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Synthesis and Template-Directed Polymerization of Adenylyl(3′–5′)adenosine Cyclic 2′,3′-Phosphate[†]

Seiichi Uesugi and Morio Ikehara*

ABSTRACT: Adenylyl(3′-5′)adenosine cyclic 2′,3′-phosphate (A-A>p) was synthesized and its polymerization was attempted under various conditions in the presence of poly(uridylic acid) and 1,3-propanediamine. Reaction at −20 °C for 16 days gave polymerized products (up to the 8-mer) in 15% yield and was proved to be dependent on the template. Reaction at 0 °C for 16 days gave more extensive (up to the 10-mer) and more efficient (35%) polymerization. The newly formed phosphodiester linkage was exclusively 2′-5′. These results are discussed in comparison with the monomer-condensation reaction.

It is very difficult to synthesize a long oligonucleotide with chain length higher than 10 by purely chemical methods. This is a particularly serious problem in the case of ribooligonucleotides. A reasonable approach for the preparation of a very long oligonucleotide is to synthesize relatively short oligonucleotides first by purely chemical methods and then to join them by another method. One of the possible ways of accomplishing the second step is to use a complementary template for holding the two reacting termini of the short oligonucleotide chains close to each other and join them chemically. This approach was first tried by Naylor and Gilham in 1966. They used poly(A)[1] as a template, oligo(thymidylic acid) with a 5′-terminal phosphate group as a fragment, and a water-soluble carbodiimide as a condensing agent in aqueous solution and obtained the dimerized product, $p(dT)_{12}$, in 5% yield. Only a few papers have been published on template-directed chemical condensation of oligonucleotides since this paper (Shabarova and Prokofiev, 1970; Uesugi and Ts'o, 1974). Uesugi and Ts'o succeeded in polymerizing oligo(2′-*O*-methylinosinic acid) with a 3′-terminal phosphate group using poly(C) and a water-soluble carbodiimide in practical yields (40–70%) for the first time. On the other hand, Orgel, Lohrmann, and their co-workers have reported detailed studies on condensation and polymerization reactions of mononucleotides in the presence of a complementary polynucleotide template (see the review by Orgel and Lohrmann, 1974). In one of these papers, Renz et al. (1971) reported that adenosine cyclic 2′,3′-phosphate (A>p) could be dimerized in the presence of poly(U) and polybasic amines and the best yield (23%) of condensation was obtained in a frozen solution.

As we have been working on the synthesis of ribooligonucleotides in our laboratory, we are interested in the chemical joining of ribooligonucleotides on appropriate templates. In order to obtain basic data on such systems, we examined the polymerization reaction of di(adenylic acid) with a terminal 2′,3′-cyclic phosphate group (A-A>p) in the presence of poly(U) and 1,3-propanediamine. We wish to report here the results and discuss the difference between the dimer polymerization and the monomer dimerization reaction. After completion of this work, a preliminary report on a template-directed polymerization reaction of hexa(adenylic acid) in a similar system was published by Usher and McHale (1976).

Experimental Procedure

General Methods. Paper chromatography was carried out by the descending technique using Toyo filter paper no. 51A in the following solvent systems: A, 2-propanol-concentrated ammonia-water (7:1:2, v/v); B, 1-propanol-concentrated ammonia-water (55:10:35, v/v); C, ethanol-1 M ammonium acetate (pH 7.5) (7:3, v/v); D, ammonium sulfate (20 g)-0.1 M NaH_2PO_4 (pH 7.0) (100 ml). Paper electrophoresis was carried out on the same paper at 35 V/cm in 0.05 M $Et_3NH_2CO_3$ buffer (pH 7.5). UV absorption spectra were obtained on a Hitachi EPS-3T or Hitachi 124 spectrophotometer. For t_m measurements, a Hitachi 124 spectrophotometer, equipped with a Komatsu Solidate SPD-H-124 thermostated cell, was used. The temperature within the cell was measured by a Shibaura MGB-III thermistor. High-pressure liquid chromatography was carried out on a Varian LCS-1000 system using a column (1 mm × 300 cm) of pellicular anion-exchange resin and gradient elution, 0.01 M KH_2PO_4 (pH 3.55) to 1.0 M KH_2PO_4 (pH 4.0) at 70 °C and 2100–2300 psi. The molar extinction coefficients (ϵ) deter-

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[†] From Faculty of Pharmaceutical Sciences, Osaka University, Suita, Osaka 565, Japan. *Received June 22, 1976.* This work was supported in part by Grant-Aid for Scientific Research from the Ministry of Education. This paper is Part 41 of the "Polynucleotides" series.

