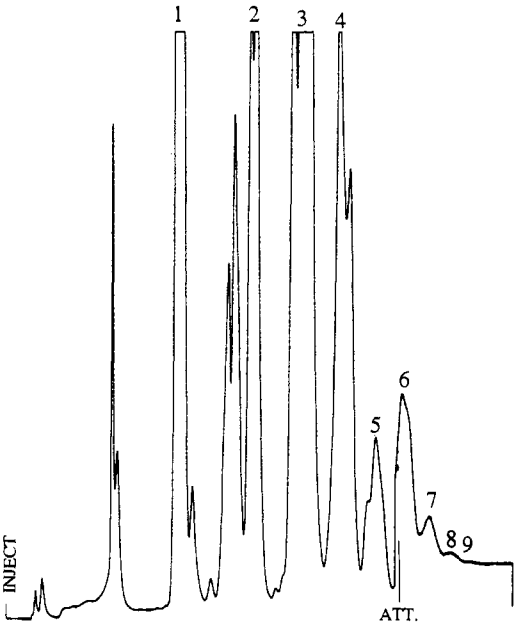

Figure 2. Anion-exchange HPLC of the reaction of 9:1 ImpA, AppA on Na^+–Vol.

The extent of ImpA condensation with polyphosphate compounds was explored using adenosine 5′-diphosphate (ppA) and adenosine 5′-triphosphate (pppA). Reaction of 9:1 mixtures of ImpA, ppA and ImpA, pppA on Na^+–Vol also resulted in oligomer formation, but as will be discussed below, the extent of ImpA condensation with these polyphosphate derivatives is much less than with $A^{5'}$ppA. Low yields of dimers and trimers and no longer oligomers are formed when the ImpA condensation reactions are performed in the absence of montmorillonite.

The chain length of the oligomer formed is dependent on the ionic strength of the aqueous medium. Reactions were routinely

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Table I. ImpA and 9:1 ImpA, Adenosine Polyphosphate Reactions[a]

	% oligomer										
reactants	1	2	3	4	5	6	7	8	9	10	11
ImpA	33	24	18	11	4.5	2.3	1.0	0.6	0.3	0.1	0.03
9:1 ImpA, A$^{5'}$ppA	40	19	24	10	3.2	0.9	0.3	0.1	0.03		
4:1 ImpA, A$^{5'}$ppA	32	25	31	8.3	1.7	0.3	0.06				
1:1 Imp, A$^{5'}$ppA	45	44	10	0.7	0.05	0.03					
9:1 ImpA, p_2A	39	28	17	8.9	3.5	1.1	0.3				
9:1 ImpA, p_3A	33	26	21	9.2	2.8	1.3	0.3	0.07			
9:1 ImpA, A$^{5'}$ppA[b]	17	31	31	14	4.8	1.4	0.4	0.1	0.04		
9:1 ImpA, A$^{5'}$ppA[c]	21	31	45	1.1	0.06	0.04	0.01				

[a] Reaction in the presence of Na^+–Vol in pH 8 aqueous solution containing 0.2 M NaCl and 0.075 M $MgCl_2$ except where noted. Anion–exchange HPLC analysis. Percentages listed are the uncorrected HPLC absorbance readings because the composition of each fraction has not been determined for higher molecular weight oligomers. [b] Reaction in 0.2 M PIPES at pH 8. [c] Reaction in water at pH 8.

Table II. Effect of the Exchangeable Cation of Montmorillonite 22A on Oligomer Formation

