MONTMORILLONITE CATALYSIS OF 30–50 MER OLIGONUCLEOTIDES: LABORATORY DEMONSTRATION OF POTENTIAL STEPS IN THE ORIGIN OF THE RNA WORLD

JAMES P. FERRIS
Contribution from the Department of Chemistry, Rensselaer Polytechnic Institute, Troy, New York, 12180–3590; phone 518–276–8493, fax 518–276–4887, e-mail: ferrij@rpi.edu

(Received 18 February 2002; accepted in revised form 27 March 2002)

Abstract. Elongation of the primer ^{32}pdA(pdA)$_8$pA proceeds by the reaction of the 5′-phosphorimidazolides of adenosine and uridine in the presence of montmorillonite clay. Daily addition of the activated nucleotides for up to 14 days results in the formation of 40–50 mers using the 5′-phosphorimidazolide of adenosine (ImpA) and 25–30 mers using the 5′-phosphorimidazolide of uridine (ImpU). The limitation on the lengths of the chains formed is not due to the inhibitors formed since the same chain lengths were formed using 2–3 times the amount of montmorillonite catalyst. The shorter oligomers formed by the addition of U monomers is not due to its greater rate of decomposition since it was found that both the A and the U adducts decompose at about the same rates. Alkaline phosphatase hydrolysis studies revealed that some of the oligomers are capped at the 5′-end to form, with ImpA, Ap^{32}pdA(pdA)$_8$pA(pA)$_n$. The extent of capping depends on the reaction time and the purine or pyrimidine base in the activated mononucleotide. Hydrolysis with ribonuclease T_2 followed by alkaline phosphatase determined the sites of the 3′, 5′- and 2′, 5′-phosphodiester bonding to the primer. The potential significance of the mineral catalyzed formation of 50 mer oligonucleotides to the origin of life based on RNA (the RNA world scenario) is discussed.

Keywords: mineral catalysis, montmorillonite, oligoadenylates, prebiotic synthesis, RNA, RNA elongation

1. Introduction

In the RNA world scenario RNA was the essential biopolymer in the first life on Earth because of its ability to store genetic information and catalyze reactions (Crick, 1968; Orgel, 1968; Cech *et al*., 1981; Guerrier-Takada, 1983; Gilbert, 1986). In vitro evolution studies have demonstrated the broad catalytic activity of RNA (Joyce *et al*., 1998) and the x-ray structure determination of the ribosome established the central role of RNA catalysis in the biosynthesis of the peptide bond of proteins (Ban *et al*., 2000; Muth *et al*., 2000). While others propose that the RNA in the first life evolved from simpler biopolymers (Orgel, 1998), I am investigating the direct formation of RNA from RNA monomers as part of a study of the role of catalysis in prebiotic synthesis (Ferris, 1993).

The synthesis of 6–14 mer oligonucleotides by the montmorillonite-catalyzed reaction of activated 5′-mononucleotides in pH 8 aqueous solution at 25 °C has

Origins of Life and Evolution of the Biosphere **32:** 311–332, 2002.

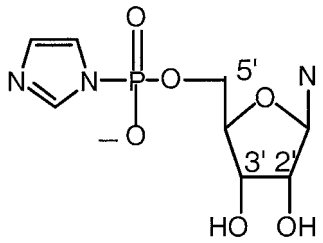

Figure 1. Activated monomers a. N = A, ImpA. b. N = U, ImpU.

been reported (Figure 1; Ferris, 1993). The reaction occurs with the 5′-activated phosphates of adenosine, guanosine, uridine, cytidine and inosine (Ferris and Ertem, 1993; Prabahar *et al.*, 1997; Ding *et al.*, 1996; Ertem and Ferris, 1997; Kawamura and Ferris, 1999).

Chain lengths of 6–14 mers are unlikely to have been sufficiently long for information storage or catalysis in the first life on Earth (Joyce and Orgel, 1999; Szostok and Ellington, 1993). The initial report on the preparation of longer oligonucleotides (40–50 mers) by the addition of adenylic acid units to a 10 mer primer was described (Ferris *et al.*, 1996). In the present report the elongation of the same primer with uridylic acid units is described. Results on the regioselectivity of phosphodiester bond formation and the extent of formation of 5′-caps, using regiospecific hydrolytic reactions, is presented for both RNAs.

2. Experimental Section

The montmorillonite Volclay, a gift from the American Colloid Company, was converted to a homoionic Na^+-montmorillonite by the titration (Banin, 1973; Banin *et al.*, 1985). Mononucleotides were purchased from Sigma and converted to the 5′-phosphorimidazolides (Joyce *et al.*, 1984). The 10 mer used as primer (dpA(pdA)$_8$-pA) was synthesized on an Applied Biosystems 391 DNA synthesizer or purchased from Operon. The 5′-terminal ^{32}P-labeling of the primers was performed using [^{32}P]ATP (Amersham) using T-4 polynucleotide kinase (New England Biolabs). Gel electrophoresis (Owl Scientific) using an EC-500 power supply (Apparatus Corporation) was performed on a 20% acrylamide-bisacrylamide denaturing gel on 16 × 28 cm. plates. Autoradiography was performed using Kodak X-OMAT film and the extent of ^{32}P-labeling was measured on a Packard Tricarb 2100TR counter. Centrifugation was performed on an Eppendorf 5415 centrifuge and solutions were concentrated to dryness using an Eppendorf Concentrator model 5301. Reagent grade chemicals were purchased.

2.1. GEL ELECTROPHORESIS

Approximately equal counts of radioactive material were loaded into the wells of the electrophoresis gel together with xylene cyanole and bromphenol blue and the gel was run for ∼2.5 hr at 1000 volts. The gel was then transferred to Saran Wrap and autoradiographed overnight at –20 °C and the film was then developed. The 10 mer, a ^{32}P-labeled primer, ^{32}pdA(pdA)$_8$pA was assigned a relative migration distance of 10. Addition of a nucleotide to the 3′-end of the primer, an increase of one nucleoside and one negative change, decreases the migration distance one unit from 10 to 11. The band moving more slowly on the gel was assigned the higher number since it has the higher molecular weight. The 5′-capped 10 mer, Ap^{32}pdA(pdA)$_8$pA, formed by the addition of one nucleoside with no change in the number of negative charges, migrated more slowly by about 1.5 units at 11.5 units. From these data it was determined that the addition of one nucleoside decreases the extent of migration by 1.5 units while the addition of one negative charge increases the extent of migration by 0.5 unit. The extent of elongation of the oligomers was estimated using the above data. In general, it was not possible to resolve the longer capped and not capped oligomers by gel electrophoresis so a band at about 15.5–16 may reflect the presence of both an uncapped 14 mer and a capped 12 mer. The areas of some of the bands were determined on a Power Macintosh G3 computer using public domain NIH Image program, version 1.61 (developed at the U.S. National Institutes of Health and available on the Internet at http://rsb.iinfo.nih.gov/nih-image/).

2.2. ENZYMATIC HYDROLYSES

Alkaline Phosphatase (APH). 5′-terminal phosphate groups were cleaved with the enzyme (0.1–0.001 units) in the standard buffer (10 mM tris, 1 mM $MgCl_2$, 1 mM $ZnCl_2$, pH 8.3) on incubation for 15 min at 37 °C, 30 min at 55 °C, followed by inactivation of the enzyme by addition of EDTA and heating for 10 min at 75 °C. Gel electrophoresis of the hydrolysis products revealed the capped oligomers.

