[Chem. Pharm. Bull. 31(2) 527—537 (1983)]

Nucleotides. XIX.[1] Synthesis and Properties of Poly-2-alkyladenylic Acids. II. Interactions with Poly (br^{5}U) or Poly (I)

MASAO HATTORI,*[,a] KAZUHARU IENAGA,[a] and WOLFGANG PFLEIDERER[b]

Research Institute for Wakan-Yaku [Oriental Medicines] Toyama Medical and Pharmaceutical University,[a] 2630 Sugitani, Toyama 930-01, Japan and Fakultät für Chemie der Universität Konstanz,[b] Postfach 5560, D-7750 Konstanz, West Germany

(Received August 10, 1982)

Contrary to earlier reports on the interaction between polyadenylic acid (poly(A)) and poly-5-bromouridylic acid (poly(br^{5}U)), poly(A) was demonstrated to form both 1:1 and 1:2 complexes with poly(br^{5}U) in 0.1 M Na^+, pH 7.3, at 25°C by reconstructing mixing curves.

Under comparable conditions, newly synthesized poly-2-methyladenylic acid (poly(m^{2}A)) and poly-2-ethyladenylic acid (poly(e^{2}A) formed only 1:1 complexes with poly(br^{5}U), possibly having non Watson-Crick base pairing due to steric hindrance between the C(2)-alkyl substituents of adenine and the C(2)-carbonyl oxygen of bromouracil in polynucleotides, whereas poly-2-isopropyladenylic acid (poly(iso-pr^{2}A)) did not interact with poly(br^{5}U).

On the other hand, with polyinosinic acid (poly(I)), poly(m^{2}A) formed a 1:2 complex with hydrogen bonding at both N(1) and N(7) of 2-methyladenine, similar to the structure of poly(A)·2poly(I), whereas poly(e^{2}A) formed only a 1:1 complex, possibly having non Watson-Crick type base pairing due to steric hindrance of a bulkier substituent which blocks pairing at the N(1) bonding site of 2-ethyladenine to hypoxanthine, and poly(iso-pr^{2}A) again gave no complex at all. The ultraviolet (UV) and circular dichroism (CD) spectra and thermal stabilities of these duplexes and triplexes were compared under various conditions.

Keywords——poly-2-methyladenylic acid; poly-2-ethyladenylic acid; poly-2-isopropyladenylic acid; polyadenylic acid; polyinosinic acid; non Watson-Crick base pairing; polynucleotide interaction

Introduction of a bulky group at the C(2)-position of polyadenylic acid (poly(A)) results in failure to develop normal Watson–Crick (A)·(U) helices,[3] but Hoogsteen[4] (or reverse Hoogsteen) type helices form instead.[5,6] In the course of investigation of such a non Watson–Crick interaction in polynucleotide systems, we have further reported the enzymic synthesis and properties of poly-2,N^6-dimethyladenylic acid (poly($m_2^{2,6}$ A)).[7] For ease of spectroscopic comparisons, alkyl substituents are preferred to other bulky groups. Since an alkyl group at the C(2)-position of adenine base would not signifciantly alter the original electric transition moments, simple comparisons of ultraviolet (UV) and circular dichroic (CD) spectral changes among homopolynucleotides or their complexes reveal relatively undisturbed pictures of the molecular interactions. For this reason, we have synthesized a series of poly-2-alkyladenylic acids and have studied some of their properties as homopolynucleotides.[8] These polynucleotides were shown to have single-stranded stacked structure under neutral conditions by the criteria of percent hypochromicity, circular dichroism and thermal melting profile, and to form stable double-helical structures under acidic conditions.[8] It is of interest that one of these polynucleotides, poly-2-isopropyladenylic acid (poly(iso-pr^{2}A)) was demonstrated to inhibit the ribonucleic acid (RNA)-directed deoxyribonucleic acid (DNA) polymerase (reverse transcriptase) activity of Moloney murine leukemia virus, though others, poly-2-methyladenylic acid (poly(m^{2}A)) and poly-2-ethyladenylic acid (poly(e^{2}A)), did not markedly affect the activity.[9]

In the present paper, the interactions of these poly-2-alkyladenylic acids with poly-5-bromouridylic acid (poly(br^{5}U)) and polyinosinic acid (poly(I)), and comparisons of their

complexes are reported in connection with the effects of a series of alkyl substituents on the complex formation.

Materials and Methods

Poly(m^2A), poly(e^2A) and poly(iso-pr^2A) were synthesized as reported in a preceding paper.[8] Poly(A) and poly(I) were purchased from Boehringer Mannheim GmbH (Germany). Poly(br^5U) was synthesized according to the method of Riley *et al.*[10] All polynucleotides used in these experiments were determined to have chain lengths of over 100 residues by alkaline digestion, followed by quantitative analysis of nucleoside and 2′(3′)-nucleotides. Extinction coefficients ($\varepsilon(p)$) are presented in values per residue on the basis of phosphate analysis.[11] A standard phosphate solution (1 μmol/ml) for quantitative phosphate analysis was obtained from Serva Feinchemica (Germany). $\varepsilon(p)$'s of poly(m^2A), poly(e^2A) and poly(iso-pr^2A) were 9100 at 257 nm, 8900 at 256 nm and 9300 at 256 nm, respectively, in 0.1 M Na^+, pH 7.3, at 25°C.[8]

UV spectra were measured with a Cary 15 spectrometer. CD was measured with a Cary 60 spectropolarimeter with a CD attachment using a 5 mm path-length cell. pH values were determined with an Orion Research digital pH meter model 801.

Melting temperatures (T_m) of polynucleotide complexes were measured with a Cary 15 spectrometer equipped with a cell holder in which the temperature was controlled by circulating water. Temperatures inside the cell were measured with a Mettler digital thermometer, TM 15, using a Glass supermax 8409 temperature sensor.

Mixing curves were constructed in various salt concentrations, pH 7.3, at 25°C by varying the ratio of each component in a separate solution for each point. The UV spectra of these mixtures were measured at 17—18 h and 7 d after mixing.

For kinetic studies of the complex formations, two complimontary polynucleotides were mixed in the presence of 0.11 M Na^+, pH 7.3, at 25°C. Complex formation was monitored by measuring UV spectral changes at appropriate wavelengths.

