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Polynucleotides. VII. Synthesis of Ribopolynucleotides Containing 8-Substituted Purine Nucleotides by Polynucleotide Phosphorylase*

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ABSTRACT: Polymerization of 8-substituted purine ribonucleoside diphosphates, such as 8-bromoadenosine diphosphate, 8-oxyadenosine diphosphate, 8-bromoguanosine diphosphate, 8-oxyguanosine diphosphate, and 8-dimethylaminoguanosine diphosphate, catalyzed by polynucleotide phosphorylase was studied. Although homopolymerization of these diphosphate analogs failed, copolynucleotides were obtained from these analogs and adenosine diphosphate or guanosine diphosphate. The rate and extent of the polymerization decreased according to the amount of analog diphosphates used in the polymerization reaction. Polynucleotides containing 8-substituted purine in either polyadenylic acid or polyguanylic acid chains had lower T_m's relative to the parent polyadenylic acid or polyguanylic acid, respectively. Melting temperature of the complex of the copolymer, (poly A,BrG)–(poly U)$_2$, was also lower than that of (poly A)–(poly U)$_2$ complex due presumably to the looping out of nucleotide analog residues.

Synthesis of ribopolynucleotides from naturally occurring ribonucleotide diphosphates as well as from a variety of analog diphosphates catalyzed by polynucleotide phosphorylase (Grunberg-Manago and Ochoa, 1955) has been extensively investigated (Steiner and Beers, 1961; Grunberg-Manago, 1963; Michelson *et al.*, 1967). Although it was generally accepted that polynucleotide phosphorylase had relatively loose substrate specificity, several ribonucleoside diphosphates, such as 6-azauridine diphosphate (Skoda *et al.*, 1959), 6-mercaptopurine 9-riboside diphosphates (Carbon, 1962), and arabinosyluracil diphosphate (Michelson *et al.*, 1962), were known to inhibit polynucleotide phosphorylase action.

Recently, methods for the introduction of various substituents into the 8 position of purine nucleosides and nucleotides have been developed (Holmes and Robins, 1964; Ikehara and Muneyama, 1965; Ikehara *et al.*, 1967; Long *et al.*, 1967). Since some of these nucleosides having 8 substituents were found to be inhibitory toward growth of the cancer cells (Kuretani and Fukuoka, 1966; Bloch *et al.*, 1966), the mode of their incorporation into ribopolynucleotides would be of interest. This paper deals with the polymerization of 8-bromo-ADP, 8-oxy-ADP, 8-bromo-GDP, 8-oxy-GDP, and 8-dimethylamino-GDP catalyzed by polynucleotide phosphorylase and with the nature of the resulting polynucleotides containing analogs. It was found that: (i) 8-Substituted ADP and 8-substituted GDP were very poor substrates for this enzyme and essentially no homopolynucleotides were formed. (ii) In the copolymerization of these analog diphosphates with ADP or GDP, the rate of the polymerization and the amount of polynucleotide synthesized decreased according to the content of analog diphosphates in the incubation mixture. (iii) The copolynucleotides containing analogs showed lower T_m's in their acid form and in the complex with poly U.

Materials

Synthesis of 8-Substituted Diphosphates (Ikehara *et al.*, 1968). 8-BrAMP and 8-BrGMP were obtained by bromination of AMP and GMP with bromine–water (1.2 equiv) in sodium acetate buffer of pH 3–4 (Ikehara *et al.*, 1967; Ikehara and Uesugi, 1968). Resulting 8-bromo compounds were isolated by ion-exchange column chromatography in the yield of 64–78%.

8-Oxy-AMP and 8-oxy-GMP were synthesized from the 8-bromo compounds obtained as above. Reflux with sodium acetate in acetic acid for 2 hr (Ikehara *et al.*, 1965) and chromatography on Dowex 1-X8 column

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TABLE I: Properties of 8-Substituted Purine Ribonucleoside Diphosphates.

Compound	Ultraviolet Absorption Properties (mμ) $\lambda_{max}^{0.1\ N\ HCl}$	$\lambda_{max}^{pH\ 7}$	$\lambda_{max}^{0.1\ N\ NaOH}$	R_{MP} in Paper Chromatography[b]	R_{MP} in Paper Electrophoresis[c]	P Analyzed Ns:Labile P_i:Total P	Yield (%)
BrADP	263.5	265	267	R_{ADP} 1.05	R_{ADP} 0.94	1.0:1.0:2.0	62
HOADP	265.4 286.5	255 (sh)[a] 270	281	R_{ADP} 0.85	R_{ADP} 1.01	1.0:0.9:2.0	44
BrGDP	263 293 (sh)	263 293 (sh)	273	R_{GDP} 1.09	R_{GDP} 0.97	1.0:2.1:2.0	69
HOGDP	247 296	247 296	258, 282	R_{GDP} 0.94	R_{GDP} 1.04	1.0:0.9:2.0	52
Me_2NGDP	266.5 294 (sh)	264 294 (sh)	271	R_{GDP} 1.44	R_{GDP} 0.98	1.0:1.0:2.0	19

[a] Sh = shoulder. [b] Performed in solvent A. [c] Performed at pH 7.5.

gave 8-oxy-AMP in 88% and 8-oxy-GMP in 32% yield.