[1] Abbreviations used are: >p, 2′,3′-cyclic phosphate group; RNase M, ribonuclease M (*Aspergillus saitoi,* EC 2.7.7.17); Ap-urea, *N*-(adenylyl)-*N,N′*-dicyclohexylurea; Bz, benzoyl; A′A, A(2′-5′)A; poly(A), poly(adenylic acid); poly(U), poly(uridylic acid); DEAE, diethylaminoethyl; UV, ultraviolet.

TABLE I: Identification of the Main Peaks in Figure 2.

		Paper Chromatography R_f		Paper Electrophoresis
Peak No.[a]	Identification	Solvent A	Solvent B	$R_{\text{Ap-A}}$[b]
1	Benzamide	0.84	0.89	0.03
4	A>p	0.45	0.63	0.65
6	Ap	0.11	0.45	1.00
	A-Ap-urea (ca. 20%)	0.68	0.85	0.68
8	A-A>p	0.18	0.52	0.82
10	A-A-A>p[c]	0.05	0.40	0.98
11	A-A-A>p	0.05	0.45	0.89

[a] The total A_{260} units of peaks 4, 6, 8, 10, and 11 were 335, 484, 495, 124, and 96, respectively. [b] Relative mobility to Ap(1.0) and A(0.0) is presented.

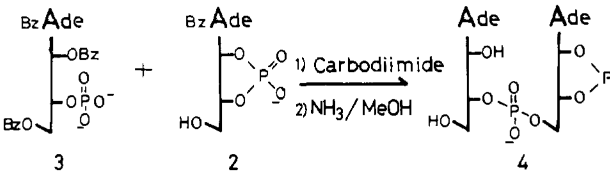

FIGURE 1: Synthesis of A-A>p.

mined by Brahms et al. for oligo(adenylic acids) (Michelson and Monny, 1967) were used for oligomers with corresponding chain lengths in these experiments. *Escherichia coli* alkaline phosphatase was purchased from Worthington Biochemical Co. and incubation was carried out in 0.1 M NH_4HCO_3 at 37 °C for 3 h with the enzyme (0.01 mg/ml). RNase M was a generous gift from Dr. M. Irie and incubation was carried out in 0.05 M ammonium acetate (pH 5) at 37 °C for 4 h with the enzyme (0.02 mg/ml). *N*-Benzoyladenosine cyclic 2′,3′-phosphate (**2**) was synthesized starting from adenosine 2′(3′)-phosphate (**1**) by benzoylation (Lapidot and Khorana, 1963), selective deblocking of the sugar benzoyl group (Ralph and Khorana, 1961), and cyclization (Lohrmann et al., 1966) according to the published procedure with some modifications. *N*, 2′,5′-*O*-Tribenzoyladenosine 3′-phosphate (**3**) was prepared as described by Lapidot and Khorana (1963).

Adenylyl(3′-5′)adenosine Cyclic 2′,3′-Phosphate (**4**). The pyridinium salt of *N*-benzoyladenosine cyclic 2′,3′-phosphate (**2**) was again passed through a column of pyridinium Dowex-50 resin and made anhydrous by repeated evaporation with pyridine. The residue and pyridinium *N*,2′,5′-*O*-tribenzoyladenosine 3′-phosphate (**3**) (74 mg, 0.1 mmol) were dissolved in pyridine and the mixture was dried by repeated evaporation with pyridine. The final residue was treated with dicyclohexylcarbodiimide (0.9 mmol) in anhydrous pyridine (0.6 ml) at 23 °C for 5 days. Fifty percent aqueous pyridine (6 ml) was added and the carbodiimide was extracted with *n*-hexane. The aqueous fraction was kept at room temperature for 20 h and precipitated urea was removed by filtration. The filtrate was evaporated to dryness in vacuo. After evaporation with pyridine, the residue was treated with 80% acetic acid (10 ml) at room temperature for 2 h. The solvent was removed in vacuo and the residue was made anhydrous by evaporation with pyridine. The final solution in pyridine (6 ml) was precipitated in ether-*n*-hexane (3:2, v/v, 200 ml) and the precipitates were treated with Et_3N (0.35 ml) and the carbodiimide (0.75 mmol) in pyridine (5 ml) at room temperature for 2 days. Water (5 ml) was added and the carbodiimide was extracted with *n*-hexane (6 ml × 3). The aqueous fraction was made anhydrous by evaporation with pyridine. The residue was treated with methanolic ammonia saturated at 0 °C (20 ml) at room temperature for 3 days. After removal of the solvent, the residue was dissolved in a dilute $(NH_4)_2CO_3$ solution (60 ml), filtered, and applied to a column (1.2 × 32 cm) of DEAE-cellulose (bicarbonate). After washing with 0.01 M ammonium carbonate, elution was carried out with a linear gradient of $Et_3NH_2CO_3$ (0.01-0.25 M, total 2 l.). Fractions (5 ml) were collected every 8 min. The elution pattern is shown in Figure 2. Peak 8 contained the desired product **4** (495 A_{260} units, ca. 20% from **2**). The fractions were combined, desalted by repeated evaporation with water and dissolved in a small volume of water. A part of this sample was purified by paper chromatography in solvent B and lyophilized. This lot was used in the polymerization at −20 °C. The remaining portion was purified by gel filtration with Sephadex G-50. This sample was converted to the ammonium salt by passing through an ammonium Dowex-50 column. This lot was used in the polymerization at 0 °C and control reaction at −20 °C. UV: $\lambda_{\max}$ (H_2O) at 258 nm, paper chromatography and paper electrophoresis as shown in Table I.