Results

I. Interactions of Poly(A) and Poly-2-alkyladenylic Acids with Poly(br^5U)

Stoichiometry——For the purpose of re-investigating the stoichiometry of the complexes between poly(A) and poly(br^5U), mixing curves were constructed in 0.1 M Na^+, pH 7.3, at 25°C. In the range of 0—50 mol% poly(br^5U), the mixtures showed continuous changes of UV absorbance with definite isosbestic points at 217.5 and 275.5 nm (Fig. 1A). With stepwise increase of the ratio from 50 to 65 mol% poly(br^5U), the isosbestic point at 275.5 nm changed to 285 nm (Fig. 1B), and in the range of 66.7—100 mol% poly(br^5U), new isosbestic points appeared at 235, 258 and 308 nm (Fig. 1C). When the absorbances at 275.5 and 285 nm were plotted (Fig.2), breaks at 50 mol% poly(br^5U) and 67 mol% poly(br^5U) were clearly observed indicating the existence of a 1:1 complex and a 1:2 complex, respectively.

In contrast to the case of the poly(A)/poly(br^5U) system, the mixtures containing various mol ratios of poly(m^2A) and poly(br^5U) showed quite different UV spectral changes (Fig.3). In the range of 0—50 mol% poly(br^5U), the UV absorbance changed continuously with isosbestic points at 217 and 277.6 nm (Fig. 3A). On increasing the ratio from 50 to 100 mol% poly(br^5U) (Fig. 3B), a new isosbestic point appeared at 261 nm, indicating that only two species existed in each polymer mixture. Plots of the mixing curves at each wavelength (Fig. 4) showed only one break at 50 mol% poly(br^5U), and no break at 66.7 mol% poly(br^5U) as observed in the case of the interaction between poly(A) and poly(br^5U). These findings indicate that poly(m^2A) formed only a 1:1 complex with poly(br^5U), as in the case of the interaction with polyuridylic acid(poly(U)).[5]

Similar results were found for the interaction between poly(e^2A) and poly(br^5U). The mixing curves at each wavelength (Fig. 5) showed only one break at 50 mol% poly(br^5U) and there was no other break indicating a 1:2 complex. This was confirmed by consideration of the isosbestic points of the UV spectra of various mixtures. Definite isosbestic points were observed at 217, 235.5 and 276 nm in the range of 0—50 mol% poly(br^5U) and at 220, 234 and 261

nm in the range of 50—100 mol% poly(br^5U) (data not presented). Poly(e^2A) was therefore concluded to form only a 1:1 complex analogous to the poly(m^2A/poly(br^5U) interaction.

On the other hand, the interaction between poly(iso-pr^2A) and poly(br^5U) was quite different from those of poly(A), poly(m^2A), and poly(e^2A). No complex formation with poly(br^5U) could be detected in the presence of 0.1, 0.2 and 0.48M sodium ion concentrations at 25°C. The UV spectra of the continuous mixtures showed isosbestic points at 220, 233.5 and 268 nm over the whole range, indicating that each mixture contained two components as independent species without any mutual interaction.

Kinetics of Complex Formation——The complex formation was kinetically measured by means of UV spectroscopy. Poly (m^2A) and poly(e^2A) as well as poly(A) formed complexes with poly(br^5U) immediately after mixing. The half-time of complex formation was less than 1 min in each case under the conditions of 0.11M Na^+, pH 7.3, at 25°C (Table I). Even after 5 d of mixing, the UV spectra of these solutions showed no further change, indicating that these were kinetic end points. On the other hand, the UV spectra of a 1:1 mixture of poly(iso-pr^2A) and poly(br^5U) did not change at all up to 5 d after mixing in comparison with that measured before mixing.

UV Spectra——Table II shows UV spectral data for these complexes. The poly(A)·poly(br^5U) duplex had λ_{max} at 257 nm and a shoulder at 280 nm, while poly-(m^2A)·poly(br^5U) and poly(e^2A)·poly(br^5U) had λ_{max} at 259 nm and an indistinct shoulder at around 275—280 nm. λ_{max}'s of these duplexes were almost equally shifted by 3—3.5 nm hypsochromically from those of the summation spectra. On the other hand, λ_{max} of the triplex of poly(A)·2poly(br^5U) was shifted by 7.5 nm hypsochromically, the shift being greater than the shift values for the duplexes. The hypochromicities caused by the complex formation were in the range of 22—26% for these duplexes but as much as 42% for the triplex. These findings indicate that the base stacking tendencies of poly (m^2A)·poly(br^5U) and poly(e^2A)·poly(br^5U) as well as of poly (A)·poly(br^5U) were all quite similar, but significantly different from that of poly(A)·2poly(br^5U).