		% oligomer								
exchangeable cation	reactants	1	2	3	4	5	6	7	8	9
Na^+	ImpA	59	23	9.6	3.5	0.9	0.2	0.05	0.01	
Li^+	ImpA	53	23	13	6.0	1.9	0.7	0.2	0.05	0.01
NH_4^+	ImpA	61	19	12	4.3	1.1	0.3	0.07	0.02	0.004
Ca^{2+}	ImpA	58	23	12	3.8	1.1	0.4	0.1	0.002	
Mg^{2+}	ImpA	88	8.5	1.0	0.09					
Na^+	9:1 ImpA, A$^{5'}$ppA	54	19	16	6.1	1.7	0.4	0.1	0.002	0.002
Li^+	9:1 ImpA, A$^{5'}$ppA	46	19	20	8.8	2.8	0.8	0.3	0.09	0.03
NH_4	9:1 ImpA, A$^{5'}$ppA	42	21	23	9.2	2.7	0.7	0.2	0.03	
Ca^{2+}	9:1 ImpA, A$^{5'}$ppA	52	21	17	6.0	1.7	0.4	0.1	0.02	
Mg^{2+}	9:1 ImpA, A$^{5'}$ppA	85	12	1.7	0.2					
UO_2^{2+}	9:1 ImpA, A$^{5'}$ppA	67[a]	1.7	0.7						

[a] A 10% yield of a product with the retention time of adenosine was also observed.

performed in the presence of 0.2 M NaCl and 0.075 M $MgCl_2$.[14] The reaction of ImpA on montmorillonite in water alone yields mainly 5′-AMP (pA) and some dimeric products (Table I). High yields of dimeric and trimeric products are obtained in water using 9:1 ImpA, A$^{5'}$ppA but much lower yields of tetramers and high molecular weight oligomers are formed. This finding demonstrates that salts are not required for the direct addition of ImpA to A$^{5'}$ppA to form dimers and trimers but they are essential for the addition of ImpA to the initially formed adducts of ImpA and A$^{5'}$ppA. There is no reaction of ImpA with A$^{5'}$ppA in water in the absence of montmorillonite.[10] Reaction of ImpA in the presence of the Na^+ form of 0.2 M PIPES buffer gave results comparable to those obtained using a 0.2 M NaCl, 0.075 M $MgCl_2$ mixture. These findings demonstrate that Mg^{2+} is not essential for reaction to occur.

Previous studies demonstrated that the use of Li^+– or Ca^{2+}–montmorillonites as catalysts resulted in higher yields of dimers and trimers than were observed when Na^+–montmorillonite was used.[9] Reactions catalyzed by other homoionic montmorillonites were reinvestigated to determine if the exchangeable cation had an effect on oligomer chain length (Table II). Little effect on oligomer yield or chain length was observed in this study when Na^+ was substituted by Li^+, NH_4^+, or Ca^{2+}. Al^{3+}– and Mg^{2+}–montmorillonites do not catalyze oligomer formation.[9,10]

Investigation of UO_2^{2+}–montmorillonite as a catalyst was of special interest because the UO_2^{2+} ion alone is an effective catalyst for the formation of 2′,5′-linked oligoadenylates using ImpA as the starting material.[15] Only small yields of 2′,5′-linked dimers and trimers were detected using UO_2^{2+}–montmorillonite (Table II). An ImpA reaction catalyzed by the same amount of soluble UO_2^{2+} as was bound to the montmorillonite gave oligomers as long as the decamer. The inhibition of oligomer formation may be due to the inhibition of the formation of a UO_2^{2+}–ImpA complex[15] required for catalysis because of the UO_2^{2+} interaction with montmorillonite. An alternative explanation is that the formation of 2′,5′-linked oligomers does not proceed efficiently on montmorillonites (see below), and since UO_2^{2+} only catalyzes the formation of 2′,5′-linkages, little reaction is observed.

Oligomer Composition. Structural analyses of the reaction products were performed after separation and collection of oligomer fractions by anion-exchange HPLC. The compositions of the dimer and trimer fractions were established by using a C-18 reverse-phase HPLC column (Table III).[9,10] The compounds were identified by comparison with authentic standards and by their APH hydrolysis products. The percentages of 3′,5′-phosphodiester bonds in the dimer and trimer fractions are given in Table IV.

The presence of the cyclic trimer in the dimer fraction (Table III) was established by its resistance to APH hydrolysis, by its retention time, which differed from those of other products, and by coinjection with an authentic sample, provided by Dr. K. J. Prabahar,[16] on a reverse-phase column.