Ribonuclease T_2 (RNase T_2), The oligomers were incubated with ribonuclease T_2 (RNase T_2) (Sigma) (0.1–0.01 units) in ammonium acetate (pH 4.3) for 2 hr at 37 °C and then 4–6 μL of 0.1 M ammonium hydroxide was added to bring the pH to 9 to inactivate the enzyme. RNase T_2 hydrolysis monitored by gel electrophoresis reveals the number of 2′, 5′-links to the primer before a 3′, 5′-link is formed. This analysis is complicated by the presence of both capped and not capped oligomers as well as the possibility for the capped oligomers to elongate from both ends of the primer. The subsequent APH hydrolysis reveals the capped hydrolysis products.

2.3. CONVERSION OF ^{32}pdA(pdA)$_8$pA TO Ap^{32}pdA(pdA)$_8$pA (CHU AND ORGEL, 1984)

To 10 μL of a solution of ^{32}pdA(pdA)$_8$pA (93,000 counts) in water was added 10 μL of 1 M $MgCl_2$, 20 μL of 0.5 M HEPES buffer (pH 7) and 10 μL of 1 M ImpA. The mixture was heated at 55 °C for 6 hr, desalted on a Nensorb (New England Nuclear) column and the oligomer fraction was concentrated to dryness in the Eppendorf spin concentrator. The product was shown by electrophoresis to consist of a mixture of approximately 25% unreacted ^{32}pdA(pdA)$_8$pA, 50% Ap^{32}pdA(pdA)$_8$pA and 25% Ap^{32}pdA(pdA)$_8$pApA. Treatment with APH removed the band assigned to ^{32}pdA(pdA)$_8$pA but not the bands assigned to Ap^{32}pdA(pdA)$_8$pA and Ap^{32}pdA(pdA)$_8$pApA.

2.4. ELONGATION OF ^{32}pdA(pdA)$_8$pA

The reactions were first performed in 1.7 mL microcentrifuge tubes. To 2 mg Na^+-montmorillonite in the microcentrifuge tube was added ^{32}pdA(pdA)$_8$pA ($\sim$140,000 counts) in 10 μL of water and 20 μL of a mixture of 0.4 M NaCl, 0.15 M $MgCl_2$ and 0.2 M HEPES (pH 8) (2x BE). Then 10 μL of 0.06 M ImpA was added and the mixture was vortexed and allowed to stand at room temperature for 24 hr. The tube was then centrifuged at 14,000 rpm and the supernatant removed with a pipette. The montmorillonite was washed by resuspending the clay-oligomer twice in 40 μL of BE by stirring with a sterile, plastic inoculation loop (Copan Diagnostics), vortexing and centrifuging at 14,000 rpm for 6 min. The elongation was initiated by the addition of 20 μL of 2X BE, 10 μL of water and 10 μL of 0.06 M ImpA and suspending the clay in the solution by stirring with an inoculation loop and vortexing the reaction mixture for $\sim$1 min. The reaction was then washed as described after the first 24 hr of reaction. At the end of the desired number of cycles the reaction solution was centrifuged and washed as described previously and to the clay-oligonucleotide mixture was added 40 μL of 0.1 M sodium pyrophosphate (pH 9) and the clay was stirred to a slurry with the inoculation loop and then vortexed for about 1 min. The tube was then centrifuged at 14,000 rpm for 15 min or longer to give 30–40 μL of supernatant. The wash was performed two more times and the eluates were combined and desalted using a Nensorb column by the procedure supplied by the manufacturer. Recently it was found that the use of Pall filter centrifuge tubes containing a filter insert tube were more effective for the generation of longer oligomers. The filter was first washed with $\sim$400 μL of distilled water and spun dry at 14,000 rpm and then 2 mg of clay was weighed into the filter tube insert. The liquid reagents were added as described above and the mixture was stirred to a slurry with an inoculation loop. The filter tube was placed in the accompanying microcentrifuge tube, was vortexed and placed in a closed container which contained sufficient water to maintain the humidity and hence minimize the evaporation of the water from the reaction solution. The same

procedure described for the reaction in the microcentrifuge tube was used for the subsequent steps in the reactions performed in the filter tubes.

3. Results

Investigation of the effect of ImpA concentration on the extent of elongation of ^{32}pdA(pdA)$_8$pA revealed that the optimum concentration was about 15 mM where 6 monomer units were added to the primer in 24 hr. Use of 1.7 and 0.17 mM resulted in the addition of four and one monomer units, respectively. A 170 mM concentration of ImpA resulted mainly in the formation of the capped primer, Ap^{32}pdA(pdA)$_8$pA and a small amount of ^{32}pdA(pdA)$_8$pApA. About 60% of the primer was eluted from the montmorillonite with this concentration of ImpA as compared to 10% when the lower concentrations were used. The inhibition of elongation is due to displacement of the ^{32}pdA(pdA)$_8$pA from the montmorillonite clay by the high ImpA concentration.

A brief investigation of the optimal amount of montmorillonite was undertaken using 4 mg instead of the usual 2 mg in microcentrifuge tubes. A slower rate of growth of oligomers was observed using the 4 mg of clay. In experiments to be described later, the primers were elongated to the same chain lengths with either amount of clay but it just took longer with the larger amount of clay. Increasing the amount of clay to 10 mg and decreasing the solution volume to 25 μL resulted in the more efficient binding of the primer to the clay surface but less elongation of the primer was observed than with 2 mg of clay and 40 μL volumes. These data suggest that with a larger amount of clay the primer is spread over a larger surface area on the clay so there is less chance for ImpA to be bound proximate to the 3′-end of the primer where it can react.

3.1. Elongation of ^{32}pdA(pdA)$_8$pA with ImpA

The conditions determined above were used to explore the length of oligomers formed in a 12 day elongation reaction using ImpA in microcentrifuge tubes. The chain length increased by about 27 mers after 6 days but no further elongation was observed after 12 days (Figure 2). The principal process in the 6–11 day time period was the reaction of the ImpA with the primer to add an Ap group to the 5′-end of the oligomers as shown by the increase in intensity of an electrophoresis band due to Ap^{32}pdA(pdA)$_8$pA. That band in the gel for the elongation reaction had the same migration distance as an authentic sample (Chu and Orgel, 1984).

It was observed during the elongation study that the amount of clay present decreased by about 25% at the end of 6 days of reaction but did not decrease further after a 12 day reaction period. A possible explanation for the absence of elongation after 6 days reaction time was the loss of some catalytic clay during the washing and pipetting procedures used in the experiments. That the decrease in the elongation was due in part to the loss of the clay catalyst was shown by the elongation