8-Dimethylamino-GMP was synthesized from 8-BrGMP by heating with dimethylamine in anhydrous methanol for 5 hr at 115–125°. Purification of the product by column chromatography gave 24% of 8-dimethylamino-GMP. The structure of these monophosphates was confirmed by their elemental analytical data, ultraviolet absorption properties, paper chromatography, and paper electrophoresis. In the case of oxy-GMP and BrGMP direct comparison with authentic samples (Ikehara and Murao, 1968) was made.

These monophosphates were converted into the phosphoromorpholidate, which was allowed to react with P_i to give diphosphates by the method described by Moffatt and Khorana (1961). The results of ultraviolet absorption, paper chromatography, paper electrophoresis, and phosphate analyses, as well as yield of diphosphates, are summarized in Table I.

GpA was synthesized according to a modified procedure described by Lohrmann *et al.* (1966) and was a gift from Dr. E. Ohtsuka. Poly A, poly U, and ADP were purchased from Sigma Chemical Co., and GDP from P-L Biochemical Co.

Polynucleotide phosphorylase was prepared from *Escherichia coli* strain B and purified 100-fold according to the procedure described by Grunberg-Manago *et al.* (1956). Specific activity of the final preparation was 43.3 μmole of P_i per hr per mg of protein. Ultraviolet absorption ratio, A_{280}/A_{260} = 1.12.

Methods

Incubation Conditions. POLYMERIZATION OF ADP IN THE PRESENCE OF ANALOG DIPHOSPHATES. The incubation mixture contained per milliliter: 50 mM Tris-HCl (pH 8.1), 100 mM KCl, 2 mM $MgCl_2$, 2 or 4 mM ADP, 0.2–0.4 mM analog diphosphate, and 0.46–1.13 units of enzyme. Incubation was carried out at 37°.

POLYMERIZATION OF GDP IN THE PRESENCE OF ANALOG DIPHOSPHATES. The incubation mixture contained per milliliter: 50 mM Tris-HCl (pH 8.5), 0.5 mM $MnCl_2$, 2 mM GDP, 0.5–2 mM analog diphosphate, and 1.15 units of enzyme. Incubation was carried out at 60°.

POLYMERIZATION OF BROMO-ADP USING GPA AS PRIMER. The incubation mixture (2 ml) contained: 3.5 mM GpA, 10 mM bromo-ADP, 50 mM Tris-HCl (pH 9.0), 10 mM $MgCl_2$, 0.05 mM EDTA, and 2.5 units/ml of enzyme. Incubation was carried out at 37° for 19 hr. The reaction mixture was applied to filter paper (Toyo filter paper No. 51A) and chromatographed in ethanol–1 M ammonium acetate (1:1, v/v) by the descending technique. Bands corresponding to GpA, BrADP, GpApBrA, and GpApBrApBrA[1] were visualized by ultraviolet lamp, cut out, and eluted with each 3 ml of water. The ratio GpA:GpApBrA:GpApBrApBrA = 22:3:2. These oligonucleotides were characterized by digestion with RNase T_1 to give Gp and with 0.2 N alkali to give Gp, Ap, (BrAp), and BrA. Properties of these oligonucleotides together with reference GpApA and GpApApA are listed in Table II.

TABLE II: Properties of Products Obtained in the Reaction of BrADP in Polynucleotide Phosphorylase System Using GpA as Primer.

Compound	Paper Chromatography (R_F)[a]	Paper Electrophoresis (R_{GPA})[b]	Ultraviolet Absorption Properties (mμ)
GpApBrA	0.33	1.42	$\lambda_{max}^{H_2O}$ 259.5
GpApBrApBrA	0.27	1.79	$\lambda_{max}^{H_2O}$ 260
GpA	0.39	1.00	
GpApA	0.32	1.58	$\lambda_{max}^{H_2O}$ 256
GpApApA	0.19	2.10	

[a] Performed in solvent A. [b] Performed at pH 7.5.