The presence of A$^{3'}$pA$^{3'}$pA and A$^{3'}$pA$^{2'}$pA in the APH hydrolysate of the trimer fraction was established by coinjection with authentic samples.[9,10] The absence of A$^{2'}$pA$^{2'}$pA in the hydrolysate was also established by use of an authentic sample. The oligomers containing the A$^{5'}$ppA group were identified by their resistance to hydrolysis by APH and by comparison with authentic samples.[10]

The tetramer and pentamer fractions formed by the reaction of ImpA alone or 9:1 ImpA, A$^{5'}$ppA mixtures are not eluted from a C-18 reverse-phase column, so their compositions were deduced by hydrolytic degradation and analysis of the hydrolysate by anion-exchange HPLC. The extent of A$^{5'}$ppA incorporation was established by APH hydrolysis. The 5′-phosphate group is cleaved from oligomers of general formula $(pA)_n$ to give $A(pA)_{n-1}$. These hydrolysis products are readily separated using anion-exchange HPLC from those which contain A$^{5'}$ppA and which are not cleaved by APH. The relative amounts of $A(pA)_{n-1}$ oligomers and A$^{5'}$p-$(pA)_n$ oligomers were readily established from their peak areas

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Table III. Compositions of Oligomer Fractions

product	reactants and product percentages: ImpA	9:1 ImpA, A$^{5'}$ppA	9:1 ImpA, ppA	9:1 ImpA, pppA
	A. "Dimers"			
pA$^{2'}$pA	33	20	13	*a*
pA$^{3'}$pA	52	43	27	*a*
A$^{5'}$ppA$^{3'}$pA	2.6	20	26 (all isomers)	*a*
cyclic(pA)$_3$	13	*b*	*a*	*a*
ppA	*b*	*b*	34	*a*
	B. "Trimers"			
pA$^{3'}$pA$^{2'}$pA	47	10	32	23
pA$^{3'}$pA$^{3'}$pA	28	10	18	9.3
A$^{5'}$ppA$^{3'}$pA$^{3'}$pA	15	63	17[c]	6.4[c]
A$^{5'}$ppA$^{3'}$pA$^{2'}$pA	4	9	*c*	*c*
(Ap)$_m$A$^{5'}$ppA(pA)$_n$ ($m+n=2$)	2	3	*c*	*c*
ppA$^{3'}$pA	*b*	*b*	13	2.9
ppA$^{2'}$pA	*b*	*b*	19	*b*
pppA	*b*	*b*	*b*	59
	C. "Tetramers"			
pA$^{3'}$pA$^{3'}$pA$^{3'}$pA	9	2	*a*	*a*
pA$^{3'}$pA$^{3'}$pA$^{2'}$pA	22	6	*a*	*a*
(pA)$_4$ isomers	37[d]	0	31	20
A$^{5'}$ppA(pA)$_3$	1	37	nd[e]	nd
(Ap)$_m$A$^{5'}$ppA(pA)$_n$ ($m+n=3$)	32	55	22[c]	22[c]
ppA$^{3'}$pA$^{3'}$pA	*b*	*b*	22	11
ppA$^{3'}$pA$^{2'}$pA	*b*	*b*	19	14
pppA$^{3'}$pA isomers	*b*	*b*	7.8[b]	24
	D. "Pentamers"			
(pA)$_5$ isomers	72	17	*a*	*a*
A$^{5'}$ppA(pA)$_4$	2	21	*a*	*a*
(Ap)$_m$A$^{5'}$ppA(pA)$_n$ ($m+n=4$)	26	62	*a*	*a*

[a] Not determined. [b] No product of this composition expected. [c] Addition products of A$^{5'}$ppA not resolved. Percentage of total product mixture given in one entry in table. [d] Percentage of isomers of unknown structure. [e] nd = not determined.