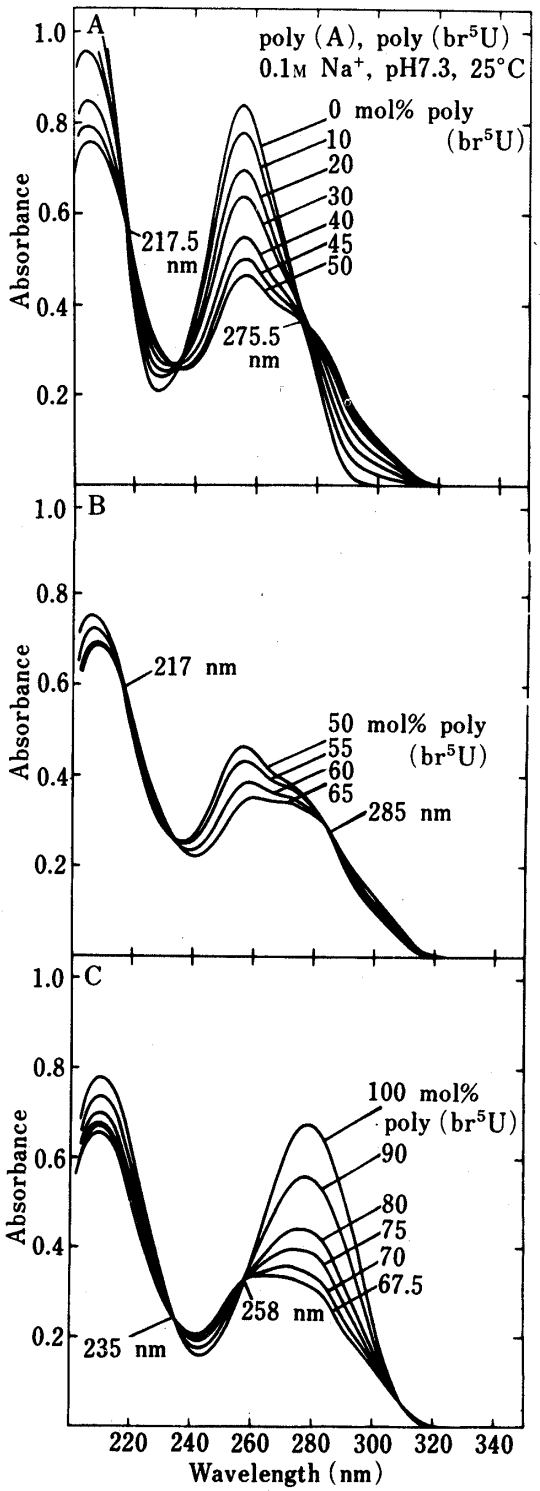

Fig. 1. UV Spectra of Mixtures of Poly(A) and Poly(br^5U) in 0.1 M Na^+ and 0.005 M Phosphate, pH 7.3 at 25°C

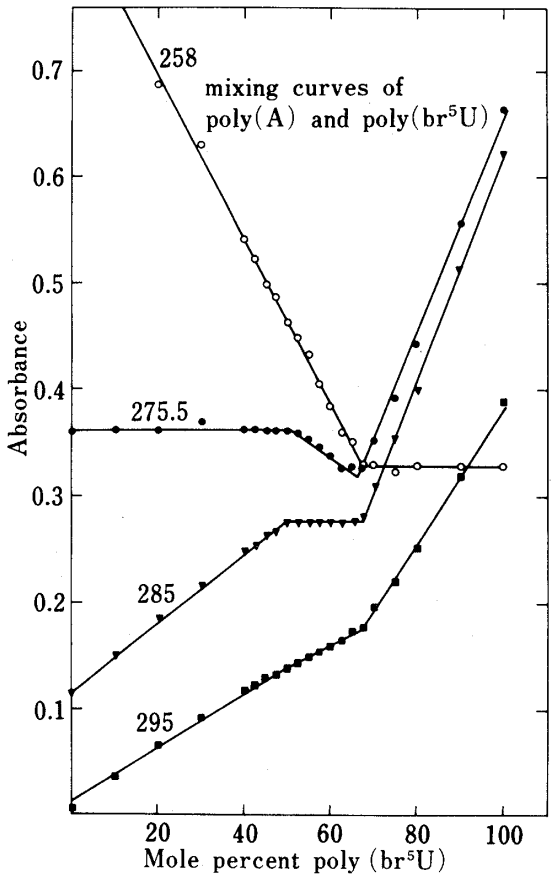

Fig. 2. UV Mixing Curves of Poly(A) and Poly (br^5U) in 0.1 M Na^+ and 0.005 M Phosphate, pH 7.3 at 25°C

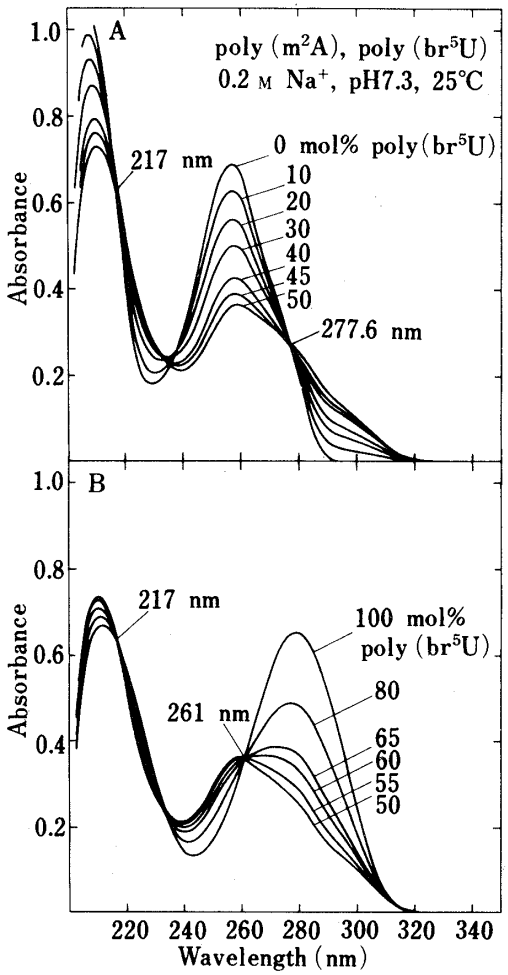

Fig. 3. UV Spectra of Mixtures of Poly(m^2A) and Poly(br^5U) in 0.2 M Na^+ and 0.005 M Phosphate, pH 7.3 at 25°C

The mixing curves were essentially identical in the presence of either 0.1 M Na^+ or 0.2 M Na^+.

CD Spectra——CD spectra of the 1:1 complexes of poly(m^2A)·poly(br^5U) and poly(e^2A)·poly(br^5U) showed peaks at 222.5 and 267—268 nm, troughs at 210 and 250 nm, and shoulders at 230 and *ca.* 295—298 nm, which were quite similar for each complex in shape and in magnitude, whereas that of the mixture of poly(iso-pr^2A) and poly(br^5U) clearly differed in these features (Fig. 6). The peak at 267—268 nm of these complexes was bathochromically shifted by 2—5 nm as compared to the summation spectra of the two components, while the corresponding peak at 262.5 nm of poly(A)·poly(br^5U) was hypsochromically shifted by 2 nm from that of the summation spectrum. Furthermore, these peaks of the former complexes were little changed in magnitude, but that of the latter was greatly increased in magnitude by the complex formation. Fig. 7 shows the CD difference spectra resulting from the complex formation, which were calculated by subtracting the CD summation spectrum of the two components from the spectrum of the complex. The general features of the difference spectra for poly(m^2A)·poly(br^5U) and poly (e^2A)·poly(br^5U) were again quite similar in shape, but were distinct from that of poly(A)·poly(br^5U).