Isolation and Characterization of Polynucleotides. The incubation conditions were as described above. The incubation was stopped at an appropriate time

[1] Abbreviations used: BrA, 8-bromoadenosine; OA, 8-oxyadenosine; BrG, 8-bromoguanosine; OG, 8-oxyguanosine; Me_2NG, 8-dimethylaminoguanosine.

TABLE III: Properties of Ribopolynucleotides Containing 8-Substituted Purine Nucleotides.

Compound	Yield (%)[a]	Ratio of Nucleotide Residue	Chain Length	$s_{20,w}$ (S)	T_m (°C)
Poly (A,G) (6:1)[b]	22.5	A:G = 74:26			59.5
Poly (A,BrA) (4:1)	25.1	A:BrA = 94:6	>100[c]		68.5
Poly (A,BrA) (2:1)	1.1	A:BrA = 83:17	>100	4.9	62.5
Poly (G,BrA) (2:1)	18.1				
Poly (A,OA) (4:1)	16.8		>100	4.7	66
Poly (A,OA) (2:1)	12.5		>100	5.2	65
Poly (G,OA) (2:1)	34.3	G:OA = 96:4			
Poly (A,BrG) (4:1)	15.8	A:BrG = 96:4			71.5
Poly (A,BrG) (2:1)	9.2	A:BrG = 82:18	>100		61.5
Poly (A,BrG) (1:1)	6.8	A:BrG = 77:23			50.5
Poly (G,BrG) (4:1)	6.0		40		
Poly (G,BrG) (2:1)	8.1	G:BrG = 82:18	40		
Poly (G,BrG) (1:1)	12.1	G:BrG = 78:22			
Poly (A,OG) (1:1)	11.9	A:OH = 97:3		7.5	72
Poly (G,OG) (1:1)	31.5				
Poly (A,Me_2NG) (2:1)	11.2		100		60.5
Poly (G,Me_2NG) (2:1)	16.7				

[a] Calculated from OD_{260} regardless of hypochromism. [b] Poly (A,G) (6:1) stands for polymer obtained from the reaction mixture containing ADP and GDP in the ratio of 6:1. [c] Estimated from the results in paper chromatography, in which absorbance of nucleoside was nearly zero.

to give maximum P_i release. The reaction mixture was extracted with one volume of 90% phenol. The water layer was extracted again with 90% phenol. The phenol layers were combined and back-extracted with a small amount of water. The water layers were combined, extracted with ether, and lyophilized. The residue was dissolved in 1.5 ml of water. If necessary, insolubles were removed by centrifugation. The solution was applied to gel filtration through a Sephadex G-50 column and eluents containing polynucleotides obtained as the first peak were lyophilized. For characterization of poly (A,BrG) and poly (A,BrA), 8 OD_{260} units was dissolved in 0.1 ml of 0.05 M glycine buffer (pH 6.5) and incubated with RNase M (Irie, 1966) (1 mg of protein) at 37° for 20 hr. For characterization of poly (G,HOA), poly (A,BrG), and poly (G,BrG), alkaline hydrolysis with 0.2 N KOH for 20 hr was employed. For characterization of poly (A,OG), 7 OD_{260} units was incubated with snake venom phosphodiesterase–*E. coli* alkaline phosphatase mixture at 37° for 10 hr. The ratio of natural nucleotides or nucleosides to analogs was obtained either by paper chromatography or by paper electrophoresis. The results were summarized in Table III.

Measurement of S Values. Polynucleotides were dissolved in 1.05 M sodium cacodylate buffer containing 0.1 M NaCl (pH 7.0). The concentration of polynucleotides was 0.2–0.3%. Centrifugation was carried out with a Hitachi UCA-IA ultracentrifuge operated at 55,430 rpm for 15 hr at 22–23°. S values were obtained from the schlieren pattern by calculation according to Schachman (1959).

Measurement of Melting Temperature. T_m's were measured with a Shimadzu AQV 50 spectrophotometer installed with a Komatsu thermostated cell. The temperature inside of the cuvet was measured by a thermister, Takara SPD-ID.

Paper Chromatography. All chromatographies were carried out on Toyo filter paper No. 51A by the ascending technique. The solvents used were (A) *n*-propyl alcohol–concentrated NH_3–water (55:10:35, v/v), (B) ethanol–1 M ammonium acetate (pH 7.0) (1:1, v/v), and (C) isopropyl alcohol–concentrated NH_3–water (7:1:2, v v).

Paper Electrophoresis. Toyo filter paper No. 51A was used. Electrophoresis at pH 7.5 was carried out in 0.05 M triethylammonium bicarbonate and at 20 or 60 V/cm for 30–90 min.