Template-Directed Polymerization Reaction at −20 °C. A solution of ammonium A-A>p (68 A_{259} units) and a solution of ammonium poly(U) (92 A_{261} units) were mixed and lyophilized. The residue was dissolved in 0.05 M 1,3-propanediamine·HCl (pH 7.5, 0.5 ml) and the pH of this solution was adjusted to 8 with 1 N ammonium hydroxide.[2] After addition of 5 μl of toluene, the mixture was kept frozen in a freezer at −20 °C for 16 days. The thawed solution was applied on a column (1.7 × 101 cm) of Sephadex G-50. Elution was carried out with 0.1 M $Et_3NH_2CO_3$ buffer (pH 7.5). Fractions (4 ml) were collected every 20 min. The absorbance of the eluent was also monitored continuously by a flow cell connected to a Hitachi 124 spectrophotometer. The elution pattern is shown in Figure 4. The yields and the results of identification of peaks are summarized in Table II. The UV spectrum of each fraction was checked and all adenosine derivatives showed $\lambda_{\max}$ at 258-259 nm. The combined fractions of each peak were desalted by repeated evaporation with water in vacuo.

Template-Directed Polymerization Reaction at 0 °C. The same reaction mixture as that of the reaction at −20 °C, except that a 0.5 M propanediamine·HCl solution was used instead of a 0.05 M solution, was prepared and kept in an ice box in a refrigerator for 16 days. The reaction mixture was then analyzed in the same manner as in the reaction at −20 °C. The

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[2] The pH's were measured at room temperature (~25 °C).

TABLE II: Yields and Identification of the Polymerization Products at −20 °C.

Peak No.[a]	Fractions Pooled	Total A_{260} Units	% Ratio[b]	Identification
I	19-24	90.0		Poly(U)
II	32-35	0.272	0.4	8-mer
III	36-39	1.236	1.8	6-mer
IV	40-42	0.780	1.1	A-A-A-Ap
V	43-46	7.910	11.6	A-A-A-A>p
VI	47-51	6.290	9.2	A-Ap
VII	52-56	50.0	73.4	A-A>p
VIII	57-62	2.700	4.0	A-A

[a] The peak numbers are shown in Figure 4. [b] The ratio of A_{260} units to that of total adenine oligonucleotides is given.

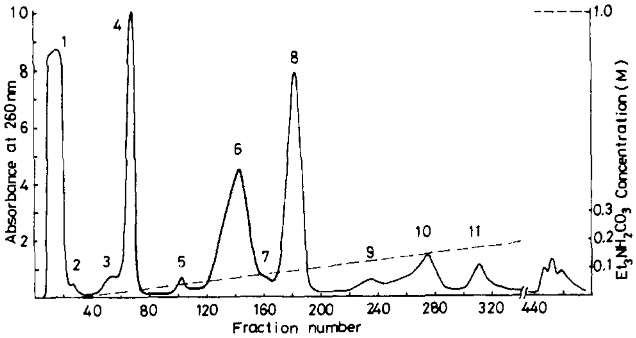

FIGURE 2: Chromatography of the products in the synthesis of A-A>p on a DEAE-cellulose column (1.2 × 32 cm). Elution was carried out using a linear salt gradient of $Et_3NH_2CO_3$ (pH 7.5) (0.01–0.25 M, total 2 l.). The identification of the main peaks is shown in Table I.