Thermal Stability——The UV-temperature melting profiles of poly(m^2A)·poly(br^5U) and poly(e^2A)·poly(br^5U) were sharp and formed sigmoid curves indicaing that these complexes dissociated cooperatively. Under conditions where a poly(A)·poly(br^5U) duplex melts with two step transitions (T_m=64 and 91°C in 0.1 M Na^+, pH 7,[10] these complexes melted with a

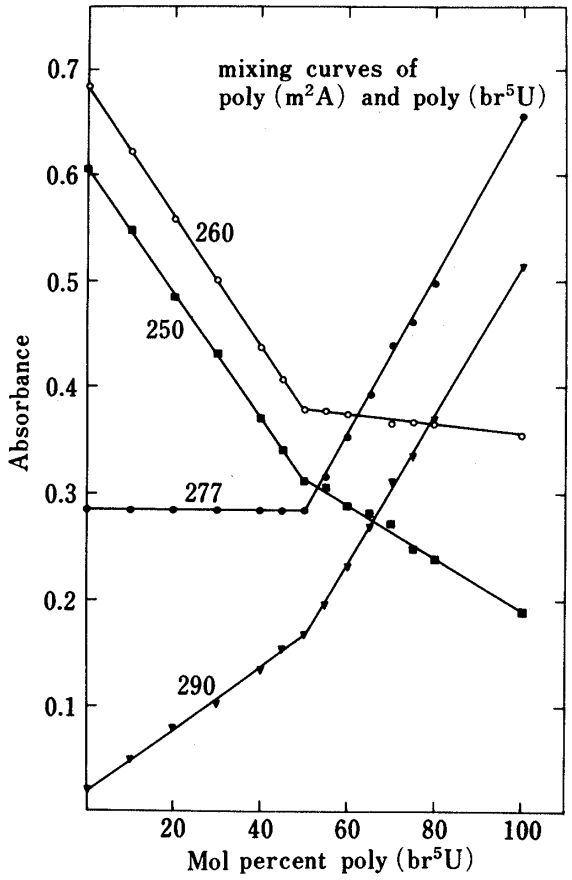

Fig. 4. UV Mixing Curves of Poly(m^2A) and Poly-(br^5U) in 0.11 M Na^+ and 0.005 M Phosphate, pH 7.3 at 25°C

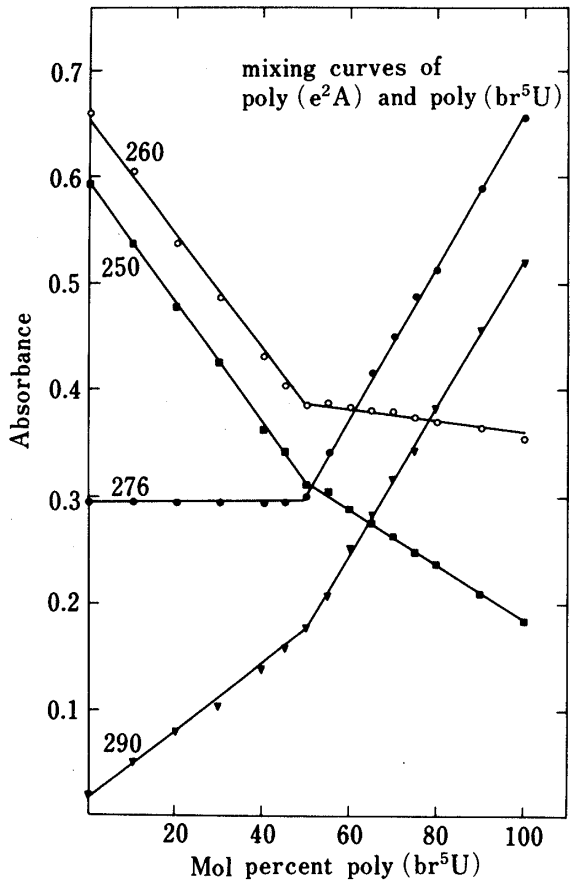

Fig. 5. UV Mixing Curves of Poly(e^2A) and Poly(br^5U) under the Same Conditions as described in Fig. 4

TABLE I. Half-time of Complex Formation in 0.11 M Na^+, pH 7.3, at 25°C

Components				Half-time for complex formation	Percent completion at 3 h after mixing
Materials	Concentration (M)	Materials	Concentration (M)		
Poly(A)	0.8×10^{-4}	Poly(br^5U)	0.8×10^{-4}	<1 min	100%
Poly(m^2A)	0.8×10^{-4}	Poly(br^5U)	0.8×10^{-4}	<1 min	100%
Poly(e^2A)	0.8×10^{-4}	Poly(br^5U)	0.8×10^{-4}	<1 min	100%
Poly(iso-pr²A)	0.8×10^{-4}	Poly(br^5U)	0.8×10^{-4}	——	0%
Poly(A)	0.4×10^{-4}	Poly(I)	0.8×10^{-4}	20 min	59%
Poly(m^2A)	0.4×10^{-4}	Poly(I)	0.8×10^{-4}	2 min	97%
Poly(e^2A)	0.4×10^{-4}	Poly(I)	0.4×10^{-4}	4 min	76%
Poly(iso-pr²A)	0.4×10^{-4}	Poly(I)	0.4×10^{-4}	——	0%

single transition at all wavelengths examined, giving T_m=49.4 and 45.2°C for poly(m^2A)·poly(br^5U) and poly(e^2A)·poly(br^5U), respectively. Fig. 8 shows the dependence of T_m upon the sodium ion concentration. The thermal stability of the complexes was a linear function of sodium ion concentration and had the same slope $dT_m/d\log[Na^+]$=21°C in both cases. The complex of poly(e^2A)·poly(br^5U) was equally destabilized by 4°C compared to that of poly(m^2A)·poly(br^5U) under various salt conditions.

II. Interactions of Poly-2-alkyladenylic Acids with Poly(I)

Stoichiometry——In order to determine the stoichiometry of the complexes of poly-2-alkyladenylic acids with poly(I), mixing curves were constructed in 0.11 M Na^+, pH 7.3, at

TABLE II. Spectroscopic Data for Polynucleotide Complexes

Complexes	UV spectra λ_{max} (nm)	ϵ (p)	$\Delta\lambda_{max}$[b] (nm)	Hypo-chromicity[c]
Poly(A)·poly(br^{5}U)[a]	206	9300		
	257	5700	−3	22%
	280sh	4200		
Poly(m^{2}A)·poly(br^{5}U)	209	10300		
	259	5000	−3.5	26%
	275sh	4100		
Poly(e^{2}A)·poly(br^{5}U)	209	10700		
	259	4900	−3.5	22%
	280sh	3700		
Poly(A)·2poly(br^{5}U)	218	8100		
	265	4200	−7.5	42%
	285sh	3500		
Poly(A)·2poly(I)	251	7900	+1	24%
Poly(m^{2}A)·2poly(I)	251	7700	+1	25%
Poly(e^{2}A)·poly(I)	252	7400	+1	19%

a) Na^+, 0.11 M; phosphate, 0.005 M, pH 7.3, 25°C.
b) $\Delta\lambda_{max}=\lambda_{max}$ (polymer)$-\lambda_{max}$ (summation)
c) hypochromicity at λ_{max} $h=(1-\epsilon_{polymer}/\epsilon_{monomer})\times 100$.