Results and Discussion

Homopolymerization of 8-Substituted Diphosphates. In contrast to our expectation, 8-bromo-ADP, 8-oxy-ADP, 8-bromo-GDP, 8-oxy-GDP, and 8-dimethylamino-GDP would not give rise to homopolynucleotides by the catalysis of polynucleotide phosphorylase. Although incubation temperature and the amount of ingredients in the reaction mixture were varied, release of P_i was negligible and polynucleotide could not be obtained. These results are rather surprising, because polynucleotide phosphorylase was thought to be able to utilize a wide variety of diphosphates as substrates. The cause of this phenomenon could be interpreted in two ways: (1) by the distortion of large substituents situated at 8 position with the enzyme active site(s), and (2) by the different confor-

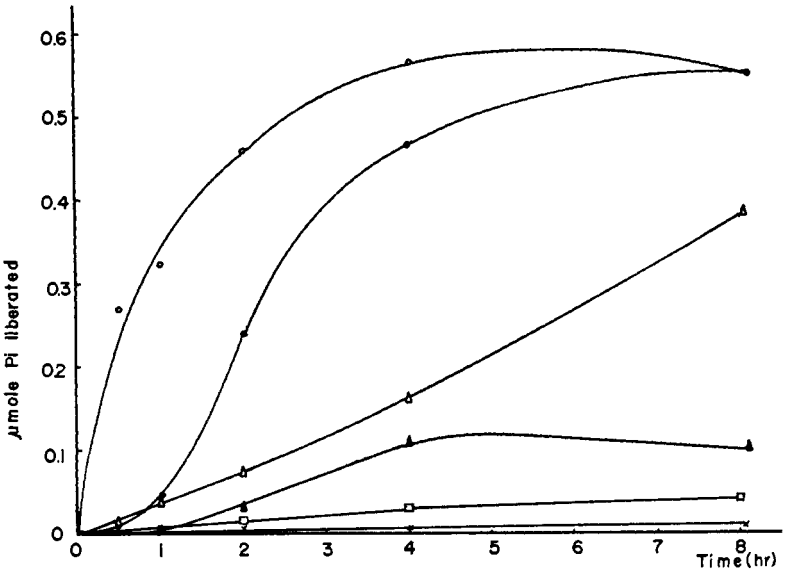

FIGURE 1: Polymerization of ADP in the presence of various amount of BrADP. The incubation mixture (0.25 ml) contained: Tris-HCl (pH 8.1), 50 mM; KCl, 100 mM; $MgCl_2$, 2 mM; ADP, 4 mM; BrADP, 0–4 mM; and polynucleotide phosphorylase, 0.0066 mg. Incubation was at 37°. (O-O-O) ADP, (●-●-●) ADP plus mM 0.4 BrADP, (Δ-Δ-Δ) ADP plus 1 mM BrADP, (▲-▲-▲) ADP plus 2 mM BrADP, (□-□-□) ADP plus 3 mM BrADP, and (×-×-×) ADP plus 1 mM BrADP.