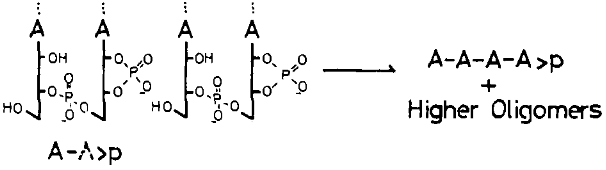

FIGURE 3: Chemical polymerization of A-A>p on a poly(U) template.

elution profile is shown in Figure 6. The yields and the results of identification of peaks are summarized in Table IV.

Results and Discussion

Synthesis of A-A>p (**4**). Adenylyl(3′-5′)adenosine cyclic 2′,3′-phosphate (**4**, A-A>p) was synthesized by condensation of *N*,2′,5′-*O*-tribenzoyladenosine 3′-phosphate (**3**) and *N*-benzoyladenosine 2′,3′-cyclic phosphate (**2**) with dicyclohexylcarbodiimide in pyridine. This type of condensation utilizing 2′,3′-cyclic phosphate for protection of the 2′-hydroxyl and 3′-phosphate was originally reported by Söll and Khorana (1965) and used by Ohtsuka et al. (1970) to synthesize triribonucleotides containing uridylyl residues at their 3′ ends. After the condensation reaction of **3** and **2** (23 °C, 5 days), the products were separated by chromatography on a DEAE-cellulose column. The elution profile is shown in Figure 2 and the yields and identification of the main peaks are shown in Table I. The desired product, A-A>p, was obtained from peak 8 in ca. 20% yield from compound **2**.

The identification of this compound was performed as follows. The compound from peak 8 was treated with 0.05 N HCl at room temperature for 30 min to open the cyclic phosphate. The product, which was isolated by paper electrophoresis, showed a smaller R_f in paper chromatography (solvent B) and a larger mobility in paper electrophoresis and these values were the same as those of an authentic A-Ap (Lapidot and Khorana, 1963). Dephosphorylation of this compound with *E. coli* alkaline phosphatase gave a compound which was identical with authentic A(3′-5′)A (Ohtsuka et al., 1974). This dephosphorylated compound was finally digested with RNase M (Irie, 1967) to give Ap and A in a 1:1 ratio. A-A>p itself was also digested with RNase M to give Ap as a sole product. As to the side products, peak 6 contained a phosphourea derivative (Dekker and Khorana, 1954; Ohtsuka et al., 1970) of A-Ap (A-Ap-urea, **5**, ca. 20%) in addition to Ap and peak 10 and 11 contained trimers with a terminal 2′,3′-cyclic phosphate group (A-A′A>p, **6**, and A-A-A>p, **7**).[3] Compound **5** gave ApA>p by mild acidic treatment (e.g., 0.1 N acetic acid at room temperature for 20 h produced about 60% conversion) and gave Ap and probably Ap-urea (R_f in solvent A was 0.86 when that of Ap is 0.17) by digestion with RNase M. The presence of A-Ap-urea in the products shows that the acidic treatment (80% acetic acid at room temperature for 2 h) in the work-up of the condensation reaction was not sufficient for complete degradation of this compound. Compound **7** from peak 11 was proved to be A-A-A>p with only 3′-5′ phosphodiester bonds because after successive treatments with 0.05 N HCl, phosphatase, and RNase M, it gave Ap and A in a 2:1 ratio. The compound **6** from peak 10 was assumed to be mainly A-A′A>p with a 2′-5′ phosphodiester linkage between the second and the third nucleoside residues because it gave Ap and 2′-5′ A-A in 1.2:1.0 molar ratio[4] after successive treatments with acid, phosphatase, and RNase M. Formation of these compounds might suggest that a terminal cyclic 2′,3′-phosphate group in a protected dinucleotide can be activated by the carbodiimide sufficiently for phosphodiester formation. A similar phenomenon has been also observed by Ohtsuka et al. (1970).