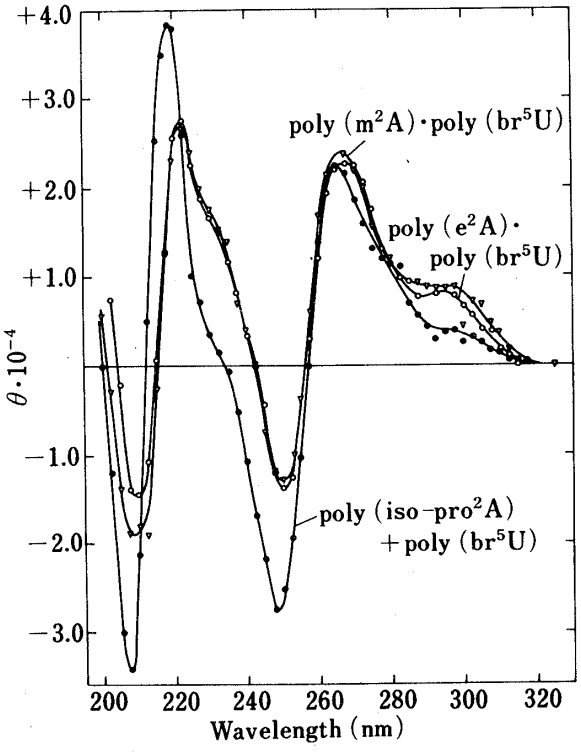

Fig. 6. CD Spectra of 1:1 Complexes and a Mixture in 0.11 M Na^+, pH 7.3, at 25°C

—△—, poly(m^{2}A)·poly(br^{5}U);
—○—, poly(e^{2}A)·poly(br^{5}U);
—●—, poly(iso-pr^{2}A)+poly(br^{5}U).

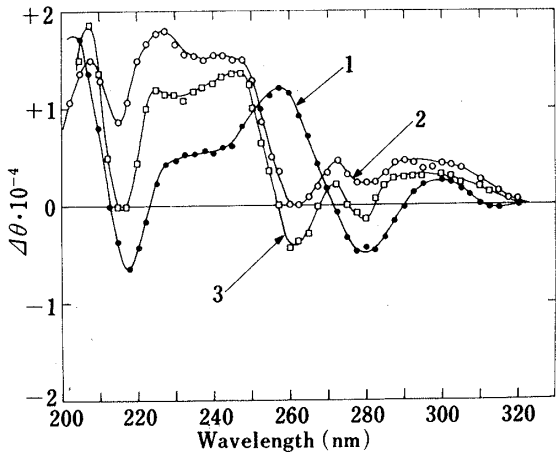

Fig. 7. CD Difference Spectra

CD spectra were measured under the conditions described in Fig. 6.
$\Delta\theta=\theta_{complex}-\theta_{summation}$
1, poly(A)/poly(br^{5}U);
2, poly(m^{2}A)/poly(br^{5}U);
3, poly(e^{2}A)/poly(br^{5}U).

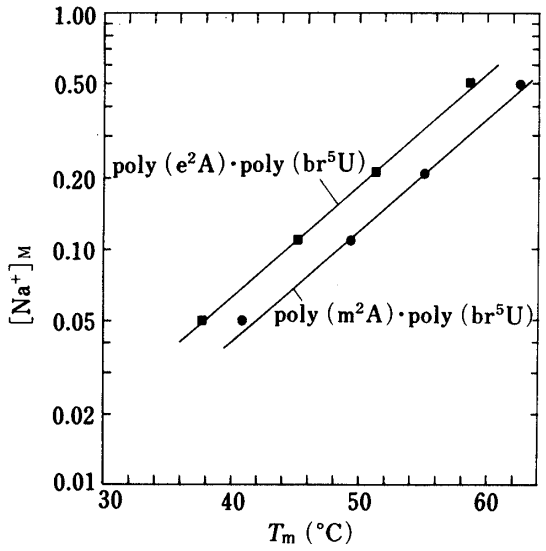

Fig8. Salt Dependence Curves of T_m's for Poly-(m^2A)·Poly(br^5U) (●) and Poly(e^2A)·Poly-(br^5U) (■)

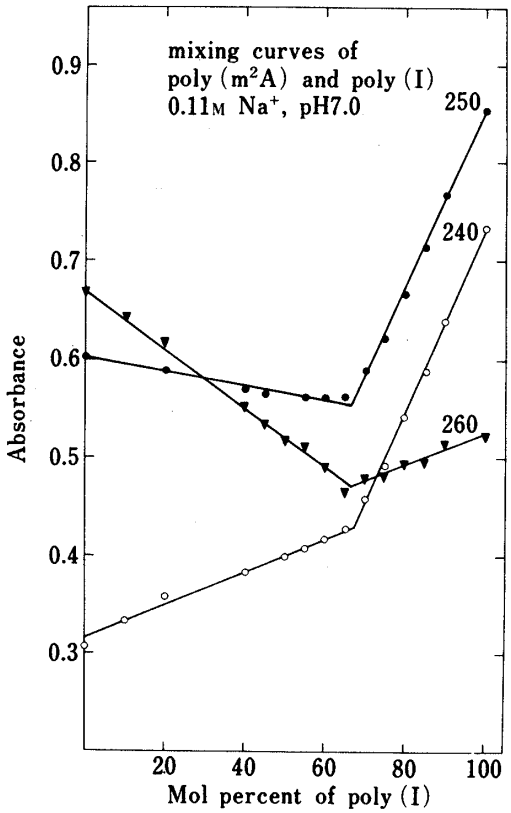

Fig. 9. UV Mixing Curves of Poly(m^2A) and Poly(I) in 0.11 M Na^+, 0.005 M Phosphate, pH 7.3, at 25°C

25°C. The mixing curves of poly(m^2A) and poly(I) showed only one break at 66.5—67.5 mol% of poly(I), and no break at 50 mol% poly(I), indicating that a 1:2 complex (1(m^2A) to 2(I)) was formed (Fig. 9). The UV spectra of the mixtures containing 0—65 mol% poly(I) were characterized by three isosbestic points at 215, 248 and 280 nm and those of mixtures containing 70—100 mol% poly(I) by two isosbestic points at 223 and 264 nm (data not presented).