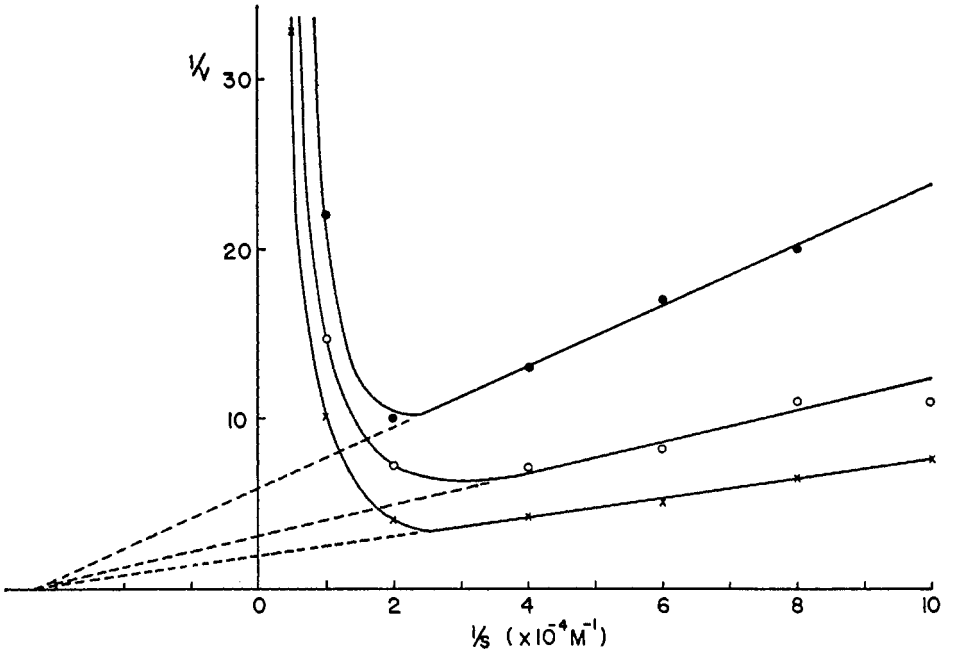

FIGURE 2: Lineweaver–Burk plot of the polymerization of ADP and BrADP. Incubation conditions were as in Figure 1. (●-●-●) 4mM ADP plus 0.4 mM BrADP, (O-O-O) 4mM ADP plus 0.2 mM BrADP, and (×-×-×) 4 mM ADP.

mation of analog diphosphates from that of naturally occurring diphosphates. The slow rate of incorporation encountered in the polymerization of formycin diphosphate (T. Fukui, 1967, unpublished experiments) suggested the possibility of the latter view. An alternate explanation assuming the presence of inhibitor(s) in the preparation of diphosphates could be eliminated by the fact that in the copolymerization with ADP or GDP, these analog diphosphates were readily polymerized and by the exchange reaction of BrADP with ^{32}P which was catalyzed by polynucleotide phosphorylase.

Copolymerization of 8-Substituted Diphosphates with ADP or GDP. As shown in Figure 1, BrADP could be polymerized in the copresence of ADP to afford a random copolynucleotide, poly (A,BrA). The rate of polymerization and the yield of polynucleotides decreased with increased amount of BrADP in the incubation mixture. This inhibitory action of BrADP could be interpreted by the slow incorporation of a BrA residue into the 3′-OH end of preexisting primer. The experiment using GpA as the primer clearly demonstrated the difficulty in the extensive chain elongation on the 3′-OH of BrA. Therefore according to increased

incorporation of BrA residue into polynucleotide, the rate of polymerization decreased. The failure in obtaining homopolymers of BrA could be interpreted in the same way. When the rate of the reaction was plotted against substrate quantity according to Lineweaver–Burk (1934) (Figure 2), the mode of inhibition appeared to be noncompetitive. However, simultaneous incorporation of inhibitor mononucleotide unit into the product should be expressed in a more complex mode.

As was previously observed in the polymerization of GDP (Fresco and Su, 1962), poly G having a large number of nucleotide units could not be obtained by the incubation of GDP in the usual condition. However, copolymerization of GDP with other nucleoside diphosphates led to the formation of a long polynucleotide (Bretcher and Grunberg-Manago, 1962). A long poly G was synthesized by Thang *et al.* (1965) using elevated temperature and Mn^{2+} ion in the incubation mixture. Therefore, the polymerization of BrADP in the presence of GDP was investigated in this condition. As shown in Figure 3, the rate of the reaction was retarded significantly as compared with that of the polymerization of GDP alone. These results suggested

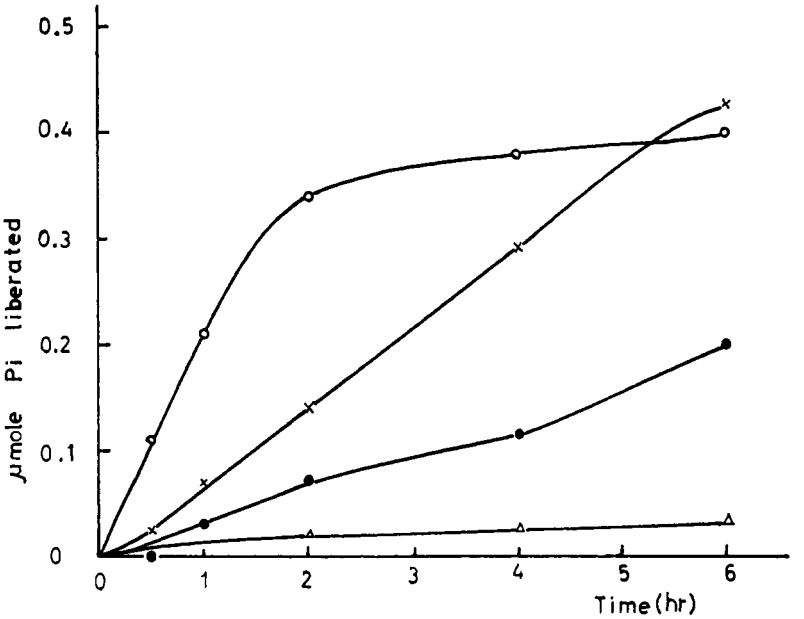

FIGURE 3: Polymerization of GDP in the presence of BrADP. The incubation mixture contained (0.25 ml): Tris-HCl (pH 8.5), 50 mM; $MnCl_2$, 0.5 mM; GDF, 2 mM; BrADP, 0–2 mM; and enzyme, 0.0036 mg. Incubation was at 60°. (O-O-O) 2 mM GDP, (×-×-×) 2 mM GDP plus 0.5 mM BrADP, (△-△-△) 2 mM GDP plus 1 mM BrADP, and (□-□-□) 2 mM GDP plus 2 mM BrADP.