Template-Directed Polymerization of ApA>p at −20 °C. It is reported by Renz et al. that the best yield of dimer formation is obtained in the reaction of A>p at −15 °C, either with or without a poly(U) template. Therefore, a polymerization reaction of A-A>p was first attempted in a frozen solution. A solution of poly(U) (20 mM) and A-A>p (10 mM) in 0.05 M 1,3-propanediamine hydrochloride (pH 8) was kept at −20 °C for 16 days. The reaction mixture was separated by chromatography on a Sephadex G-50 column. The elution profile is shown in Figure 4. Eight different peaks were also observed in a continuous chromatogram (not shown here) which was recorded using a flow cell in a spectrophotometer. Yields and results of identification of these peaks are shown in Table II. As markers, the elution positions of poly(U), A-Ap (obtained by acid hydrolysis of A-A>p), and A>p were determined separately. Poly(U) was eluted at the same position as peak I and A-Ap at the same position as peak VI. A>p came out at a position (tube no. 56-58) between peak VII and VIII. Peaks VI, VII, and VIII were identified as A-Ap, A-A>p and

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[3] For compound **6** only, the abbreviation -A′A- is used to distinguish a 2′-5′ phosphodiester linkage from the (3′-5′) counterpart. Elswhere, -A-A- can represent either a 2′-5′ or a 3′-5′ linkage.

[4] The A(2′-5′)A can be distinguished from the 3′-5′ isomer by paper chromatography in solvent D.

[5] The relative yield of the polymerization products can be as high as 38% when hypochromicities of the oligomers are taken into account.

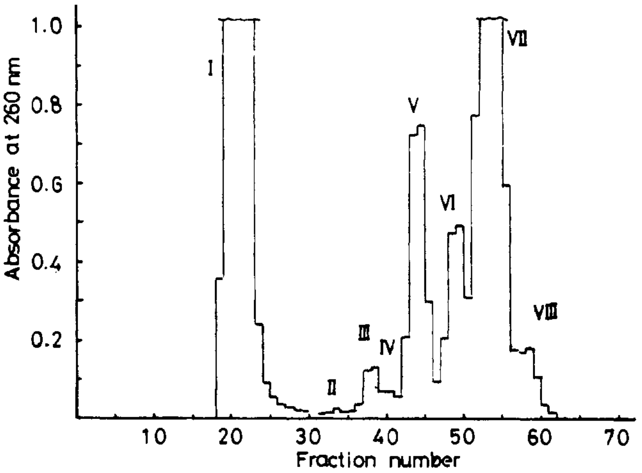

FIGURE 4: Elution profile of the polymerization products at −20 °C. A column (1.7 × 101 cm) of Sephadex G-50 was used and elution was carried out with 0.1 M $Et_3NH_2CO_3$ (7.5). The yields and identification of these peaks are shown in Table II.

TABLE III: Paper Chromatographic Properties of the Polymerization Products and Related Compounds.

Compound	R_f of Solvent B
A	0.68
Ap	0.43
A-A>p	0.52
A-A-A-A>p	0.26
A-A-A-Ap	0.13
A-A-A-A	0.26
A-A-A-A-A-A>p	0.13
A-A-A-A-A-Ap	0.07
A-A-A-A-A-A	0.12

A-A by direct comparison with authentic sample in paper chromatography and electrophoresis. Products in peaks IV and V were identified as A-A-A-Ap and A-A-A-A>p as follows. The compound from peak V was treated with 0.05 N HCl at 30 °C for 15 min to give a product that showed an R_f value lower than that of the original compound and the same as that of an authentic A-A-A-Ap in paper chromatography. This product was treated with phosphatase to give a product that showed the same R_f value as that of A-A-A-A in paper chromatography (see Table III). This dephosphorylated product was subjected to alkaline hydrolysis and the result was analyzed by high-pressure liquid chromatography. The ratio of (2′-AMP + 3′-AMP)/A was found to be 2.9. From these results, the original compound was proved to be tetra(adenylic acid) with a terminal cyclic 2′,3′-phosphate group. The compound from peak IV showed the same R_f value as that of A-A-A-Ap and gave a product having the same R_f value as that of A-A-A-A upon phosphatase treatment. The compound from peak IV was also identified to be A-A-A-A-A-A>p (6-mer) in a similar manner by successive treatment with dilute acid and phosphatase. The product in each step showed the same R_f value as those of authentic A_6p and A_6, respectively. The compound from peak II was identified as an octamer (8-mer) by t_m measurement of the complex with poly(U). The t_m's of 1A:2U complexes of 4-, 6-, and 8-mer with poly(U) were 27, 41, and 50 °C, respectively, in 0.01 M $MgCl_2$-0.01 M phosphate buffer (pH 7.6) maintaining the oligomer-strand concentration at 1.3×10^{-6} M. The $1/T_m$ (*K*) vs. $1/n$ plot gave a straight line, thus confirming the correctness of the chain-length (*n*) determinations. The linear relationship between $1/T_m$ and $1/n$ has been predicted by theoretical analysis (Magee et al., 1963; Damle, 1970) and also confirmed experimentally in many polymer-oligomer complexes (Ts'o, 1974). In conclusion, under the present conditions at −20 °C for 16 days, about 15% of A-A>p was polymerized to give 4-mer (12.7%), 6-mer (1.8%), and 8-mer (0.4%). The extent of hydrolysis of the terminal 2′,3′-cyclic phosphate is about 10% both in the 2-mer and the 4-mer, though about 30–50% hydrolysis was observed in the case of the A>p condensation.