A new situation, however, arose from the interaction of poly(e^2A) and poly(I). The mixing curves (Fig. 10) showed only a break at 50 mol% poly(I) in 0.11 M Na^+ at 7.3 after 30 h of mixing , providing a 1:1 stoichiometry of (e^2A):(I). This was further confirmed by the isosbestic behavior of the UV spectra taken of the mixtures with 0—50 mol% and 50—100 mol% poly(I) (*i.e.*, isosbestic points at 250 and 280 nm, and at 227 and 260 nm, respectively).

Unlike poly(A), poly(m^2A) and poly(e^2A), poly(iso-pr^2A) did not form any complex with poly(I) in 0.1 and 0.2M Na^+, pH 7.3, at 25°C. The UV spectrum of the 1:1 mixture did not change even after 7 d when compared to the summation spectrum of the two components.

Kinetics of Complex Formation———The kinetics of complex formation (Table I) was measured from the time-dependent UV spectral change of each mixture. Poly(m^2A) formed a triplex with poly(I) more rapidly than poly(A) *i.e.*, the half-time of the former complex was 2 min, and that of the latter was 20 min. On the other hand, poly(e^2A) formed duplex with poly(I) with the half-time of 4 min, and poly(iso-pr^2A) did not interact with poly(I) even after 5 d of mixing.

UV Spectra———The UV spectra of poly(A)·2poly(I) and poly(m^2A)·2poly(I) both had λ_{max} at 251 nm, which was shifted by 1 nm bathochromically compared to the respective summation spectra of the two components (Table II). The hypochromicity caused by the complex formation was approx. 25% in both cases. These findings suggested that these triplexes are quite similar to each other in structure regardless of the presence or absence of a

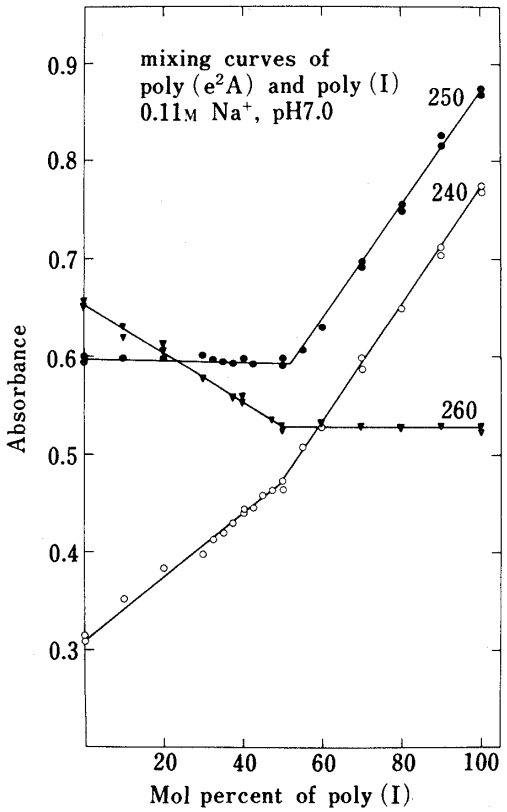

Fig. 10. UV Mixing Curves of Poly(e^2A) and Poly(I) in 0.11 M Na^+, 0.005 M Phosphate, pH 7.3, at 25°C

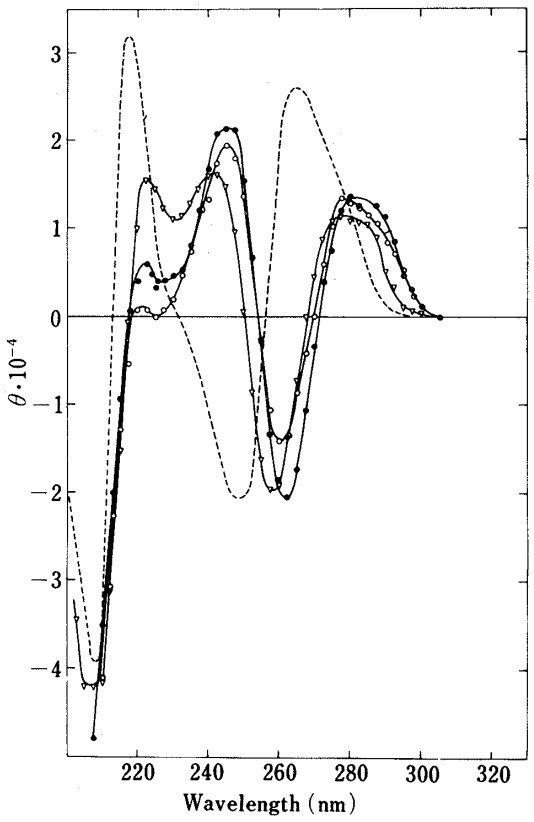

Fig. 11. CD Spectra of Complexes and a Mixture in 0.11 M Na^+ and 0.005 M Phosphate, pH 7.3, at 25°C

—○—, poly(A)·2poly(I);
—●—, poly(m^2A)·2poly(I); —▽—, poly(e^2A)·poly(I);
------, poly(iso-pr^2A)+poly(I).

methyl substituent at the C(2)-position of the adenine base in the polynucleotides. Unlike these triplexes, the UV spectra of poly(e^2A)·poly(I) had λ_{max} at 252 nm and the hypochromicity was 19%, less than those of poly(A)·2poly(I) and poly(m^2A)·2poly(I).

