Template-Directed Synthesis Using the Heterogeneous Templates Produced by Montmorillonite Catalysis. A Possible Bridge Between the Prebiotic and RNA Worlds

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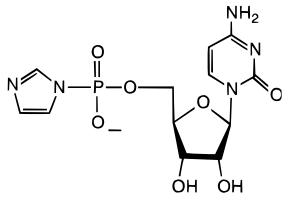
Abstract: The synthesis of oligoguanylates [oligo(G)s] is catalyzed by a template of oligocytidylates [oligo(C)s] containing 2′,5′- and 3′,5′-linked phosphodiester bonds with and without incorporated C$^{5'}$ppC groupings. An oligo(C) template containing exclusively 2′,5′-phosphodiester bonds also serves as a template for the synthesis of complementary oligo(G)s. The oligo(C) template was prepared by the condensation of the 5′-phosphorimidazolide of cytidine on montmorillonite clay. These studies establish that RNA oligomers prepared by mineral catalysis, or other routes on the primitive earth, did not have to be exclusively 3′,5′-linked to catalyze template-directed synthesis, since oligo(C)s containing a variety of linkage isomers serve as templates for the formation of complementary oligo(G)s. These findings support the postulate that origin of the RNA world was initiated by the RNA oligomers produced by polymerization of activated monomers formed by prebiotic processes.

Introduction

The RNA world was proposed as the first stage in the origin of life where catalysis and information storage was performed by RNA.[1] In this postulate, the requisite RNA oligomers were initially formed by the polymerization of mononucleotides present in the prebiotic world. These oligomers may have been replicated by template-directed synthesis;[2−4] a process that was eventually catalyzed by ribozymes.[5]

The need for catalysis in the prebiotic synthesis of biopolymers on the primitive Earth has been discussed.[6,7] Previous research has demonstrated that montmorillonite, a clay mineral with a layered structure, catalyzes the formation of RNA oligomers from activated monomers in pH 8 aqueous solution at room temperature.[8] The effect of the nature of the phosphate activating group on the regioselectivity and the chain length of oligoadenylates, and the kinetics of the oligoadenylate formation were also reported.[9] The montmorillonite-catalyzed self-condensation of activated mononucleotides was extended to 5′-phosphorimidazolide of uridine (ImpU), and the mechanism of the reaction was investigated.[10]

In this study, we investigated the formation of oligocytidylates, oligo(C)s, from 5′-phosphorimidazolide of cytidine (ImpC) and nonenzymatic template-directed synthesis of oligo(G)s on these oligo(C)s. Some of these findings were reported in a preliminary paper.[11] Oligo(C)s are also formed by the UO_2^{2+}-catalyzed oligomerization of ImpC.[12]



ImpC

Experimental Section

Volclay (structure of which is shown in ref 13) was converted to Na^+ montmorillonite $[Si_{3.89}Al_{0.11}]^{IV}[Al_{1.57}Fe^{3+}{}_{0.17}Fe^{2+}{}_{0.02}Mg_{0.27}]^{VI}O_{10}(OH)_2$ by the titration method.[14] The following were prepared by literature procedures or modifications thereof: 5′-phosphorimidazolide of cytidine (ImpC),[15,16] guanosine 5′-phospho-2-methylimidazole (2-MeImpG),[15] *P*^{1},*P*^{2}-dicytidine-5′,5′-pyrophosphate (C$^{5'}$ppC),[17] pC$^{5'}$ppC,[18] pC$^{5'}$ppCp,[18] a mixture of 3′,5′- and 2′,5′-cytidine diphosphate (pCp).[18] The reaction of ImpC on Na^+ montmorillonite was carried out as described for ImpA[8] (Figure 1). HPLC analyses were performed on reverse phase and anion-exchange HPLC columns.[8] The binding of ImpC to Na^+ montmorillonite was performed as described for ImpA.[19]