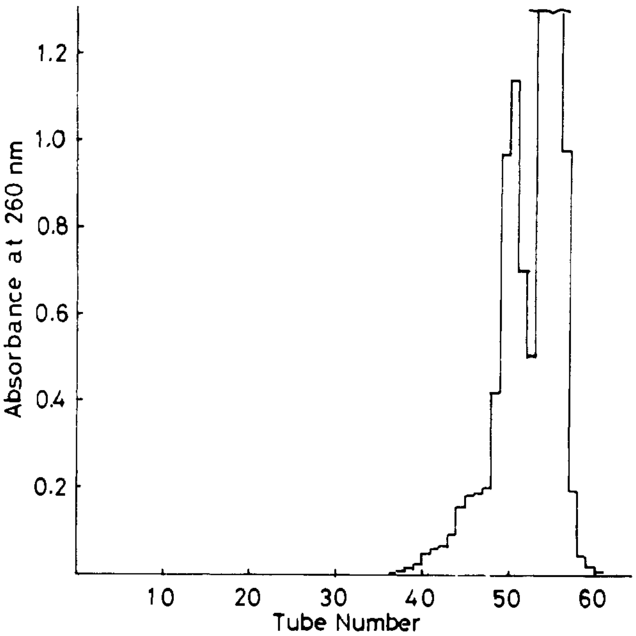

FIGURE 5: Elution profile for the control reaction without poly(U). The conditions are the same as described in Figure 4.

Next, the effect of the template on the polymerization reaction was examined by a control reaction without poly(U). The elution profile from the same column of Sephadex G-50 is shown in Figure 5. About 5% of the total absorbance at 260 nm was found in the 4-mer region. But reexamination of the A-A>p sample itself showed about 3.5% contamination of 4-mer, which might be formed during prolonged storage of the sample in a freezer. Thus, the extent of template-independent condensation under the present conditions was less than 2%, proving that the polymerization reaction was, indeed, template directed. This result is in sharp contrast to the results of the monomer-condensation reaction which was almost independent of the presence of template in the freezing experiment (Renz et al., 1971). As it was also reported that the phosphodiester linkage formed in the condensation reaction of A>p was exclusively 2′-5′ (Renz et al., 1971), the newly formed phosphodiester bond in A-A-A-A>p was also examined. The 4-mer was directly digested with RNase M and the products were analyzed by high-pressure liquid chromatography. Peaks of Ap and A(2′-5′)Ap were observed in about a 2:1 molar ratio. This result indicates that the new phosphodiester bond in A-A-A-A>p has exclusively the 2′-5′ linkage as is the case with the A>p condensation. In a helical complex of A>p or A-A>p with poly(U), the free 5′-hydroxyl group of one molecule and the $O_{3'}$-P bond (not the $O_{2'}$-P bond) of the cyclic phosphate ring of the next molecule may be arranged in line as in the case of poly(A)·poly(U) complexes. Then, if the condensation goes by the so-called "in-line" mechanism, the leaving group will be $O_{3'}$ and a 2′-5′ phosphodiester bond will be the result. A similar discussion has been presented by Usher (1972).