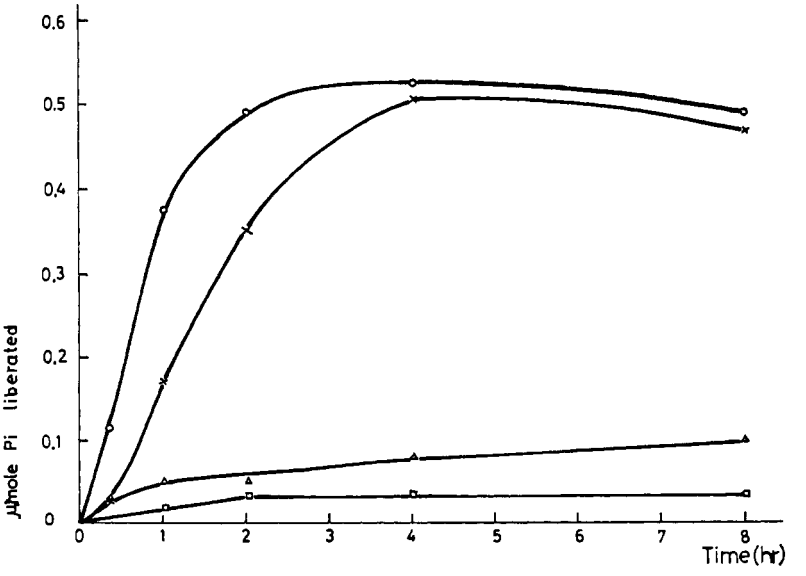

FIGURE 4: Polymerization of ADP in the presence of 8-substituted GDP. The incubation mixture contained (0.5 ml): Tris-HCl (pH 8.5), 50 mM; HCl, 100 mM; $MgCl_2$, 2 mM; ADP, 4 mM; 8-substituted GDP, 2 mM; and enzyme, 0.0036 mg. Incubation was at 37°. (O-O-O) ADP, (×-×-×) ADP plus HOGDP, (△-△-△) GDP plus BrGDP, and (□-□-□) GDP plus Me_2GDP.

TABLE IV: Ultraviolet Absorption Properties of Polynucleotides Containing 8-Substituted Nucleotides.

Compound	λ_{max}[a] (mμ)	Ratio of Absorbance		
		A_{270}/A_{260}	A_{280}/A_{260}	A_{300}/A_{260}
Poly A	256.5	0.67	0.32	
Poly (A,BrA) (4:1)[b]	258	0.71	0.32	
Poly (A,OA) (4:1)	257	0.67	0.31	
Poly (A,OA) (2:1)	257	0.67	0.32	
Poly (A,BrG) (2:1)	257	0.70	0.35	
Poly (A,BrG) (1:1)	257.5	0.72	0.37	
Poly (A,OG) (1:1)	256.5	0.66	0.31	
Poly (A,Me_2NG) (2:1)	257	0.68	0.33	
Poly G	253		0.53	0.16
Poly (G,BrA) (2:1)	255		0.59	0.11
Poly (G,OA) (2:1)	253.5		0.55	0.15
Poly (G,BrG) (4:1)	253.5		0.53	0.14
Poly (G,BrG) (2:1)	254		0.59	0.16
Poly (G,BrG) (1:1)	255		0.59	0.17
Poly (G,OG) (1:1)	253		0.54	0.18
Poly (G,Me_2NG) (2:1)	253		0.53	0.17

[a] Measured in 0.0125 M sodium cacodylate at pH 7.0. [b] Poly (A,BrA) (4:1) stands for polynucleotide obtained by the incubation of ADP and BrADP in the ratio of 4:1.

that the same type of reaction as observed in poly (A,BrA) had occurred in the case of poly (G,BrA) polymerization.

Figure 4 showed the same retarding tendency of analog GDPs having Br-, HO-, and dimethylamino groups in the 8 position in polymerization of ADP. Analogs of ADP having 8-Br and 8-OH also inhibited the polymerization of GDP.