Characterization of the Reaction Products of ImpC on Na^+ Montmorillonite, General Procedure. The reaction mixture (65−75 μL) was injected on anion-exchange column, and monomer, dimer, and trimer fractions were collected into 2 mL RNase-free polyethylene

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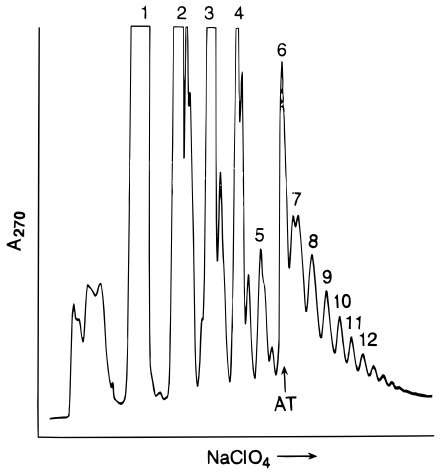

Figure 1. The anion-exchange HPLC separation of the oligomers formed from the reaction of 1 mL of 15 mM ImpC on 50 mg of Na^+-Vol montmorillonite in 0.2 M NaCl and 0.075 M $MgCl_2$, pH 8, at room temperature for 5 days. AT = change in attenuation.

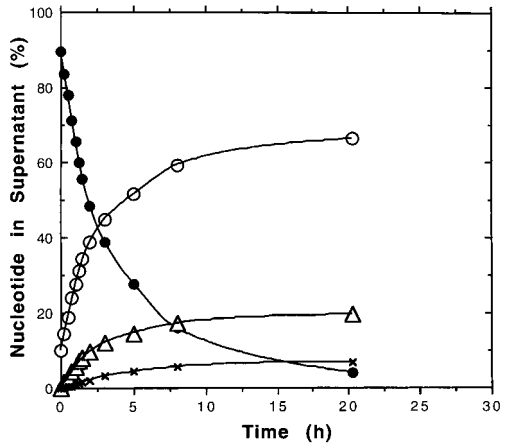

Figure 2. The reaction course of the montmorillonite-catalyzed self-condensation of ImpC. The nucleotide amounts in supernatant were taken directly from the area percentages of the products read on anion-exchange HPLC: ●, ImpC; ○, monomers; △, dimers; ×, trimers.

centrifuge tubes. Reinjections of the collected fractions indicated that they were 95−99% pure. The collected fractions were then dialyzed using Spectra/Por 6 MWCO 1000 tubing for 15 h at 2 °C, freeze-dried, dissolved in 400−1600 μL of 2 mM Tris solution at pH 8, and digested with alkaline phosphatase (APH). Amount of enzyme used was 0.1−0.7 unit depending upon the oligomer concentration and the volume of the solution to be hydrolyzed. Hydrolysis was carried out for 2 h at 37 °C. The APH-hydrolyzed products were then separated from the unhydrolyzed oligomers by anion-exchange HPLC, dialyzed, and freeze-dried, except in the monomer case where the unhydrolyzed portion was used as such for further analysis.

The APH used to cleave the terminal phosphate groups was prepared by dissolving 33 units of enzyme in 1.0 mL of 0.1 M TRIS buffer, pH 8, to give a final enzyme concentration of 0.033 unit/μL.

The RNase T_2 was prepared by dissolving 100 units of enzyme in 1.0 mL of 0.015 M of ammonium acetate solution at pH 4−5 to give an enzyme concentration of 0.1 unit/μL. The pH of the APH-hydrolyzed solutions (700−1500 μL) was adjusted to 4−5 by the addition of a few microliters of diluted acetic acid before RNase T_2 hydrolysis. The amount of RNase T_2 used was 0.7−2.5 units depending upon the oligomer concentration and the volume of the solution to be hydrolyzed. Hydrolysis was carried out for 2 h at 37 °C. More RNase T_2 (16−20 units) was required for the hydrolysis of cyclic dimer isomers than for linear dimers. Hydrolysis of authentic oligocytidylates with RNase T_2 demonstrated that use of excess of enzyme does not result in the over hydrolysis of the oligo(C) cleavage products. This result differed from the hydrolysis of oligoadenylates, where excess enzyme resulted in the slow hydrolysis of pA$^{5'}$ppAp and pA$^{5'}$ppA.[8]