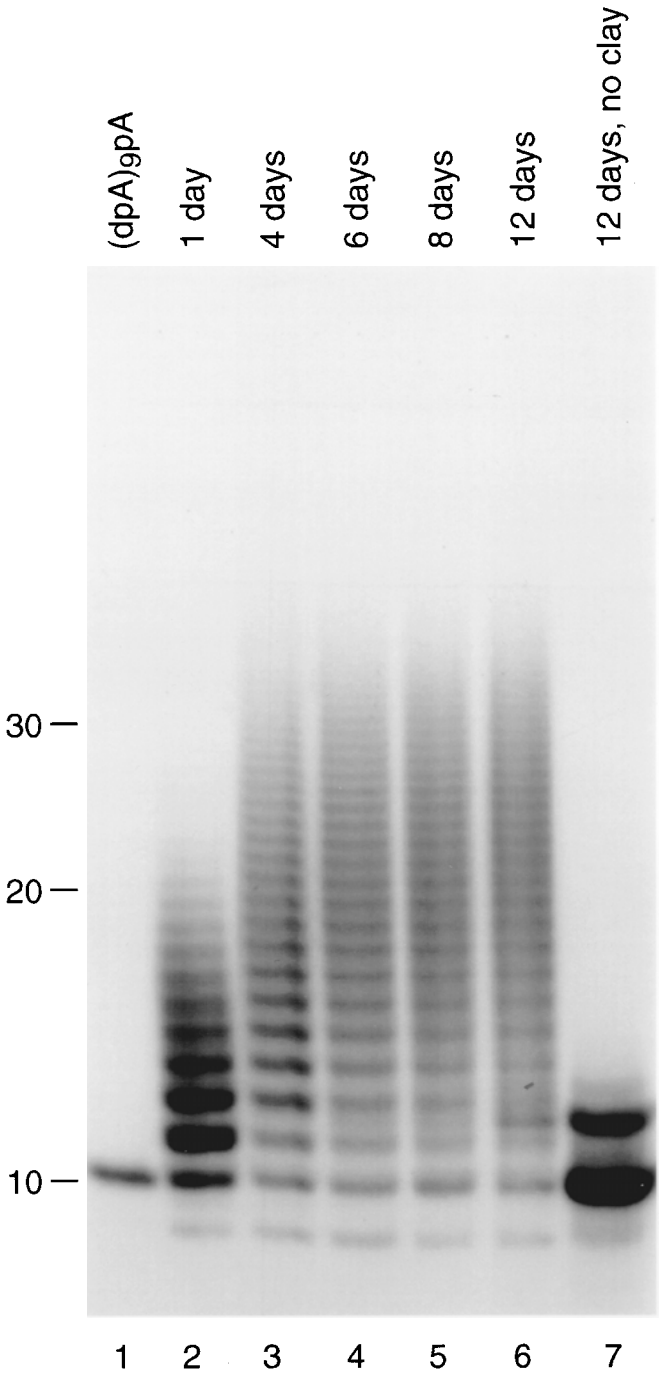

Figure 2. Gel electrophoresis of the elongation of ^{32}pdA(pdA)$_8$pA with ImpA in microcentrifuge tubes. Lane 1, ^{32}pdA(pdA)$_8$pA; lanes 2–6 elongation in the presence of montmorillonite; lane 7, elongation in the absence of montmorillonite.

of the oligomers formed after 4 days using fresh samples of montmorillonite. The chain lengths of oligomers formed in the presence of fresh clay to which ImpA and ImpU were added for an additional 4 days were longer than those formed for in a 8 day reaction using the same clay sample (Figure 3).

The loss of clay was eliminated by performing the elongation reactions in the 0.45 micron insert in Gelman spin tubes. Preliminary tests confirmed that the clay particles did not pass through the filter when a clay slurry was centrifuged at 14,000 rpm and that the primer did not bind to the nylon filter. The elongation procedure used for the spin tubes was essentially the same as that for the microcentrifuge tubes with the exception that the elongation reaction occurred in the filter tube insert and the clay phase was separated by the liquid phase by centrifugation with the clay remaining in the filter tube. The spin tubes were placed in a closed container, which had sufficient water to maintain a constant level of humidity to minimize the evaporative loss of water from the spin tubes. Elution of the oligomers from the clay using 0.1 M $Na_4P_2O_7$ required 15–40 min centrifugation. Use of the filter tubes made it possible to add about 40 monomer units to the primer, ^{32}pdA(pdA)$_8$pA, to give 40–50 mers after 14 days reaction (Ferris *et al*., 1996). This elongation to 50 mers was repeated using Pall Filtron tubes with a 0.45 micron filters since the Gelman spin tubes are no longer available (Figure 4). The longer oligomers obtained using spin tubes (Figure 4) versus microcentrifuge tubes (Figure 2) suggests that the shorter chain lengths using microcentrifuge tubes reflect losses of the clay catalyst during the removal of the supernatant by pipetting.

There is an upper limit of elongation to 40–50 mers using the spin tubes (Figure 4). The use of 4 instead of the usual 2 mg of Na^+-montmorillonite resulted in less elongation after a 3, 6, and 9 day reactions (lanes 1–6) but comparable chain lengths were observed after a total of 12 days reaction (lanes 7, 8). The same elongation limit was attained in other studies (data not shown) by adding fresh clay to the reaction after 3 days and after 3 and 6 days and carrying out the elongation reactions for 12 days. Since the oligonucleotide elongation with ImpA did not proceed past the 40–50 mers using more clay or with the addition of fresh clay, the limit on the extent of elongation is probably not due to an inhibitor that is formed in the course of the elongation reaction. The addition of fresh clay would have at least partially overcome the effect of inhibitors formed in the reaction mixture.

3.2. Elongation of ^{32}pdA(pdA)$_8$pA with ImpU

It was only possible to add about 20 U monomer units to ^{32}pdA(pdA)$_8$pA in a 6 day elongation time using spin tubes and no further elongation was observed on extended elongation to 10 days (Figure 5). Decomposition of the oligomers appears to be the main reaction in the 8–10 day time period.