Therefore, large substituents in position 8 of these analogs exerted equally significant inhibitory effects on polymerization of natural diphosphates, ADP or GDP, though the analog residues were incorporated to a certain extent into copolynucleotides.

Ultraviolet Absorption Properties of Polynucleotides Containing Analogs. The ultraviolet absorption properties of copolynucleotides were summarized in Table IV. In polynucleotides of the poly A series, λ_{max} was in the range of 256.5–268 mμ, which was close to the λ_{max} of poly A (256.5 mμ). However A_{270}/A_{260} significantly differs from the parent poly A according to the content of analog residues. The same tendencies were found also in poly G analog series.

Thermal Stability of the Secondary Structure of Copolynucleotides. Poly A has the so-called "acid form" in acidic media due to the protonation at N^1 of the adenine ring and stabilization of the double-helical structure (Fresco and Klemperer, 1959; Rich *et al.*, 1961). Therefore a study of the influence of replacing the A or G residue in the poly A or poly G chain by 8-substituted analogs on thermal stability seems to be of interest.

As shown in Figure 5, T_m's of the acid form of copolynucleotides derived from poly A were lowered according to the content of analogs. In poly (A,BrA), replacement of 17% of A by BrA lowered the T_m for 12.5° compared with that of poly A. In copolynucleotides containing OA, BrG, OG, and Me_2NG, lowering of the T_m's was also observed. When the A residue in poly A was replaced by BrG or OG to the extent of 18 and 3%, the T_m's were lowered for 13.5 and 3°, respectively.

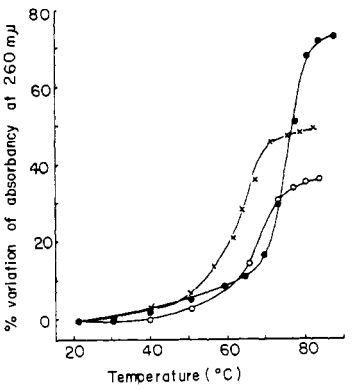

FIGURE 5: Temperature-absorbance profile of poly A and poly (A,BrA). (●—●—●) poly A, (○—○—○) poly (A,-BrA) (4:1), and (×—×—×) poly (A,BrA) (2:1).

The cause of this lowering effect could not be elucidated as yet. A large substituent in the 8 position of the analog would labilize the double-helical structure of the poly A acid form. The cooperative melting of these polynucleotides suggested that the incorporation of analogs was random.

The T_m of copolynucleotides containing BrG, OG, and Me_2NG residues in the poly G chain was also studied. In these cases all T_m's were above 95° and

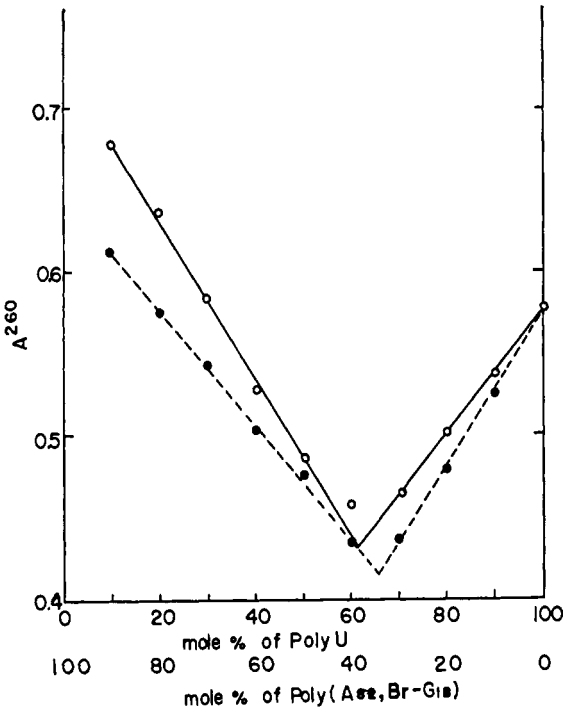

FIGURE 6: Absorbance of the solution containing poly (A,G) and poly U in various ratios. (O—O—O) In the presence of) 0.1 M NaCl–0.05 M sodium cacodylate (pH 7.0) and in the initial solution containing 0.01 M $MgCl_2$ and (●—●—●) 17 hr after mixing in the presence of Mg^{2+}.