Preparation of Oligo(C) Templates. ImpC was dissolved in 0.2 M NaCl, 0.075 M $MgCl_2$, and 0.1 M HEPES solution at pH 8.0 to give a final concentration of 0.014 mmol/mL. Each 4.0 mL of this solution was added to 6.5 mL round-bottomed polyethylene centrifuge tubes (Nalgene) containing 200 mg of Na^+ montmorillonite. Eleven such reactions were set up. Reactions were run for 5 days at 25 °C and centrifuged, and the supernatants were removed, combined, and kept at −20 °C for 3 h until used. To each clay precipitate was added 2 mL of 0.1 M NH_4OH. The clay was suspended by vortexing, and the mixture was kept for 1.5−2 h at 2 °C and centrifuged. The reaction supernatants and washes were combined for size exclusion chromatography using a Sephadex column.

Isolation of a Heterogeneous Template Containing Hexamers and Higher Oligomers. The combined reaction supernatants and washes were applied to a column, 3.8 cm diameter, 61 cm length, containing DNA grade Sephadex G-25, prepared by Dr. Kamaluddin following the procedure of Hohn and Schaller.[20] Elution was carried out by using 0.02 M NH_4HCO_3 solution. The collected fractions were analyzed by anion-exchange chromatography. Those fractions containing only hexamer and higher oligomers were combined and freeze-dried. Fractions containing shorter oligomers along with hexamer and higher oligomers were freeze-dried separately and dissolved in 1.4 mL of water. This solution (30−75 μL) was injected on analytical anion-exchange column, and hexamer and higher oligomers were collected. The collected fractions were combined and dialyzed using MWCO 1000 tubing for 15 h at 2 °C. This dialyzed oligomer solution was used to dissolve the freeze-dried oligocytidylates separated on the Sephadex column, and the final solution contained only hexamers and higher oligomers. The resulting mixture was concentrated to 0.325 mL at 12−15 °C using a rotary evaporator. The UV absorbance of this solution was measured at 270 nm to calculate the oligomer concentration, and the solution was evaporated to dryness for further use as template.

Preparation of Templates Containing Only 2′,5′-Linked Phosphodiester Bonds. The pentamer and higher oligomers were isolated from the ImpC reaction by a procedure similar to that described for the heterogeneous template. The 3′,5′-linkages in these oligomers were hydrolyzed by treatment with 100−300 units of RNase T_2. The amount of RNase T_2 required to hydrolyze the 3′,5′-linkages was determined by investigation of the amount of enzyme required to hydrolyze a comparable amount of all 3′,5′-linked $(Cp)_n$ oligomers formed by the NaOH cleavage of 1 mg of poly(C) for 35−40 min as described in the following paragraph. It was determined that 30−50 units of enzyme were required to cleave the 3′,5′-linked oligo(C)s, consequently a 2−6-fold excess of enzyme was used to cleave the 3′,5′-linkages in the heterogeneous oligomers. The 2′,5′-linked pentamers and higher oligomers were separated by the same procedure as that used to isolate the heterogeneous template.

Preparation of Templates Containing Only 3′,5′-Linked Phosphodiester Bonds. Polycytidylic acid (poly(C), 1 mg) was dissolved in 1.0 mL of 0.1 M NaOH solution and partially hydrolyzed by heating the mixture for 35−40 min at 70 °C. Fractions containing $(Cp)_{6-8}$ were collected from anion-exchange HPLC, dialyzed with Spectra/Por MWCO 1000 tubing for 15 h at 2 °C and freeze-dried.