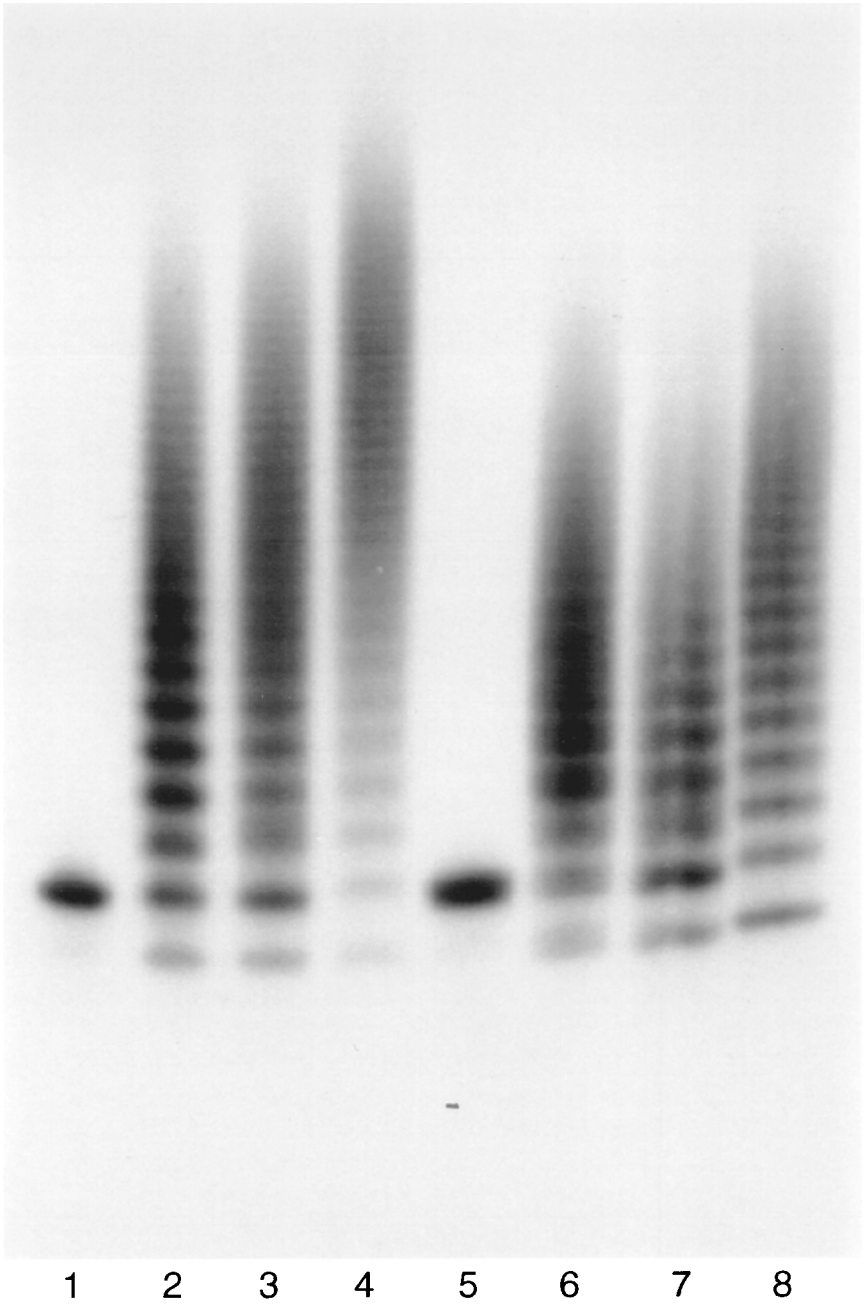

Figure 3. Role of fresh montmorillonite on elongation of ^{32}pdA(pdA)$_8$pA in microcentrifuge tubes. Lane 1, ^{32}pdA(pdA)$_8$pA; lane 2, elongation with ImpA for 2 days; lane 3, elongation for 8 days; lane 4, elongated for 4 days and product eluted from montmorillonite and bound to fresh montmorillonite and elongated for 4 days. Lane 5, ^{32}pdA(pdA)$_8$pA; lane 6, elongated for 4 days with ImpU; lane 7, elongated for 8 days; lane 8, elongated for 4 days, eluted from montmorillonite and bound to fresh montmorillonite and elongated 4 days.

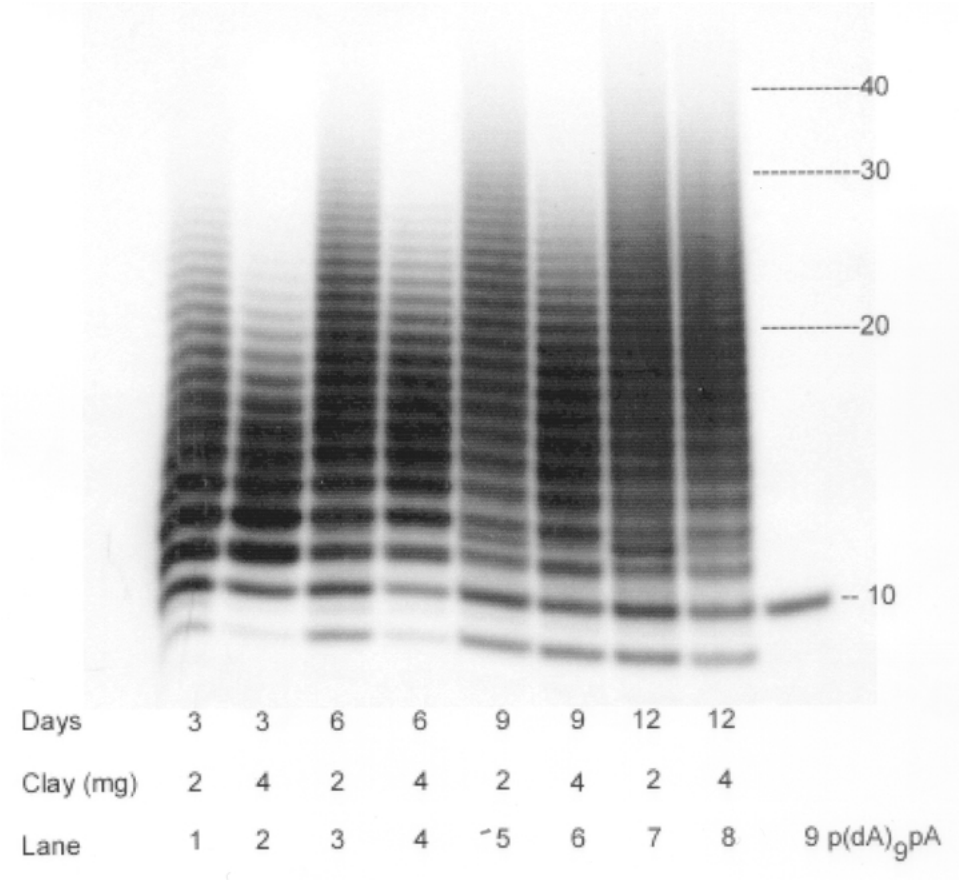

Figure 4. Gel electrophoresis of the elongation products of ^{32}pdA(pdA)$_8$pA using ImpA with 2 and 4 mg of montmorillonite catalyst in Pall filter spin tubes.

3.3. Decomposition studies

An investigation of the stability of the oligomers formed by the addition of U and A monomer units to ^{32}pdA(pdA)$_8$pA was performed to determine the relative rates of decomposition. Oligomers containing up to ~20 mers, formed by 2 elongation cycles of ImpA and ImpU to the primer in microcentrifuge tubes, were bound to Na^+-montmorillonite for 5 and 10 days and the products were analyzed by gel electrophoresis (Figures 6 and 7). Visual observation of the gels suggested that both oligomers decomposed at the same rate when bound to clay. In the case of the decomposition of the primer with A units added, the decomposition appeared to be greater with clay present than with it absent (Figure 6, compare lanes 3 and 5). Semi-quantitative analysis of the extent of decomposition was obtained by excising the '10 mer; and the band moving faster than the '10 mer' band ('9 mer') individually from the gel. The remaining bands on the gel were extracted

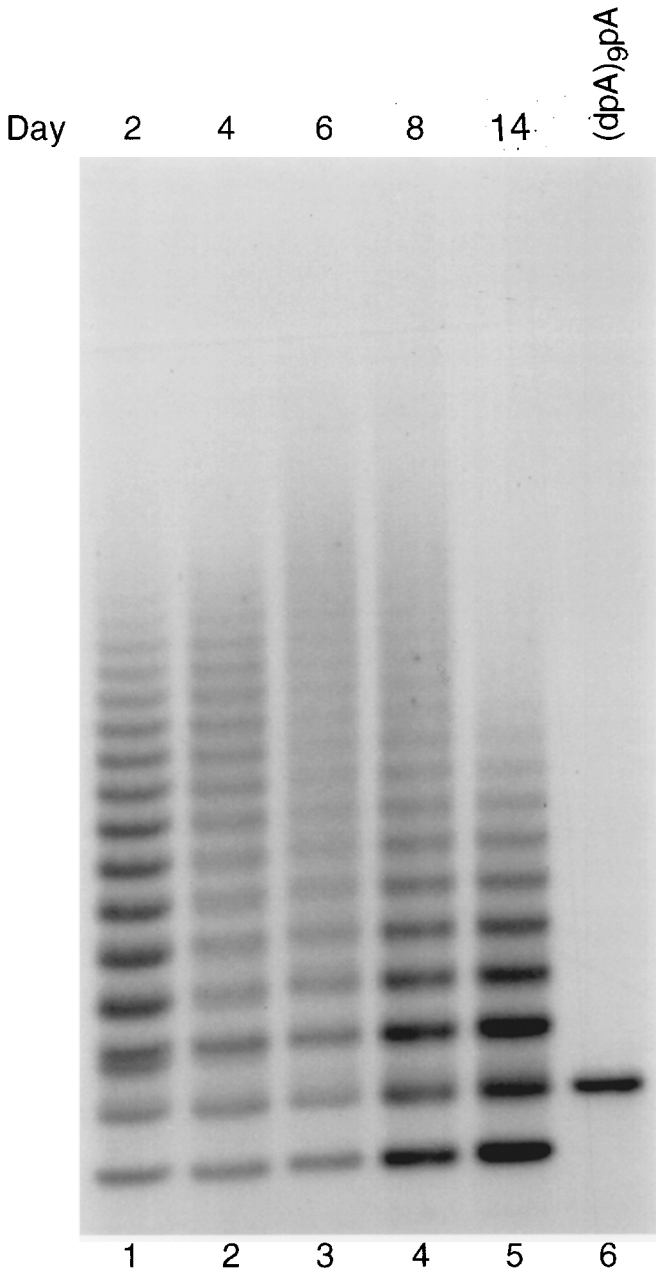

Figure 5. Gel electrophoresis of elongation products of ^{32}pdA(pdA)$_8$pA with ImpU using spin tubes. Lane 6 ^{32}pdA(pdA)$_8$pA.