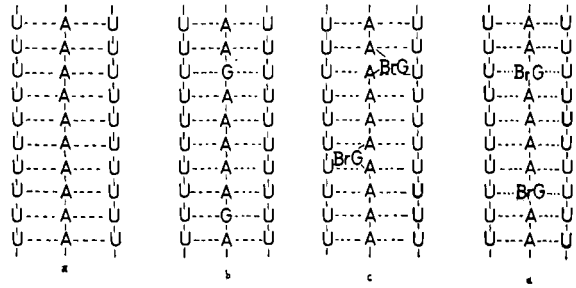

FIGURE 7: Schematic representation of triple-stranded complex with or without loop. (a) Formed from poly A and poly U in conditions I and II. (b) Formed from poly (A,G) and poly U in I and II. (c) Formed from poly (A,BrG) and poly U in I and early stage in II. (d) Formed from poly (A,BrG) and poly U in II. Condition I: 0.1 M NaCl plus 0.01 M sodium cacodylate (pH 7.0). Condition II: 0.1 M NaCl plus 0.01 M sodium cacodylate (pH 7.0) plus 0.01 M $MgCl_2$.

significant differences between poly G and copolynucleotides could not be detected.

Formation of Complex by Poly (G,BrA) with Poly U. As was studied by a number of investigators, a stable triple-stranded complex, (poly A)–(poly $U)_2$, was formed between poly A and poly U in a solution containing 0.1 M NaCl at pH 7.0. As shown in Figure 6, poly (A,BrG), in which the ratio of A:BrG was 77:23, formed a triple-stranded complex with poly U in the ratio of poly (A,BrG):poly U = 39:61. This value was consistent with the calculated value of 38:62, assuming the looping out of the BrG residue from (poly A,G)–(poly $U)_2$ structure as illustrated in Figure 7c. In the presence of Mg^{2+}, this helix with loop in the initial mixture inverted gradually to the triple-stranded helix without loop as in Figure 7d after standing for 17 hr. Therefore, introduction of the Br atom in position 8 of the G residue seems to labilize G–U hydrogen bonding in the triple-stranded complex and result in the formation of the complex with loop. The possibility of the formation of the hydrogen bonding between G and U was predicted by Crick (1966) and substantiated by experiments by Tsuboi *et al.* (1967) in the double-helical complex, poly (A,G)–poly U.

When the T_m of this (poly A,BrG)–(poly U) complex was compared with that of the (poly A)–(poly $U)_2$ complex in the same condition, lowering of 5–8° was observed (Figure 8). This is in accordance with the observation (Tsuboi *et al.*, 1967) that the complex with loop is more labile than the normal helical complex.

The results described in this paper clearly demonstrated that large substituents in the 8 position of purine ribonucleoside diphosphates changed their nature as the substrate of polynucleotide phosphorylase and resulted in the inhibition of polymerization. Although these nucleotide analogs were incorporated into polynucleotide in the copolymerization with ADP or with GDP, the extent of the incorporation and the rate of reaction were retarded significantly. Furthermore, thermal stability of the polynucleotide containing analogs decreased relative to that of the parent polynucleotide.

Whether these effects are due to the sterical distortion of large substituents in the 8 position or to the total conformation change in these analogs should be clarified by further investigations. The fact that some of the 8-substituted purine nucleosides have inhibitory effects against growth of the cancer cells might be of interest in relation to the present observations.

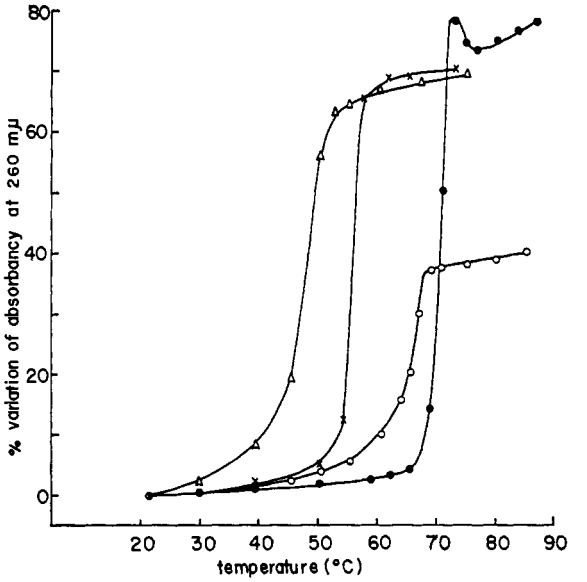

FIGURE 8: Temperature–absorbance profile of triple-stranded complex. (Poly A)–(poly U) and (poly (A,BrG)–(poly $U)_3$. (×—×—×) (Poly A)–(poly $U)_2$ in condition I of this figure, (△—△—△) (poly (A,BrG)–(poly $U)_2$ in condition I, (●—●—●) (poly A)–(poly $U)_2$ in condition II, and (O—O—O) (poly A,BrG)–(poly $U)_2$ in condition II.

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