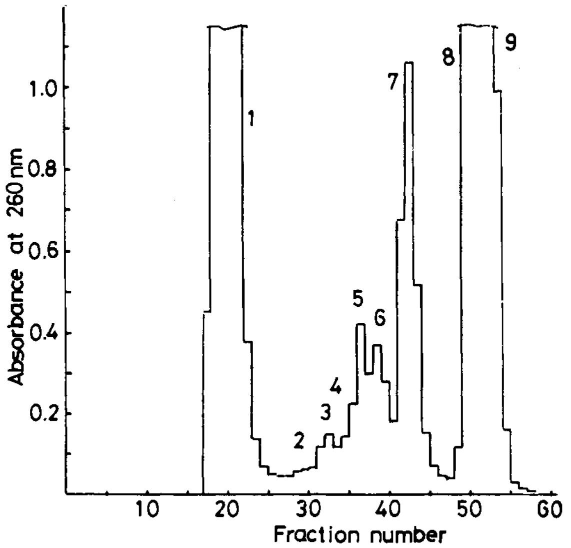

FIGURE 6: Elution profile of the polymerization products at 0 °C. The conditions are the same as described in Figure 4.

TABLE IV: Yields and Identification of the Polymerization Products at 0 °C.

Peak No.[a]	Fractions Pooled	Total A_{260} Units	% Ratio[b]	Identification
1	18-28	84.4		Poly(U)
2	29-31	0.740	1.3	10-mer
3	32-33	1.04	1.8	8-mer
4	34-35	1.03	1.8	6-mer, opened[c]
5	36-38	3.74	6.5	6-mer, cyclic
6	39-40	2.58	4.5	A-A-A-Ap
7	41-47	10.76	18.8	A-A-A-A>p
8	48-51	13.0	22.7	A-Ap
9	52-56	24.4	42.6	A-A>p

[a] The peak numbers are shown in Figure 6. [b] The ratio of A_{260} units to that of total adenine oligonucleotides is given. [c] 6-mer with a terminal phosphate group.

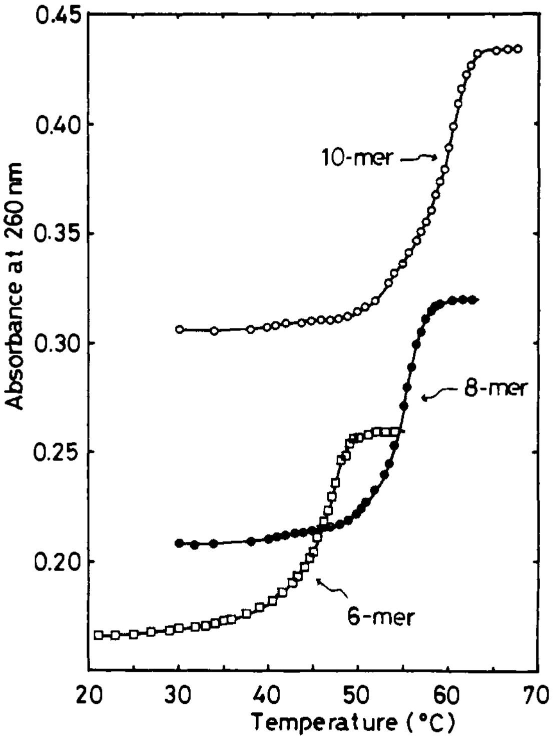

FIGURE 7: UV absorption-temperature profile for the complexes of 6-, 8-, and 10-mer with poly(U). Obtained in 0.01 M $MgCl_2$-0.01 M sodium cacodylate (pH 7.0) with oligomer-strand concentration at 1.3 μM: (□-□) complex of 6-mer; (●-●) complex of 8-mer; (○-○) complex of 10-mer.

Template-Directed Polymerization of A-A>p at 0 °C. A polymerization reaction was tried next at 0 °C. A solution of poly(U) (20 mM) and A-A>p (10 mM) in 0.5 M diaminopropane hydrochloride (pH 8) was kept at 0 °C for 16 days. This time the concentration of diaminopropane was increased tenfold for stabilization of the complex and to facilitate comparison with the data from the monomer condensation. The reaction mixture was separated on the same column of Sephadex G-50 under the same conditions. The elution profile is shown in Figure 6. Nine different peaks were noted in a continuous chromatogram, on the basis of which appropriate fractions were pooled. Yields and results of identification of these peaks are shown in Table IV. Peaks 8 and 9 were identified to be of A-Ap and A-A>p by comparison with authentic samples in paper chromatography and electrophoresis. Peaks 6 and 7 were identified to be of A-A-A-Ap and A-A-A-A>p by comparison in paper chromatography and electrophoresis with the samples obtained in previous experiments at −20 °C. The compound from peak 5 gave A_6p by mild acidic hydrolysis and gave A_6 by subsequent phosphatase treatment. The compound from peak 4 showed the same R_f value as that of A_6p and was subjected to phosphatase treatment to give A_6 upon examination by paper chromatography in solvent B. The chain lengths of the compounds from peaks 2 and 3 were estimated to be 10 and 8 by measuring t_m's of the 1A:2U complexes with poly(U). This time, 0.01 M sodium cacodylate buffer (pH 7.0) was used as solvent because some precipitation (probably of magnesium phosphate) was observed shortly after complete melting of the complex in the $MgCl_2$-phosphate buffer system. The oligomer-strand concentration was the same as that in the previous experiments. The complexes of the compounds from peaks 2-5 gave t_m's of 59, 47, and 45.5 °C, respectively (the melting profiles are shown in Figure 7). The t_m's for peak 4 and 5 complexes are about the same, indicating that their chain lengths are identical. The 6-mer with terminal cyclic phosphate should give a higher t_m than the 6-mer with an open terminal phosphate (Ts'o, 1974) because of less charge repulsion between the strands in the complex. The higher t_m observed for the complex of the 6-mer with an open terminus may be due to some contamination of higher oligonucleotide in peak 4. The $1/T_m$ (K) vs. $1/n$ plot for the complexes of the oligomers with a terminal cyclic phosphate again gave a straight line.