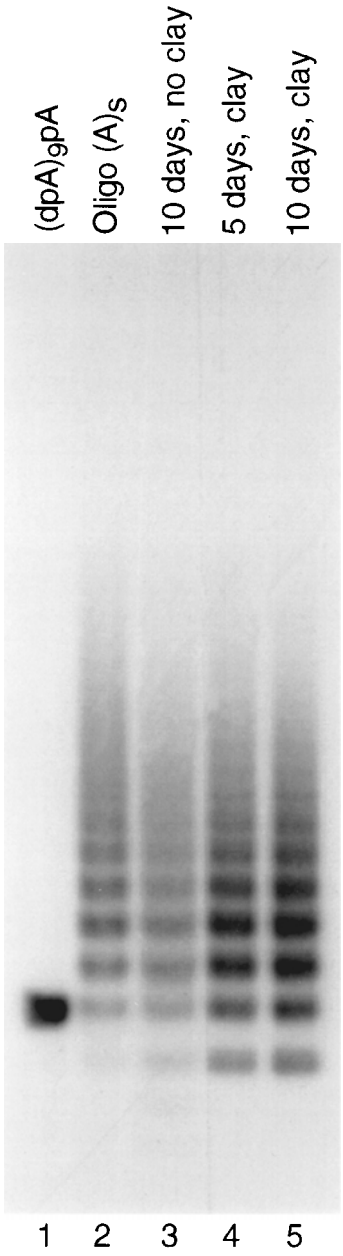

Figure 6. Monitoring the decomposition of oligomers elongated with ImpA. Lane 1, ^{32}pdA(pdA)$_8$pA; lane 2, elongation of ^{32}pdA(pdA)$_8$pA with ImpA for 2 days; lane 3, 2 day elongation product dissolved in buffer-electrolyte, in the absence of montmorillonite, for 10 days; lane 4, oligomers formed in 2 days bound to the montmorillonite stand for 5 days in the buffer-salt reaction mixture; lane 5, oligomers formed in 2 days bound to the montmorillonite for 10 days in buffer-electrolyte reaction mixture.

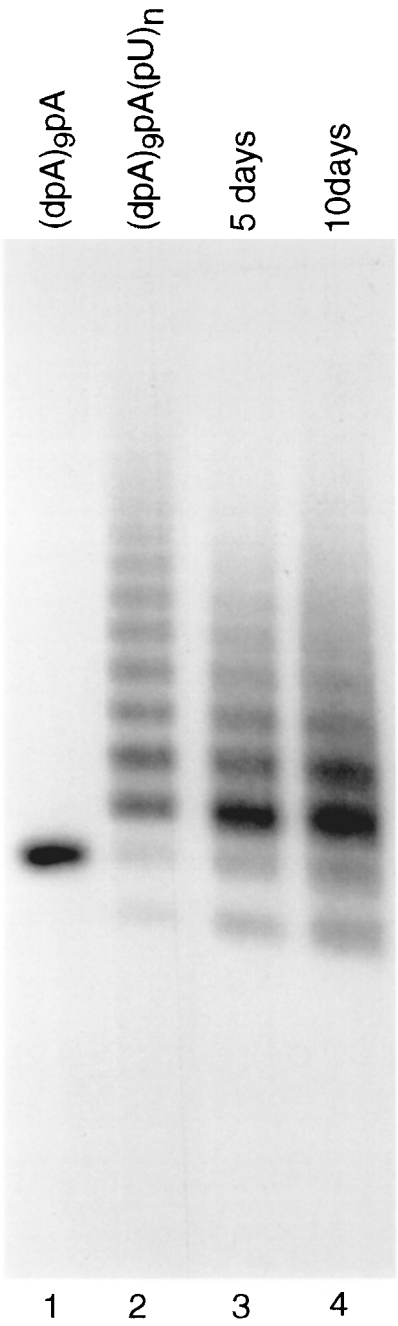

Figure 7. Monitoring the decomposition of oligomers elongated with ImpU. Lane 1, ^{32}pdA(pdA)$_8$pA; lane 2, elongation of ^{32}pdA(pdA)$_8$pA with ImpU for 2 days; lane 3, oligomers formed in 2 days bound to the montmorillonite for 5 days in buffer-electrolyte reaction mixture; lane 4, oligomers formed in 2 days bound to the montmorillonite for 10 days in buffer-electrolyte reaction mixture.

TABLE I

Decomposition of Adenylate and Uridylate Elongation Products of of ^{32}pdA(pdA)$_8$pA on Montmorillonite[a]

	Adenylate		Uridylate	
Time	^{32}P (%)	Change (%)	^{32}P (%)	Change (%)
Initial		–		
9 mer	4.3	–	4.5	–
10 mer	9.5		5.6	–
5 Days				
9 mer	9.1	4.8	8.2	3.7
10 mer	12.3	2.8	8.2	2.6
10 Days				
9 mer	7.3	3.0	9.6	5.1
10 mer	12.8	3.3	9.4	3.8

[a] The extent of hydrolysis of the adenylate and uridylate adducts of the primer when bound to montmorillonite was determined from the gels shown in Figures 6 and 7, respectively. The fastest moving band (9 mer) and next fastest moving band (10 mer) lanes 2, 4 and 5 in Figure 6, were each eluted from the gel. The structures of the 9 mer and 10 mer are assumed to be ^{32}pdA(pdA)$_8$pAp and Ap^{32}pdA(pdA)$_8$pAp, respectively. The initial counts in from lane 2 were subtracted from the counts for the 9 mer and 10 mer in lanes 4 and 5. The percentage of 9 mer ands 10 mer was calculated from the total counts in lanes 4 and 5. The same procedure was used to calculate the percent decomposition of the uridylate oligomers shown in Figure 7 using lanes 2, 3 and 4.

together and each of the three eluates was counted. These data were used to determine the percent decomposition of the oligomers. Structures ^{32}pdA(pdA)$_8$pAp and Np^{32}pdA(pdA)$_8$pAp (N = A or U) were assigned to the 9 mer and 10 mer, respectively on the basis of their migration distances relative to that of the primer. The 9 – and 10 – mer bands increase at about 3–5% for both the A and the U addition products when bound (Table I). The absence of a significant difference in the rates between the oligomers of A and U indicates that the shorter chain lengths formed in the Na^+-montmorillonite catalyzed addition of ImpU is not due to the more rapid decomposition of the U oligomers.