Template-Directed Synthesis.[21] The heterogeneous template was dissolved in 10 μL of a solution containing 0.1 M 2-MeImpG in 1 M NaCl, 0.2 M $MgCl_2$, and 0.2 M HEPES, pH 8, to give a final template concentration of 0.025 M (monomer equivalent), and the mixture was allowed to stand for 6 days at 2 °C.[21] A control 2-MeImpG reaction was also performed where no template was used.

The 2′,5′-linked template was dissolved in 4 μL of 0.1 M 2-MeImpG solution in HEPES electrolyte (1 M NaCl, 0.2 M $MgCl_2$, and 0.2 M HEPES, pH 8) to give a final template concentration of 0.025 M (monomer equivalent)[21] and kept for 6 days at 2 °C. A control 2-MeImpG reaction was also set up in the absence of template.

The 3′,5′-linked template $(Cp)_{6-8}$ was dissolved in 40 μL of 0.1 M 2-MeImpG solution in 1 M NaCl, 0.2 M $MgCl_2$, and 0.2 M HEPES, pH 8, to give a final template concentration of 0.025 M (monomer equivalent)[21] and kept for 6 days at 2 °C.

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Table 1 . Percentages of APH-Hydrolyzed (Linear) and APH-Unhydrolyzed Oligomers

oligomer fraction	linear oligomers (%)	cyclic and C$^{5'}$ppC-containing oligomers (%)
monomers	67	33
dimers	85	15
trimers	78	22
tetramers	74	26
pentamers	74	26

Results and Discussion

Oligomers containing up to 14 monomer units are formed in the reaction of ImpC on Na^+ montmorillonite in a pH 8 aqueous solution containing Na^+ and Mg^{2+} (Figure 1). The oligomers formed from ImpC are longer than those produced from ImpA, ImpU, or ImpG. The half-life for ImpC loss is 2.5 h at 25 °C (Figure 2).

Although the longest oligomers are formed from ImpC, only 9% of the ImpC is bound to montmorillonite after 2 h. The binding of ImpA and ImpU is 53 and 9%, respectively, under the same conditions. The binding trend for the activated nucleotides parallels that for 5′-AMP, 5′-CMP, and 5′-UMP, which is 19, 15, and 11%, respectively, at pH 6.8.[19] These binding studies show that the length of oligomers formed depends on the nature of the binding at the catalytic sites and not on the total amount of binding to the Na^+ montmorillonite.

ImpC is formed by the reaction of imidazole with cytidine triphosphate, a possible route for the formation of phosphoramidates on the primitive earth.[16]

Characterization of the Reaction Products. Structure analysis of the oligo(C)s formed was performed on fractions isolated by anion-exchange HPLC. Oligomers with the same number of negative charges elute from the column as one peak or cluster of peaks with very close retention times. Since preliminary experiments indicated that a complex mixture of isomers was present in each fraction, the separated fractions were further fractionated by hydrolyzing them with APH and separating the hydrolyzed products from the unhydrolyzed ones by anion-exchange HPLC. APH cleaves the 5′-phosphate grouping in linear oligonucleotides but does not degrade cyclic oligomers or those oligomers containing the pyrophosphate (C$^{5'}$ppC) grouping (Table 1). Since removal of the terminal phosphate grouping reduces the number of negative charges on the linear oligomers by 2, it is possible to separate them from the cyclic- and pyrophosphate-containing oligomers using anion-exchange HPLC (Table 1). The structures of these linear oligomers, which constitute about 75% of each fraction, were determined after their separation from the unhydrolyzed oligomers in the fraction.