Table IV. Oligomer Structural Data

fraction	% 3′,5′-phosphodiester bonds formed	pA:A$^{5'}$ppA ratio of oligomers[a]	terminal A$^{5'}$ppA:internal A$^{5'}$ppA ratio[b]
	A. ImpA Reactions		
dimers	68	33	*c*
trimers	72	3.6	9.5
tetramers	nd[d]	2.1	0.03
pentamers	nd	2.5	0.07
	B. 9:1 ImpA, A$^{5'}$ppA Reactions		
dimers	80	3.2	*c*
trimers	89	0.3	24
tetramers	nd	0.09	0.7
pentamers	nd	0.2	0.25

[a] Determined from anion-exchange analysis after APH hydrolysis. [b] Determined from pA:A5′ppA oligomer ratio pA$^{5'}$ppA:pA$^{5'}$ppAp ratio after KOH hydrolysis. [c] Not expected in the dimer fraction. [d] nd = not determined.

after correction for A$^{5'}$ppA hyperchromicity[10] and for the additional adenosine grouping in the A^{5}p(pA)$_n$ oligomers (Table IV).

It was possible to obtain structural information on the oligomers obtained in the tetramer fractions of the ImpA and ImpA, A$^{5'}$ppA reactions by anion-exchange HPLC of the APH hydrolysate. Two oligomers of composition A$^{3'}$pA$^{3'}$pA$^{3'}$pA and A$^{3'}$pA$^{3'}$pA$^{2'}$pA were present in the tetramer fraction of the 9:1 ImpA, A$^{5'}$ppA reaction which were resolved on the anion-exchange HPLC column. The separation reflects binding to the organic matrix of the ion-exchange resin; the 3′,5′-linked oligomers bind more strongly to a C-18 reverse-phase column than do those with 2′,5′-links. The structure A$^{3'}$pA$^{3'}$pA$^{3'}$pA was assigned by comparison

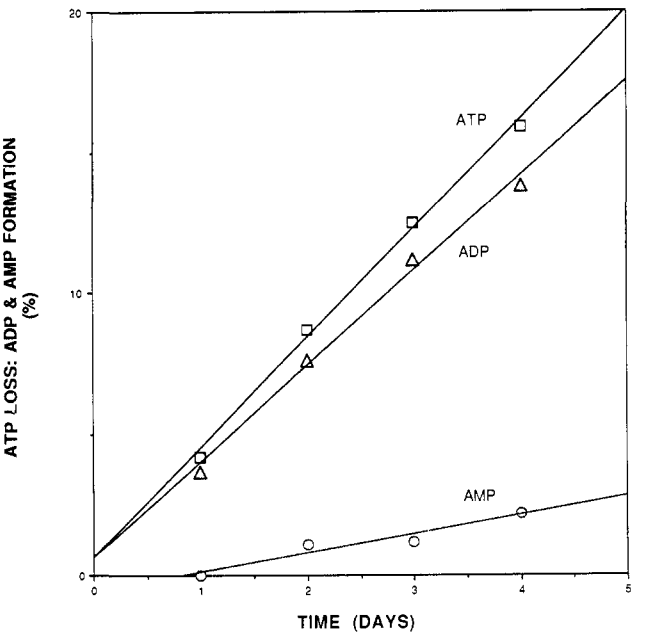

Figure 3. Hydrolysis of pppA to ppA and pA on Na$^+$–Vol.

with an authentic sample and that of A$^{3'}$pA$^{3'}$pA$^{2'}$pA to the second peak in that group with the shorter retention time (Table III). The failure to detect 2′,5′-linked products other than A$^{2'}$pA on RNase T_2 hydrolysis (see below) is in agreement with this structural assignment.

In contrast to the ImpA, A$^{5'}$ppA reaction, the APH hydrolysate of the tetramer fraction of the ImpA reaction indicated the presence of other isomers in addition to A$^{3'}$pA$^{3'}$pA$^{3'}$pA and A$^{3'}$pA$^{3'}$pA$^{2'}$pA (Table III). The presence of oligomers with internal 2′,5′-links was established by the detection of A$^{2'}$pAp as a product of RNase T_2 hydrolysis (see below). It was possible to determine the amounts of A$^{3'}$pA$^{3'}$pA$^{3'}$pA and A$^{3'}$pA$^{3'}$pA$^{2'}$pA present by anion-exchange HPLC, but the absence of authentic standards precluded identification of the other tetrameric oligomers. It was not possible to provide quantitative data on the amounts of the isomers in the tetramer fraction which contain A$^{5'}$ppA units because they were not separable by anion-exchange HPLC.