3.4. Characterization of elongation products

The oligomers were characterized by hydrolysis with alkaline phosphatase (APH) and ribonuclease T_2 (RNase T_2) hydrolysis. APH was used to differentiate those

oligomers in which the 5′-terminal phosphate group was capped by the addition of an 5′-Ap grouping to give $Ap^{32}pdA(pdA)_8pAp(N)_n$ and those which are not capped. Those oligomers that are not capped disappear from the gel after hydrolysis (Figure 8). The primer (lane 6) does not give a band while the bands in lanes 3 and 5 reveal those oligomers with 5′-caps. The gel shows that a substantial number of the oligomers formed by reaction with ImpA are capped (lanes 3 and 5 for 2 and 8 days, respectively). The extent of capping after 2 and 8 days was estimated by excising lanes 2 and 3 and 4 and 5 and measuring their radioactivity counts. From the ratios of 3:2 and 5:4 it was determined that 34 and 63% of the oligomers were capped after 2 and 8 days of elongation.

The same study was performed using the elongation products obtained with ImpU (Figure 9). Here the bands remaining from the APH hydrolysis products were much fainter and it was determined that the percent of capped oligomers after 2 and 8 days of elongation was 14 and 24 %, respectively.

The types of phosphodiester bonds formed between the primer and oligomers bound to it were determined by RNaseT_2 hydrolysis and the subsequent APH hydrolysis of the oligomers formed after 2 elongation cycles. The hydrolysis products that contain P-32 were separated by gel electrophoresis (Figures 10 and 11). A reaction scheme consistent with the electrophoretic data of the hydrolysis products of the ImpA addition reaction is given in Figure 12. The fastest moving band in lane 3 of Figure 10 was shown to be $Ap^{32}pdA(pdA)_8pA$ by comparison with an authentic sample. The band in lane 2 of Figure 10, migrating faster than the primer (lane 1), was assigned to the 3′-phosphorylated primer structure formed by the hydrolysis of the 3′, 5′-linked bond between the primer and the nucleotide bound to it at the 3′-position. Similar reasoning was used to deduce the structures of the other products in each band in the gels from the hydrolysis products.

The percentages of some of the bonds formed between the primer and the monomers was obtained by measurement of the areas of the bands in the RNase T_2 and APH hydrolyses hydrolysis products in the autoradiograms using the NIH Image program. The data derived from these measurements (Table II) for the higher percent of 3′, 5′-phosphodiester bond formation is consistent with previous observations with short oligoadenylates where it was observed that the reaction of adenosine nucleotides yield a greater proportion of 3′, 5′-phosphodiester bonds than do uridine nucleotides (Ferris and Ertem, 1993; Ding *et al.*, 1996; Ertem and Ferris, 2000).

4. Discussion and Conclusions

The addition products of the Na^+-montmorillonite-catalyzed reaction of ImpA to the 10 mer primer yields products with chain lengths as long as 40–50 mers. Oligomers as long as 30 mers are formed from the addition of ImpU to the same primer. Oligomers that contain 5′-caps are present, with a greater number of these

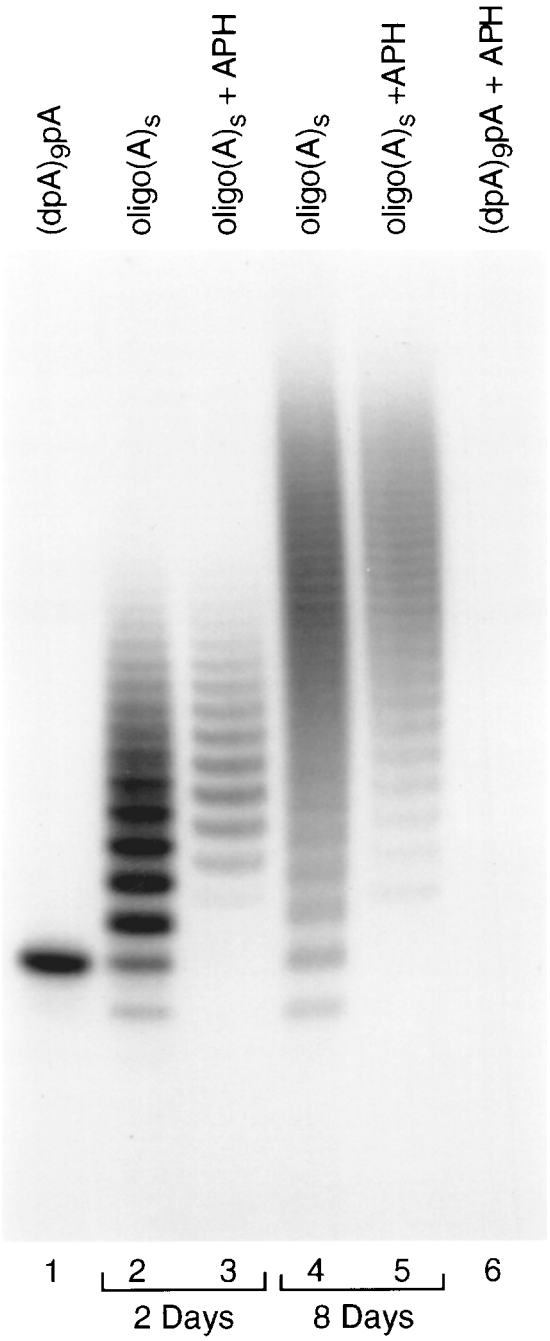

Figure 8. ^{32}P-containing ImpA elongation products after reaction with APH. Lane 1, ^{32}pdA(pdA)$_8$pA; lane 2, elongation with ImpA for 2 days; lane 3, APH hydrolysis of oligomers formed in the 2 days elongation reaction, lane 4, elongation with ImpA for 8 days; lane 5, APH hydrolysis of oligomers formed in the 8 day elongation reaction; lane 6, APH hydrolysis of ^{32}pdA(pdA)$_8$pA (no visible product).

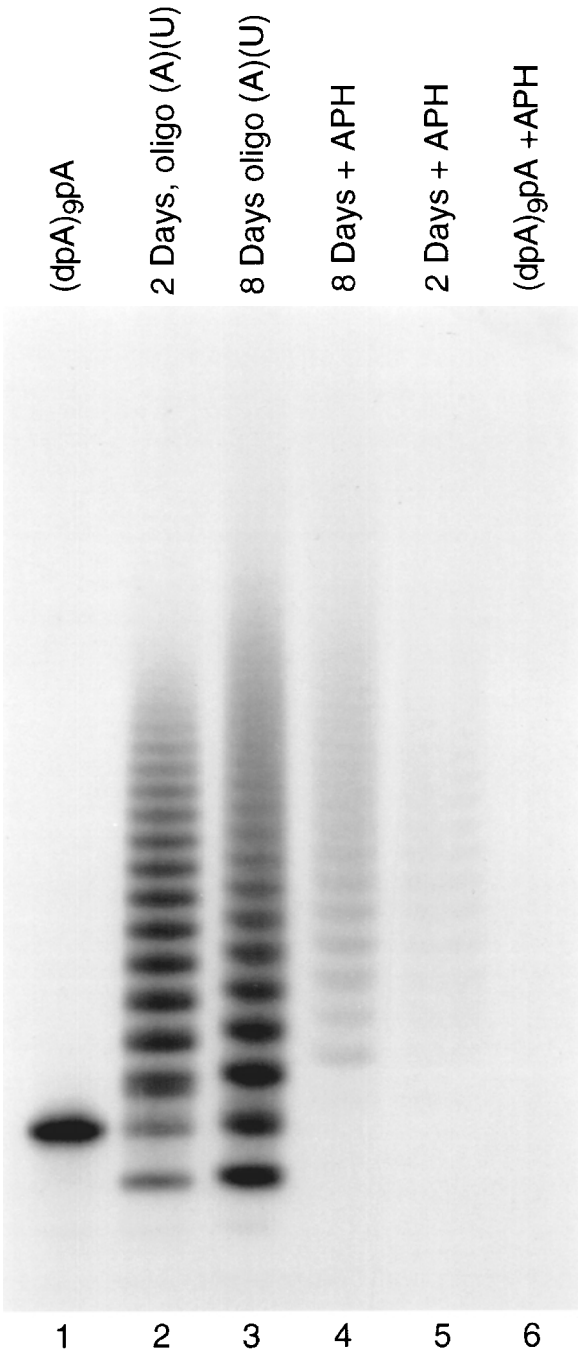

Figure 9. ^{32}P-containing ImpU elongation products after reaction with APH. Lane 1, ^{32}pdA(pdA)$_8$pA; lane 2, elongation with ImpU for 2 days; lane 3, elongation with ImpU for 8 days; lane 4, APH hydrolysis of oligomers formed in the 8 day elongation reaction, lane 5, APH hydrolysis of oligomers formed in the 2 day elongation reaction; lane 6, APH hydrolysis of ^{32}pdA(pdA)$_8$pA (no visible product).