The structures of the oligomers not hydrolyzed with APH were also investigated using selective enzymatic cleavage reactions (Table 2). First, they were treated with RNase T_2 to cleave the 3′,5′-linkages, and the cleavage products were analyzed by anion-exchange HPLC. The number of negative charges on each hydrolysis product was determined from its HPLC retention time, and tentative structural identifications were made on the basis of the number of negative charges on each cleavage product (Tables 2 and 3). The RNase T_2 hydrolysis products were further digested by APH and analyzed by anion-exchange HPLC (in the case of dimer, also by reverse phase HPLC). After each hydrolysis step, the products formed (Table 2) were coinjected with authentic samples of C, C$^{2'}$pC, pCp, C$^{5'}$ppC, pC$^{5'}$ppC, and pC$^{5'}$ppCp to facilitate the identification of the cleavage products. The relative amounts of isomers present in a particular fraction were calculated from their peak areas on the chromatogram after correction for the number of groups in each isomer. However, the increased complexity of the fractions with increasing chain length, and the absence of 2′,5′-linked authentic standards, made it impossible to obtain

Table 2. Structures of the Linear Oligomers (which do not contain A$^{5'}$ppA groups) Present in the Dimer Fraction and Their RNase T_2 Hydrolysis Products

structures of fractions before RNase T_2 hydrolysis	structures of products after RNase T_2 hydrolysis	structures of products after RNase T_2 followed by APH hydrolysis
[pC$^{3'}$pC]$^{3-}$	[pCp]$^{4-}$ + C^0	2C^0
[pC$^{2'}$pC]$^{3-}$	[pC$^{2'}$pC]$^{3-}$	[C$^{2'}$pC]$^{1-}$
Structures of A$^{5'}$ppA-Containing and Cyclic Oligomers Present in the Dimer Fraction and Their RNase T_2 Hydrolysis Products[a]		
structures of fractions before RNase T_2 hydrolysis	structures of products after RNase T_2 hydrolysis	structures of products after RNase T_2 followed by APH hydrolysis
[C$^{5'}$ppCp$^{3'}$C]$^{3-}$	[C$^{5'}$ppC$^{3'}$p]$^{4-}$ + C^0	[C$^{5'}$ppC]$^{2-}$ + C^0
[C$^{5'}$ppC$^{2'}$pC]$^{3-}$	[C$^{5'}$ppC$^{2'}$pC]$^{3-}$	[C$^{5'}$ppC$^{2'}$pC]$^{3-}$
[c-pC$^{3'}$pC$^{3'}$pC$^{3'}$]$^{3-}$	3[Cp]$^{2-}$	3 C^0
[c-pC$^{2'}$pC$^{2'}$pC$^{2'}$]$^{3-}$	[c-pC$^{2'}$pC$^{2'}$pC$^{2'}$]$^{3-}$	[c-pC$^{2'}$pC$^{2'}$pC$^{2'}$]$^{3-}$
[c-pC$^{2'}$pC$^{2'}$pC$^{3'}$]$^{3-}$	[C$^{2'}$pC$^{2'}$pC$^{3'}$p]$^{4-}$	[C$^{2'}$pC$^{2'}$pC]$^{2-}$
[c-pC$^{2'}$pC$^{3'}$pC$^{3'}$]$^{3-}$	[C$^{2'}$pC$^{3'}$p]$^{3-}$ + [Cp]$^{2-}$	[C$^{2'}$pC]$^{1-}$ + C^0

[a] Products carrying the same number of negative charges appear as one peak or cluster of peaks on the HPLC chromatogram (c = cyclic oligomers).

Table 3. Structures of Oligocytidylates Formed by Montmorillonite Catalysis[a]

	composition (%)	3′,5′-links (%)
Monomers		
hydrolyzed by APH		
pC	100	
not hydrolyzed by APH		15
C$^{5'}$ppC	2.4	
pC$^{2'}$pC$^{2'}$	76	
pC$^{3'}$pC$^{2'}$	12	
pC$^{3'}$pC$^{3'}$	8.5	
Dimers		
hydrolyzed by APH		26
pC$^{2'}$pC	73	
pC$^{3'}$pC	26	
not hydrolyzed by APH		
C$^{5'}$ppC$^{3'}$pC		
C$^{5'}$ppC$^{2'}$pC		
pC$^{2'}$pC$^{2'}$pC$^{2'}$		
pC$^{2'}$pC$^{2'}$pC$^{3'}$		
pC$^{2'}$pC$^{3'}$pC$^{3'}$		
Trimers		
hydrolyzed by APH		44
pC$^{2'}$pC$^{2'}$pC	30	
pC$^{2'}$pC$^{3'}$pC	36	
pC$^{3'}$pC$^{2'}$pC	22	
pC$^{3'}$pC$^{3'}$pC	18	
not hydrolyzed by APH		
C$^{5'}$ppCpC$^{2'}$pC		
C$^{5'}$ppC$^{2'}$pC$^{2'}$pC		
pC$^{2'}$pC$^{2'}$pC$^{2'}$pC$^{2'}$		
pC$^{2'}$pC$^{2'}$pC$^{3'}$pC$^{3'}$		
pC$^{2'}$pC$^{3'}$pC$^{3'}$pC$^{3'}$		