CD Spectra——Fig. 11 shows the CD spectra of the complexes and the summation spectrum of the two components. Poly(m^2A)·2poly(I) had peaks at *ca.* 222, 247 and *ca.* 280 nm and troughs at 208, 225—228 and 261 nm. Though the peaks and troughs of poly(m^2A)·2poly(I) were greater in magnitude and slightly bathochromically shifted from those of poly(A)·2poly(I), the general features of the spectra were quite similar to each other in shape, suggesting that the complexes must have very similar structural geometry. On the other hand, the CD spectrum of the 1:1 complex of poly(e^2A) and poly(br^5U) differed from those of the above 1:2 complexes. The peaks at 243 and 278 nm and the trough at 258 nm were

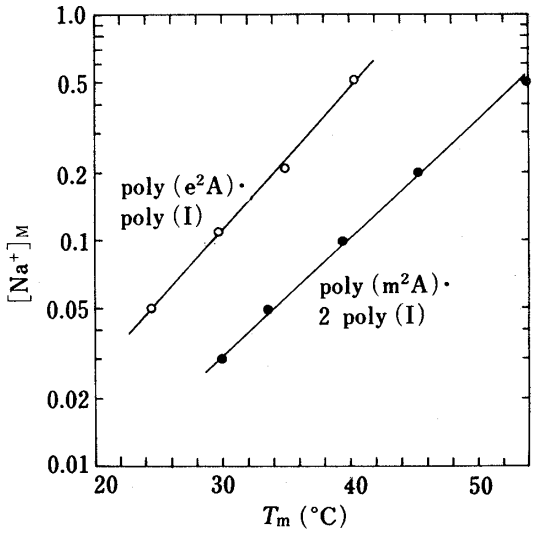

Fig. 12. Salt Dependence Curves of Poly(m^2A)·2Poly(I) (●) and Poly(e^2A)·Poly(I) (○)

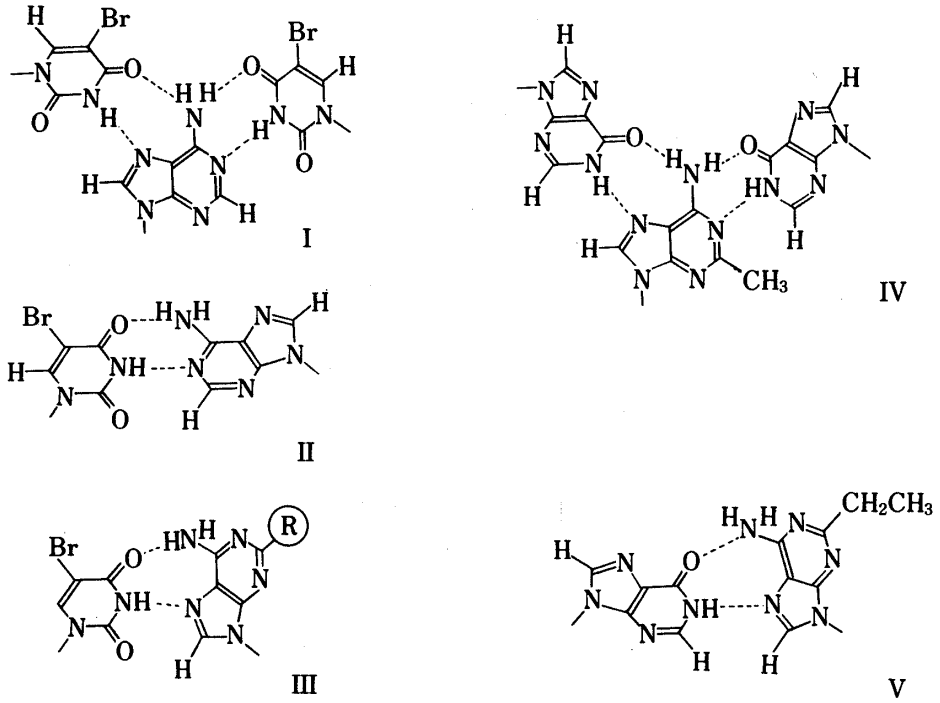

Fig. 13. Hydrogen Bonding Schemes of 1:1 and 1:2 Complexes

I, a triplex between poly(A) and poly(br^{5}U) (Watson-Crick and Hoogsteen arrangements).
II, a duplex between poly(A) and poly(br^{5}U) (Watson-Crick arrangement).
III, a duplex between poly-2-alkyladenylic acids and poly(br^{5}U) (Hoogsteen arrangement).
IV, a triplex between poly(m^{2}A) and poly(I) (Watson-Crick and Hoogsteen arrangements).
V, a duplex between poly(e^{2}A) and poly(I) (Hoogsteen arrangement).

shifted hypsochromically compared to those of poly(m^{2}A)·2poly(I), and the relative heights of the peaks at 223 and 243 nm were almost equal.

Thermal Stability——The UV-temperature melting profiles of poly(m^{2}A)·2poly(I) and poly(e^{2}A)·poly(I) consisted of sharp and sigmoid curves with transition breadths of 5 and 6°C, respectively in 0.1M Na^+, at pH 7.3, indicating that these complexes dissociated cooperatively into the separate polynucleotide chains. Under these conditions, T_m=40.5°C for poly(m^{2}A)·2poly(I) and T_m=29.8°C for poly(e^{2}A)·poly(I) were found. The difference of T_m's of about 10°C is fairly large compared with that of the corresponding poly(m^{2}A)·poly(br^{5}U) and poly(e^{2}A)·poly(br^{5}U). Fig. 12 shows T_m's in various sodium ion concentrations. T_m's of both complexes depended upon sodium ion concentration in solution, and were linear functions with slopes of $dT_m/d\log[Na^+]$=20 and 16°C, respectively.

Discusson

The stoichiometry of a complex between poly(A) and poly(br^{5}U) has been reported by many investigators.[10-13] They concluded from their observations that poly(A) forms only a triple-stranded complex with poly(br^{5}U), having the stoichiometry of 1(A) to 2(br^{5}U) under neutral conditions (Fig. 13, I). However, Riley *et al.*[10] suggested that a duplex (Fig. 13, II) could also be formed under neutral solutions as a stable species in an equimolar mixture of poly(A) and poly(br^{5}U), though they never detected an inflection at 50 mol% poly(br^{5}U) in the mixing curves. For determining the stoichiometry of polynucleotide complexes, it is most important to prepare a series of mixtures containing an accurate amount of each component, and to choose appropriate wavelengths indicating the respective complex formations at equilibrium; the latter is sometimes facilitated by consideration of isosbestic points in the UV

spectra of the mixtures. With respect to these points, we have reinvestigated the stoichiometry of the interaction between poly(A) and poly(br^{5}U) by constructing various mixing curves, and found that poly(A) forms both 1:1 and 1:2 complexes with poly(br^{5}U) in 0.1 M Na^+, pH 7.3, at 25°C, under equilibrium conditions.