The compositions of the dimer to tetramer fractions of the 9:1 ImpA, A$^{5'}$ppA and 9:1 ImpA, pppA reactions were also determined by anion-exchange HPLC analysis following APH hydrolysis. APH cleaves 5′-terminal di- and triphosphate groupings (Table III).[17] The relative amounts of A$^{5'}$ppA, ppA, and pppA and the relative yields of ImpA adducts to these polyphosphate compounds made it possible to establish that their rates of reaction with ImpA decrease in the order A$^{5'}$ppA > ppA > pppA. For example, 0.8% A$^{5'}$ppA is recovered in the 9:1 ImpA, A$^{5'}$ppA reaction[10] while 34% of the reaction product mixture is ppA and 59% pppA in the reaction of these polyphosphates with ImpA (Table III).

The reaction of 9:1 ImpA, pppA is complicated by hydrolysis of pppA on montmorillonite. The reaction of pppA on Na$^+$–Vol in the absence of ImpA results in the formation of ppA (Figure 3). The rate of pppA hydrolysis is much slower in the absence of Na$^+$–Vol than in its presence (Figure 3). ppA undergoes a much slower hydrolysis than pppA on Na$^+$–Vol to form pA (Figure 3).

Control studies established that ImpA did not form adducts with ppA or pppA when Na$^+$–Vol was absent. Low yields of A$^{5'}$p$_3$A and A$^{5'}$p$_4$A were formed, as determined by their resistance

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Table V. Oligomer Hydrolysis Products[a]

	"trimers"		"tetramers"		"pentamers"	
nucleotide	RNase T_2	KOH	RNase T_2	KOH	RNase T_2	KOH
	A. ImpA Reactions					
A	25	56	22	43	13	46
$A^{2'}pA$	20	*b*	9.2	*b*	8.6	*b*
$A^{2'}pA^{2'}pA$	*b*	*b*	3.4	*b*	2.2	*b*
Ap	25	10	3.5	27	34	24
$A^{2'}pAp$	2.1	*b*	5.7	*b*	5.6	*b*
pAp, $A^{5'}ppAp$	27	20	14	12	11	5.8
$pA^{5'}ppA^{2'}pA$	0.5	*b*	1.1	*b*	0.7	*b*
$pA^{5'}ppAp$	trace	2.4	0.7	5.3	trace	4.3
	B. ImpA, $A^{5'}ppA$ Reactions					
A	42	65	44	69	17	67
$A^{2}pA$	9.0	*b*	18	*b*	9.1	*b*
Ap	19	9	21	14	38	18
$A^{2'}pAp$	2.9	*b*	2.6	*b*	7.3	*b*
pAp, $pA^{5'}ppA$	22	12	11	6	16	4.3
$pA^{5'}ppA^{2}pA$	1.0	*b*	0.9	*b*	0.9	*b*
$pA^{5'}ppAp$	1.6	3.4	1.2	4.5	3.0	6.4

[a] Molar concentrations except for the pAp, $pA^{5'}ppA$ mixtures which are the uncorrected percentages recorded by the HPLC. [b] Listed product not expected to be present and was not detected.

to APH hydrolysis and coinjection with authentic standards. $A^{5'}p_3A$ and $A^{5'}{}_4A$ react with ImpA in the presence of montmorillonite.[18]

Individual oligomer fractions from the ImpA and 9:1 ImpA, $A^{5'}ppA$ reaction were also hydrolyzed by RNase T_2, an endonuclease that cleaves 3',5'-phosphodiester bonds to the corresponding 5'-OH and 3'-phosphate[19] (Table V). The hydrolysis products were identified by comparison with authentic samples using anion-exchange HPLC. The observation of adenosine as a major hydrolytic product in both the ImpA and 9:1 ImpA, $A^{5'}ppA$ reactions is consistent with the presence of 3',5'-linkages at the 3'-ends of the oligomer chains. The high proportions of Ap and pAp, $pA^{5'}ppA$ are also consistent with extensive internal 3',5'-linkages. The principal 2',5'-linked degradation product is $A^{2'}pA$, which must be located at the 3'-terminus of the oligomers. Smaller amounts of $A^{2'}pA^{2'}pA$ were detected in the tetramer and pentamer fractions of the ImpA reaction but were not found in the 9:1 ImpA, $A^{5'}ppA$ reactions. This 2',5'-linked trimer must also be at the 3'-terminus of the oligomers.