[a] The absence of a quantitation indicates that it was not possible to obtain a percent yield and the product was identified only by the retention time of its enzymatic cleavage products

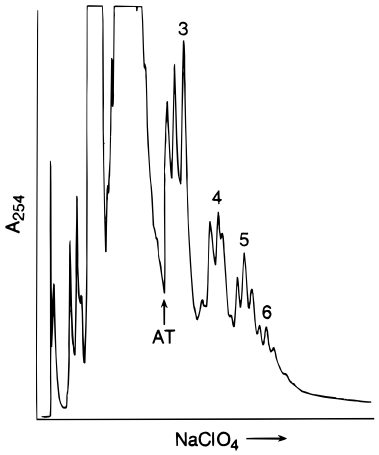

Figure 3. Anion-exchange HPLC profile of the oligoguanylates formed on the heterogeneous template. Template-directed synthesis was performed in 10 μl of solution containing 0.025 M oligo(C)s, 0.1 M 2-MeImpG, 1 M NaCl, 0.2 M $MgCl_2$, and 0.2 M HEPES, pH 8, for 6 days at 2 °C. AT = change in attenuation.

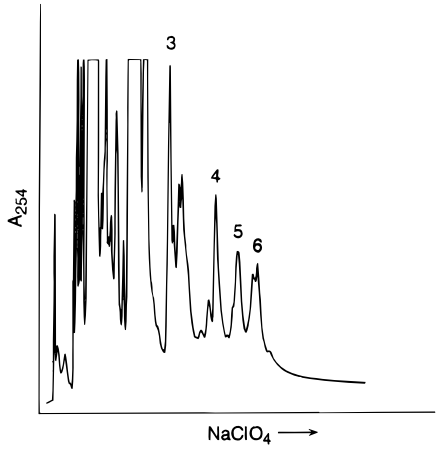

Figure 4. Anion-exchange HPLC profile of the oligoguanylates formed on the 3′,5′-$(Cp)_{6-8}$ template. Template-directed synthesis performed in 40 μL of solution containing 0.025 M template, 0.1 M 2-MeImpG, 1 M NaCl, 0.2 M $MgCl_2$, and 0.1 M HEPES, pH 8, for 6 days at 2 °C. This reaction was run as a control.

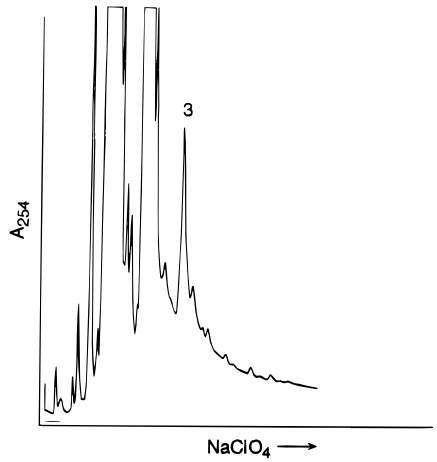

Figure 5. Anion-exchange HPLC profile of the oligoguanylates formed in the absence of any template. Reaction was performed in 20 μL of solution 0.1 M 2-MeImpG, 1 M NaCl, 0.2 M $MgCl_2$, and 0.1 M HEPES, pH 8, for 6 and 19 days at 2 °C in duplicate experiments. This reaction was run as a control.

quantitative data on the composition of the trimer and higher fractions (Table 3).