Our studies were then extended to the interactions between a series of poly-2-alkyladenylic acids and poly(br^{5}U) under comparable conditions. From the mixing curve experiments, it can be concluded that poly(m^{2}A) and poly(e^{2}A) form only 1:1 complexes with poly(br^{5}U), whereas poly(iso-pr^{2}A) is not able to form any complex even in 0.5 M Na^+, at 25°C.

As with the complexes of poly(m^{2}A)·poly(U)[5] and poly(m$_2^{2,6}$A)·poly(U),[7] bulky alkyl substituents could block Watson–Crick (A)·(U) pairing by direct steric interference of the 2-methyl or 2-ethyl group of the adenine moiety with the C(2)-carbonyl group of the uracil moiety, favouring Hoogsteen double helices formation (Fig. 13, III). As is apparent from a comparison of T_m's of poly(m^{2}A)·poly(br^{5}U) and poly(e^{2}A)·poly(br^{5}U), T_m=49.4°C and T_m=45°C, respectively, and the fact that poly(iso-pr^{2}A) does not complex with poly(br^{5}U) at room temperature, the Hoogsteen type pairing tends to be destabilized by an increase in the bulkiness of the substituents at the C(2)-position. Though Watson–Crick and Hoogsteen double helices are hardly distinguishable from each other from their UV spectra and hypochromic features, they can be clearly distinguished by comparing CD difference spectra, as described above. This is the first report on the CD diagnostic features for Watson–Crick and Hoogsteen base pairing.

In addition to the work on the poly(A)/poly(br^{5}U) system, several investigations have been undertaken on the interactions between poly(A) and poly(I). Rich[14] has reported that a 1:1 complex of poly(A)·poly(I) is formed in 0.05 M Na^+ ion solution at pH 6.8 in 31 min, followed by 1:2 complex formation (1(A) to 2(I)) at the equilibrium state, whereas the only 1:1 complex is present at lower salt concentration such as in 0.01 M Na^+ after 18 h. However, Howard *et al.*[15] have reexamined the stoichiometry and kinetics of the poly(A) and poly(I) system,showing that a 1:1 complex does not exist at any polymer ratio or salt concentration, and the 1:2 complex has to be regarded as the only detectable species under equilibrium conditions.

For comparison with the poly(A) and poly(I) system, we have investigated the interactions between a series of poly-2-alkyladenylic acids and poly(I). The mixing curve experiments confirmed the formation of only a 1:2 complex of poly(m^{2}A) with poly(I), and this was confirmed by a careful consideration of the isosbestic points in the UV spectra of continuous mixtures. These findings coincide well with the previously reported results on poly(A)/poly(I) and poly(m^{2}A)/poly(I) interactions.[15] In contrast to the interaction of poly(m^{2}A) with poly(I), the mixing curves indicated that poly(e^{2}A) forms only a 1:1 complex and poly(iso-pr^{2}A) forms no complex with it under the condition examined. Based on the above findings, we might conclude that introduction of a methyl group at the C(2)-position of the aglycone in poly(A) does not interfere with base pairing to hypoxanthine in poly(I), which includes hydrogen bonding at the N(1)-position in the usual fashion (Fig. 13, IV)(a CPK spacefilling model shows direct but not repulsive contact between the C(2)-CH_3 of 2-methyladenine and C(2)-H of hypoxanthine). However, the presence of an ethyl group at C(2) will prevent base pairing at the adjacent N(1)-atom for steric reasons (the CPK model shows repulsive contact between the C(2)-CH_2-CH_3 and C(2)-H). Therefoer, poly(m^{2}A) forms a triple structure with poly(I) in a Watson–Crick as well as a Hoogsteen type interaction (Fig. 13, IV), similar to the proposed structure for poly(A)·2poly(I),[14,15] whereas poly(e^{2}A) stabilized only the latter type in the form of a duplex structure (Fig. 13, V), which is the first example of such a structure in the poly(A)/poly(I) system. Poly(iso-pr^{2}A), however, does not form any complex with poly(I) at 25°C at all, indicating a more profound intramolecular influence of the isopropyl group, possibly involving significant conformational changes in the helical structure.

Acknowledgement We thank the Alexander von Humboldt Foundation for a fellowship, the Deutsche Forschunggemeinschaft for financial support, and also Mrs. M. Bischler for measuring the CD spectra.

References

1) Nucleotides, XVIII: D. Flockerzi, W. Schlosser and W. Pfleiderer, *Justus Liebigs Ann. Chem.*, in press.
2) A part of this work was presented at the 5th Symposium on Nucleic Acids Chemistry (Mishima, 1977). M. Hattori, K. Ienaga and W. Pfleiderer, *Nucleic Acids Res.*, specail publication, 3, s-129 (1977).
3) J.D. Watson and F.H.C. Crick, *Nature* (London), **171**, 156, 740 (1953).
4) K. Hoogsteen, *Acta Crystallogr.*, **12**, 822 (1959).
5) M. Ikehara, M. Hattori and T. Fukui, *Eur. J. Biochem.*, **31**, 329 (1972).
6) F. Ishikawa, J. Frazier, F. B. Howard and H. T. Miles, *J. Mol. Biol.*, **70**, 475 (1972).
7) M. Hattori, M. Ikehara and H.T. Miles, *Biochemistry*, **13**, 2754 (1974).
8) M. Hattori, K. Ienaga and W. Pfleiderer, *Justus Liebigs Ann. Chim.*, **1978**, 1796.
9) E. De Clercq, T. Fukui, N. Kakiuchi, M. Ikehara, M. Hattori and W. Pfleiderer, *Cancer Lett.*, **7**, 27 (1979).
10) M. Riley and A.V. Paul, *J. Mol. Biol.*, **50**, 439 (1970).
11) C.H.Fiske and Y. SubbaRow, *J. Biol. Chem.*, **66**, 375 (1925).
12) A.M.Michelson, J. Dondon and M. Grunberg-Manago, *Biochem. Biophys. Acta*, **55**, 529 (1962).
13) J. Mossoulie, A.M. Michelson and F. Pochon, *Biochem. Biophys. Acta*, **114**, 16 (1966).
14) A. Rich, *Nature* (London), **181**, 521 (1958).
15) F.B. Howard, M. Hattori, J. Frazier and H.T. Miles, *Biochemistry*, **16**, 4637, (1977).