An authentic sample of $A^{2'}pAp$ was not available for direct comparison with the RNase T_2 product assigned to that structure. Its structure is consistent with its retention time on anion-exchange HPLC columns and the loss of this peak on treatment of the RNase T_2 hydrolysate with APH. The observation of $A^{2'}pAp$ is consistent with the presence of some internal 2',5'-linkages in the oligonucleotide chains.

The ratios of the RNase T_2 degradation products provide insight into the structures of the oligomers. The ratio of the amount of adenosine to the combined amounts of $A^{2'}pA$ and $A^{2'}pA^{2'}pA$ is a measure of the ratio of 3',5'-:2',5'-phosphodiester links at the 3'-terminus of the chain. This ratio is in the 1.2–1.7 range in the ImpA reactions and is 4–1.9 in the 9:1 ImpA:$A^{5'}ppA$ reactions. The ratio of the amounts of Ap to $A^{2'}pAp$, indicative of the ratio 3',5'-:2',5'-internal links, ranges from 12 to 3.5 in the ImpA reactions and from ∞ to 5.3 in the 9:1 ImpA:$A^{5'}ppA$ reactions. These ratios show that there is a much lower probability for the 2',5'-phosphodiester bond to be at an internal position than at the 3'-terminus in the oligomer chains. These results support the conclusion that the elongation of the 2',5'-linked phosphodiester bond proceeds more slowly than does that of the 3',5'-phosphodiester bond because they document a preponderance of 2',5'-links at the 3'-terminus of the oligomers.

The oligomers containing pyrophosphate groups were apparent by the formation of $A^{5'}ppAp$ and $pA^{5'}ppAp$ upon treatment with RNase T_2 (Table V). The HPLC peaks assigned to these compounds disappeared and a peak due to $A^{5'}ppA$ appeared after treatment with APH (data not shown). Coinjection experiments showed that pAp and $A^{5'}ppAp$ have identical retention times on anion-exchange HPLC columns, so that it is not possible to differentiate between these compounds. HPLC retention time data suggested that $pA^{5'}ppA^{2'}pA$ was also formed in small amounts by RNase T_2 hydrolysis. The low yield of $pA^{5'}ppA^{2'}pA$ as compared to the sum of the $pA^{5'}ppA$ and $pA^{5'}ppAp$ yields is consistent with the observation that $A^{5'}ppA$ initiates the formation of 3',5'- in preference to 2',5'-phosphodiester bonds.[10]

Control experiments demonstrated that RNase T_2 catalyzes the slow hydrolysis of $A^{5'}ppAp$, $pA^{5'}ppAp$, and pAp.[10] Consequently, oligomer hydrolysis was also performed with KOH[20] to obtain a clearer idea of the proportions of $pA^{5'}ppAp$ and $A^{5'}ppAp$ formed since neither is cleaved by KOH. KOH also cleaves $A^{5'}$-ppA groupings attached to the rest of the oligomers with 2',5'-linkages, bonds that are not cleaved by RNase T_2. The yields of degradation products are less precise than those obtained with the use of RNase T_2 because they must be corrected for the response of the HPLC detector to the high concentrations of K^+ salts resulting from the use of 1 M KOH. The product ratios are accurate and the $pA^{5'}ppAp$:$pA^{5'}ppA$, pAp ratios were significantly higher when KOH was used in place of RNase T_2 (Table V).

The $A^{5'}ppAp$:$pA^{5'}ppAp$ ratio was determined from the KOH hydrolytic data (Table V) and the percent $A^{5'}ppA$ incorporated into each oligomer fraction as determined by APH hydrolysis (Table IV). This ratio is less than 1 for the tetramer and pentamer fractions, indicative of the addition of ImpA to both adenosine groupings of $A^{5m}ppA$ in the process of oligomer formation.