The oligomers formed from the reaction of ImpU and ImpC on montmorillonite are both linked mainly by 2′,5′-phosphodiester bonds. This result is in marked contrast with the oligomers formed from ImpA which are mainly 3′,5′-linked. These data suggest that pyrimidine nucleotides are bound in a different orientation at the catalytic sites on montmorillonite than the corresponding purine derivatives. Further studies are required to establish the generality of this conclusion.

Template-Directed Synthesis. The oligomers formed by montmorillonite catalysis would have had to have been capable of generation of new RNA oligomers if they initiated the processes leading to the RNA world. Orgel and co-workers[22,23] have demonstrated that the template-directed synthesis of oligo(G)s proceeds efficiently in the reaction of guanosine 5′-phospho-2-methylimidazole (2-MeImpG) on 3′,5′-linked poly(C).[22] In particular, they demonstrated that the template-directed reaction of 2-MeImpG on 3′,5′-$(pC)_n$ and 3′,5′-$C(Cp)_{n-1}$ oligomers ($n = 4-7$) yields almost exclusively the corresponding 3′,5′-linked oligo(G)s and 3′,5′-linked oligo(G)s with a 3′-terminal 2′,5′-linked nucleotide.[21]

In the present study, oligocytidylates formed by montmorillonite-catalyzed self-condensation of ImpC were used as templates for the synthesis of oligo(G)s to determine if the variety of bonding present in the templates inhibits the formation of the complementary G oligomers. The HPLC of the product mixture formed in the reaction of 2-MeImpG on the oligo(C)s containing heterogeneous template is given in Figure 3. Two additional reactions were performed as controls. In the first control reaction, which is a repeat of the work by Grzeskowiak and Orgel,[21] a mixture of 3′,5′-$(Cp)_{6-8}$ oligomers, isolated from the alkaline hydrolysis products of 5′-polycytidylic acid, was used as templates in place of the oligomers synthesized on Na^+ montmorillonite (Figure 4). In the second control reaction, the oligomerization of 2-MeImpG was carried out in the absence of template (Figure 5), and the HPLC elution profiles of the oligo(G)s formed in these three reactions were compared. The oligo(G)s formed by template-directed synthesis in the first control reaction, where the template was 3′,5′-$(Cp)_{6-8}$, gave mainly single peaks with HPLC retention times corresponding to 3′,5′-linked oligomers[21] formed by alkaline hydrolysis of poly(G) (Figure 4).[24] In the second control reaction (Figure 5), where no template was used, a single peak corresponding to the 2′,5′-linked trimer was the longest oligomer formed.[26] The anion-exchange HPLC of the oligomers formed on the template prepared by Na^+ montmorillonite catalysis had three peaks for each of the trimer to hexamer fractions (Figure 3). The presence of three peaks in each fraction suggests that oligo(G)s containing both 2′,5′- and 3′,5′-linked isomers were formed.[21] In fact, the shortest retention time within the trimer fraction formed on template is the same with the retention time of 2′,5′-linked trimer isomer formed in the control reaction run in the absence of template. While exclusively 3′,5′-linked oligo(C) template produces mainly single peaks corresponding to 3′,5′-linked oligo(G)s, the presence of a variety of isomers in each fraction of the products formed on the heterogeneous template suggests that 2′,5′-linked oligo(C)s did not inhibit the

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(23) For a review, see: Orgel, L. E. *Nature* **1992**, *358*, 203−209.