In conclusion, polymerization products are obtained in about 35% yield under these conditions at 0 °C for 16 days. The yields of each oligomer are 23.3 (4-mer), 8.3 (6-mer), 1.8 (8-mer), and 1.3% (10-mer), respectively. The extents of hydrolysis of the terminal cyclic phosphate are 35% in the 2-mer and about 20% in the 4-mer and 6-mer. The degrees of hydrolysis in oligonucleotides are considerably smaller than that (ca. 40%) observed in the condensation of A>p under similar conditions (Renz et al., 1971).

Conclusions

As described above, template-directed polymerization occurs in the presence of 1,3-propanediamine hydrochloride (pH 8) in 15% yield at −20 °C for 16 days and in 35% yield at 0 °C for 16 days. The polymerization reaction of A-A>p at −20 °C is different from the condensation reaction of A>p at −15 °C in some respects. In the reaction of the dimer in a frozen solution, (1) a polymerization occurs, (2) it is dependent on the presence of poly(U), and (3) the yield is not better than that obtained at 0 °C. On the other hand, only a dimerization occurs, it is almost independent of the template, and the yield is best in a frozen solution, in the case of the monomer reaction. As to the first point, it could be due to the rather facile hydrolysis reaction (often over 50%) of (2′-5′) A-A>p and/or an inability of (2′-5′) A-A>p to form a phosphodiester bond because of some geometrical reason, in the monomer reaction. As to the second point, the template-independent dimerization of the monomer may be due to some special characteristic of a monomer-amine complex in a frozen solution. As to the third point, the lack of a template-independent condensation in the dimer reaction and the necessity for some degree of conformational flexibility in the phosphodiester-forming reaction may be the main reasons. The better yield at 0 °C in the dimer reaction is a somewhat unexpected result because, in the polymerization of a oligomer of 2′-*O*-methylinosinic acid in the presence of poly(C), a better yield was obtained in a reaction involving freezing.

The yield of the A-A>p polymerization at 0 °C is about twice as large as those obtained in the monomer reactions at 0 °C. The t_m of the 2(poly(U))·A-A>p complex under the conditions of polymerization should be much higher than room temperature because the corresponding t_m of the monomer under similar conditions (25 mM A>p, 0.5 M propanediamine) is reported to be ca. 30 °C (Renz et al., 1971). The more stable complex of the dimer with the template at relatively high temperatures may produce better yields in condensations. Further improvement in yield may be expected using longer oligomers as the polymerization unit.

The t_m values of the complexes of the product oligomers with poly(U) in the cacodylate buffer are comparable to those of the corresponding complexes of oligoadenylic acids with only 3′-5′ phosphodiester linkages in similar conditions (Michelson and Monny, 1967). This may indicate a close similarity between the structures of these two different complexes. An oligonucleotide containing only a very small number of 2′-5′ linkages, as in the case of polymerization or condensation products from a long oligonucleotide unit, may have almost the same structure and function as those of the corresponding oligonucleotide with only 3′-5′ linkages. The present results suggest that these methods could be utilized for construction of such oligonucleotides for physical or biological studies.

After completion of this work, a preliminary report on a polymerization reaction of hexa(adenylic acid) with a terminal cyclic 2′,3′-phosphate in the presence of poly(U) was published by Usher and McHale (1976) but their yield seems rather low, considering the chain length of their initial condensation unit.

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