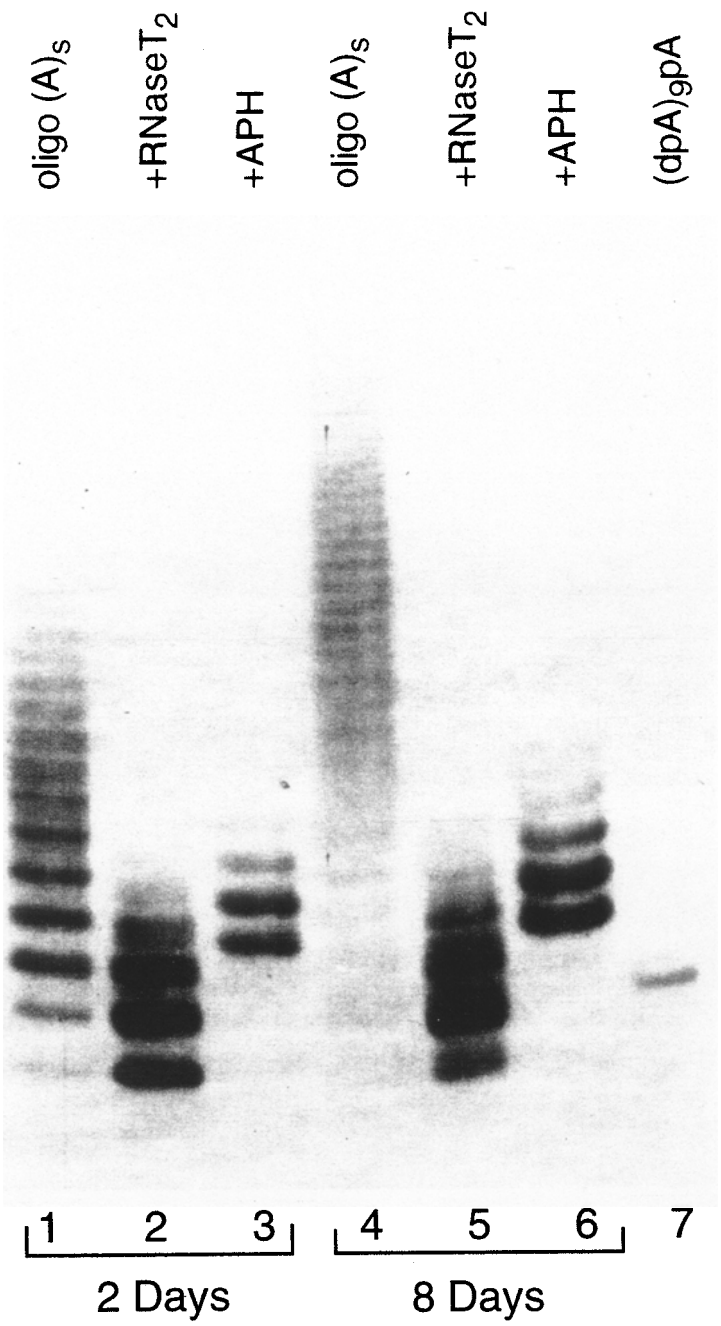

Figure 10. Reaction of ImpA elongation products with RNase T_2 with the subsequent hydrolysis with APH. Lane 1, elongation with ImpA for 2 days; lane 2, hydrolysis of the elongation product with RNase T_2 lane 3, APH hydrolysis of the RNase T_2hydrolysis products; lane 4, elongation with ImpA for 8 days; lane 6, APH hydrolysis of the RNase T_2 hydrolysis products; lane 7, $^{32}pdA(pdA)_8pA$.

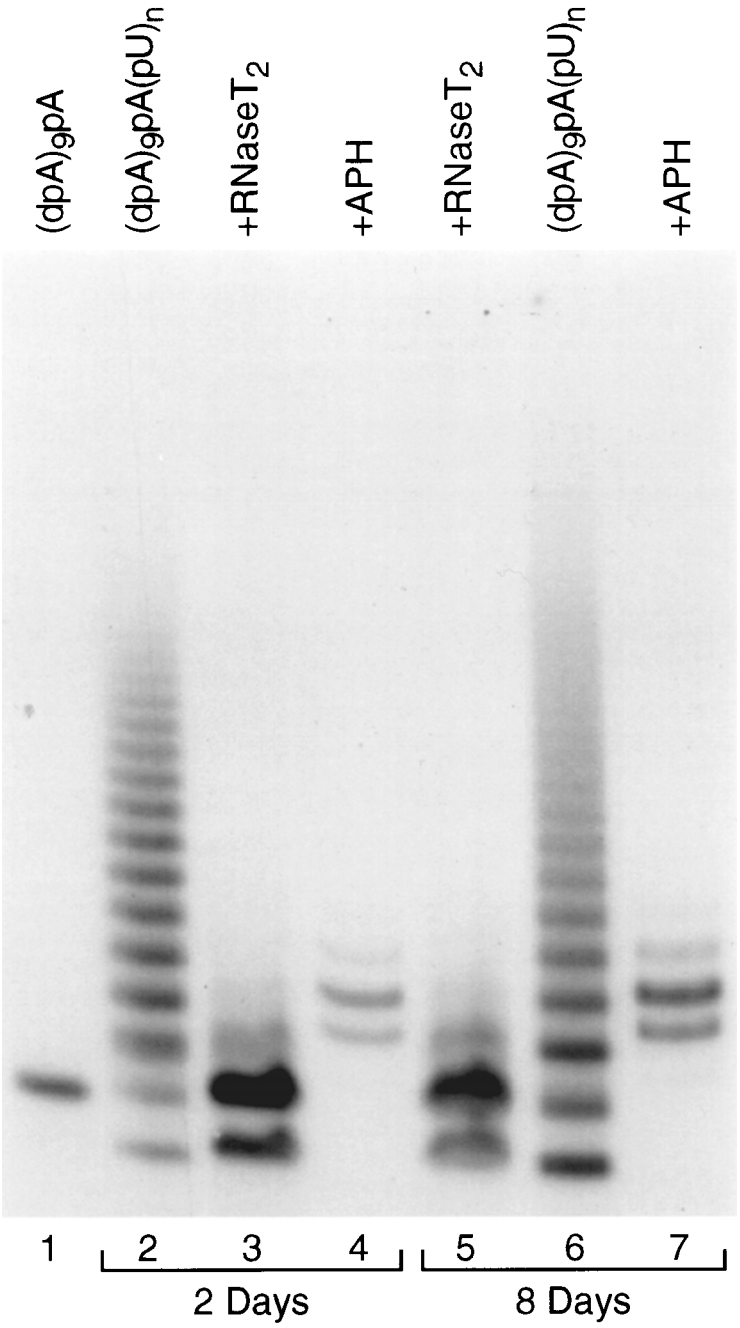

Figure 11. Reaction of ImpU elongation products with RNase T_2 with the subsequent hydrolysis with APH. Lane 1, $^{32}pdA(pdA)_8pA$; lane 2 elongation with ImpU for 2 days; lane 3, hydrolysis of the elongation product with RNase T_2; lane 4, APH hydrolysis of the RNase T_2 hydrolysis products; lane 5, RNase T_2 hydrolysis of the 8 day elongation products; lane 6, elongation with ImpU for 8 days; lane 7, APH hydrolysis of the RNase T_2 hydrolysis products.