(24) Coinjection demonstrated that there is no difference in the retention times, using the HEMA IEC BIO Q anion-exchange column,[8] of the 2′- and 3′-phosphorylated trimers and higher oligomers obtained by the base hydrolysis of poly(G) and the 5′-phosphorylated trimers and higher oligomers obtained by the nuclease S1 hydrolysis[25] of poly(G).
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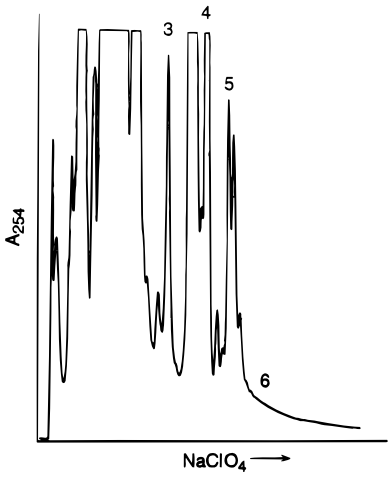

Figure 6. Anion-exchange HPLC profile of the oligoguanylates formed on exclusively 2′,5′-template. Template-directed synthesis performed in 4 μL of solution containing 0.025 M template, 0.1 M 2-MeImpG, 1 M NaCl, 0.2 M $MgCl_2$, and 0.1 M HEPES, pH 8, for 19 days at 2 °C.

template directed synthesis of oligo(G)s, but instead they served as templates for their synthesis.

The template-directed formation of oligoguanylates using 2′,5′-linked oligocytidylates which were obtained by selective hydrolysis of 3′,5′-linked oligocytidylates formed on montmorillonite was then investigated. The anion-exchange HPLC analysis of the reaction mixture formed on 2′,5′-template (Figure 6) exhibited an elution pattern that had some peaks in common with the HPLC of the product mixture obtained using the oligo(C) template before the 3′,5′-linkages were cleaved (Figure 3). Some of the oligo(G)s in Figures 3 and 6 have the same retention times as the corresponding 3′,5′-$(pG)_n$ oligomers prepared by the hydrolysis of poly(G) by nuclease S1.[25] As noted previously, only the 2′,5′-trimer is formed by the reaction of 2-MeImpG in the absence of a template.

Previous studies are consistent with the proposal that template-directed synthesis on 2′,5′-linked templates could yield the complementary 3′,5′-linked oligomers. The Pb^{2+}-catalyzed reaction of ImpG on 3′,5′-poly(C)s yields 2′,5′- and 3′,5′-linked oligo(G)s.[27,28] This finding indicates that 2′,5′-linked RNA oligomers form stable complexes with the complementary 3′,5′-linked oligomers in the presence of Pb^{2+}. In addition, studies of the stability of RNA double helices indicate that a hybrid complex between the complementary strands, where one is 2′,5′-linked and the other is 3′,5′-linked, is more stable than the corresponding double helix in which both strands are 2′,5′-linked.[29] Since complementary 3′,5′-linked RNAs form the most stable double helices,[29] it is conceivable that 3′,5′-linked RNAs would be formed from an initial 2′,5′-linked template after multiple cycles of template-directed syntheses.

The failure of the oligo(U)s formed by the interaction of A[5′]ppA with ImpU to catalyze the template-directed synthesis of oligo(A)s[10] is consistent with the observation that template synthesis in the A−U system is much less efficient than that in the G−C system.[30] Nonenzymatic template directed synthesis in the A−U system on the primitive Earth required catalysis beyond that provided by the template.

These studies establish that montmorillonite catalyzes the conversion of ImpC to oligo(C)s. The multiple types of bonding present in these oligo(C)s do not inhibit the template-directed synthesis of the corresponding oligo(G)s. In fact, the 2′,5′-linked oligomers in the synthetic oligo(C)s catalyze the formation of oligo(G)s. It can be concluded from these studies that oligo(C)s formed by montmorillonite catalysis, or by almost any other chemical process, could have initiated the formation of complementary oligomers on the primitive earth. This finding, together with the direct synthesis of oligonucleotides from mononucleotides by montmorillonite catalysis, provides support for a bridge between the prebiotic and RNA worlds.

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Supporting Information Available: Experimental description of the preparation of starting materials, reference compounds and some reaction procedures (7 pages). See any current masthead page for ordering and Internet access instructions.

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