The absence of $pA^{2'}pA^{2'}pA$ in the trimer fraction and the small proportion of $A^{2'}pA^{2'}pA$ in the APH–RNase T_2 hydrolysis products of the tetramer and pentamer fractions indicate that elongation of an oligomer with a 2',5'-phosphodiester bond on the 3'-terminus is slower than that of one with a 3',5'-terminus. This conclusion is supported by RNase T_2 hydrolysis data in which the pA:$pA^{2'}pA$ molar ratio (Table V) is higher than the A:$A^{2'}pA$ molar ratio. The pA:$pA^{2'}pA$ ratio is proportional to the 3',5'- to 2',5'-ratio of endonucleotide bonds while the A:$A^{2'}pA$ ratio is the proportional to the 3',5'- to 2',5'-phosphodiester bond ratio at the terminus.

A qualitative study of the hexamers and heptamers indicated that they were also cleaved extensively with RNase T_2. Some of the same degradation products observed in the RNase T_2 hydrolysis of the tetramer and pentamer were detected. These limited data suggest that the hexamers and heptamers have structural units and a proportion of 3',5'-links that are very similar to those of the shorter oligomers.

Conclusions

Some montmorillonites catalyze the formation of oligoadenylates from ImpA in pH 8 aqueous solution. Na^+–Volclay enhances the rate of dimer formation 1000-fold when compared with the rate of dimer formation it its absence.[21] Montmorillonites with alkali metals or NH_4^+ or Ca^{2+} as exchangeable cations are the most effective catalysts for oligomer formation. Montmorillonites with other exchangeable cations are ineffective.[22] Sodium and calcium are prevalent in the seawater and are the principal cations associated with contemporary montmorillonites, so they are likely to have been the principal cations associated with montmorillonite on primitive Earth.[23] The surface acidity

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of the montmorillonite is an important aspect of the observed catalysis, since it results in both the binding[24] and activation of ImpA.[9]

The oligomers formed by the self-condensation of ImpA contain a 2:1 ratio of 3′,5′- to 2′,5′-linked phosphodiester bonds. The regiospecificity of 3′,5′-phosphodiester bond formation increases to 4:1 when $A^{5'}$ppA is added to the reaction mixture. $A^{5'}$ppA is more effective than ppA or pppA in initiating oligomer synthesis and the formation of 3′,5′-phosphodiester bonds. The addition of ImpA to $A^{5'}$ppA is faster than the self-condensation of ImpA, as judged from the ratio of $A^{5'}$ppA-containing oligomers to those composed only of pA groups. For example, in the reaction of 9:1 ImpA, $A^{5'}$ppA, the extent of incorporation of $A^{5'}$ppA is greater than the initial 9:1 reactant ratio. In the case of trimer, tetramer, and pentamer oligomers, the ratio of those containing $A^{5'}$ppA to those which do not is in the 3.3–11:1 range (Table VI).

Oligomers with 3′,5′-linkages on the 3′-terminus of the oligonucleotide chain elongate more rapidly than those with 2′,5′-linkages. This results in a relatively higher proportion of oligomers with 3′-terminal 2′,5′-links than those with internal 2′,5′-linkages. This finding predicts that the longer oligomers will have a higher overall percentage of 3′,5′-phosphodiester bonds because these elongate more efficiently. This prediction is consistent with the extensive cleavage of the hexamers and heptamers by RNase T_2.

It may not be possible to establish if RNA formed from simple monomers produced in prebiotic processes on primitive Earth or if RNA formation was preceded by the formation of simpler precursor polymers.[25] The present research suggests that mineral catalysis may have been important in the formation of RNA or pre-RNA oligomers which contained the phosphodiester bond and that catalysis may have had an important role in circumventing other problems currently associated with the prebiotic synthesis of RNA from simple monomers.[3]

Finally, it should be noted that research on the oligomerization of unblocked nucleotides to oligomers is of potential synthetic utility. The development of methodology of the regiospecific polymerization of mononucleotides to oligomers in aqueous solution is experimentally much simpler and much less hazardous environmentally than current synthetic methodology.[25]

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