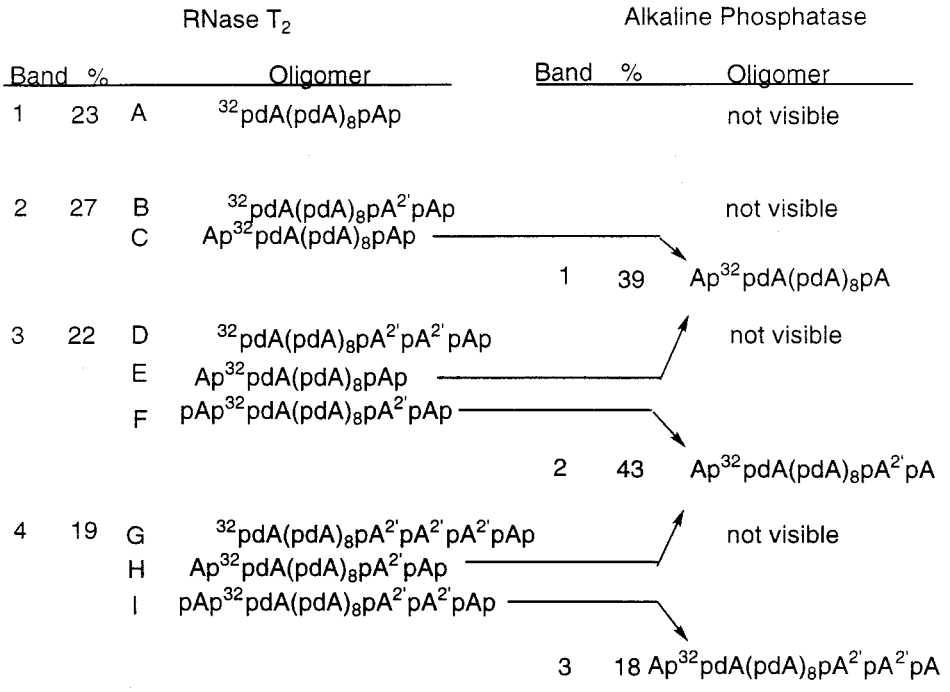

Figure 12. Structures of products of enzymatic hydrolysis of the ImpA elongation products after two elongation cycles. Capped oligomers can elongate from either end of the primer but for simplicity the isomers shown only have the nucleotides added to the 3′-end of the primer. Predicted migration distances based on ^{32}pdA(pdA)$_8$pA = 10 and Ap^{32}pdA(pdA)$_8$pAp ~11.5 as standards. Two additional negative charges from either standard results in migration one unit faster and one nucleoside additional results in migration ~1.5 units slower. It is not possible to resolve ~0.5 unit differences so the bands may consist of mixtures. For example in Figure 10 lane 2, band 1, migration distance = 9; band 2, migration distance = 9.5–10; band 3, migration distance = 10.5–11; band 4, 11.5–12.

TABLE II

Oligomer binding to primer after two feeding cycles[a,b]

	ImpA Addition Addition (%)	ImpU (%)
3′, 5′-phosphodiester bonds	37	33
Capped with one 2′, 5′-phosphodiester bond	15	7
Capped with two 2′, 5′-phosphodiester bonds	6	3

[a] Data derived from percentages in Figures 10 and 11 and the experimental determination of the percent capped oligomers for ImpA of 34% and ImpU of 14%.

[b] Bonds directly to the primer before a 3, 5′-phosphodiester bond occurs in the oligonucleotide chain.

caps on the oligomers formed from ImpA than from ImpU. It was possible to determine the extent of 3′, 5′-and 2′, 5′-phosphodiester bonding to the primer but it was not possible to determine the nature of the links in the oligomer chain that are not bound to the primer. Extrapolation from studies on the formation of oligomers directly from ImpA and ImpU would predict that the oligomers formed from ImpA would have a greater number of 3′, 5′-links and those from ImpU more 2′, 5′-links ((Ferris and Ertem, 1993; Ding *et al*., 1996).

It was not possible to extend the elongation products formed from ImpA much beyond the 50 mers and those from ImpU beyond the 30 mers. This limit does not appear to be due to the inhibition of catalytic sites on the montmorillonite because the addition of fresh clay or the use of greater amounts of clay did not result in the formation of longer oligomers. A likely explanation is that 2′, 5′-links on the end of the growing chain add another nucleotide more slowly than the corresponding 3′, 5′-phosphodiester bond (Ferris and Ertem, 1993). Since each elongation step forms both 2′, 5′- and 3′, 5′-phosphodiester bonds chain elongation will proceed less effectively as the proportion of newly formed phosphodiester bonds decreases.

The slower rate of elongation of 2′, 5′-linkages may also explain why the ImpU elongation products are shorter than those formed from ImpA. Since ImpU tends to form more 2′, 5′-linkages (Ding *et al*., 1996), its elongation products will terminate sooner than those formed by ImpA addition to the primer.

The efficient elongation of short oligomers by their reaction with activated monomers on montmorillonite clay highlights the potential significance of mineral catalysis to the origins of life (Ferris, 1993, 1985). While it is not known if montmorillonite catalysis initiated the RNA world, the mineral catalysis observed in this study and previous studies (Ferris *et al*., 1996) illustrates the potential role of mineral catalysis for the formation of the biopolymers of the first life in an aqueous environment.

This route to oligonucleotides provides a prebiotic route to the RNA world via mineral catalysis. A mineral that binds and catalyzes the synthesis of biopolymers downstream from a source of activated monomers could have served as the site for the first living system. The RNAs formed on and bound to the surface of the mineral could have interacted with each other to catalyze the ligation and replication of RNA. The integrity of this simple system would have been maintained by the binding of the longer RNAs to the mineral surface.

The generation of RNAs with chain lengths greater that 40 mers would have been long enough to initiate the first life on Earth (Joyce and Orgel, 1999; Szostak and Ellington, 1993). These oligomers would have been sufficiently long for replication without loss of the core information of the RNA. The RNA would have also been sufficiently long enough to fold into a three dimensional structure capable of binding and catalyzing the reactions of other RNA molecules.

Acknowledgement

This research was initiated during a sabbatical leave at the Salk Institute and was completed at RPI. Dr. Leslie Orgel provided laboratory facilities and many helpful and stimulating discussions. I thank Aubrey Hill, Jr. and Dr. Barbara Chu at Salk for their expert guidance and help with many of the experimental procedures used in this research. Derrick Meinhold and Charmi Miller at RPI measured the gel band areas in Figures 9 and 10 using NIH Image. The research at Salk was funded by the NASA NSCORT/Exobiology Grant NAG5-4546 and NASA grant NAGW-2881 while the research at RPI was funded by National Science Foundation Grant CHE-0076281 and the NY Center for Studies on the Origins of Life funded by NASA Grant NAG5-7598 and NASA Exobiology grant NAG5-9607.

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