Structure of Polynucleotide Complex with Non-Complementary Nucleosides.[1] II. Poly I, U + Poly C

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Three copolymers of riboinosinic acid and ribouridylic acid (poly I,U) with uridylic acid contents, 20, 32, and 44% were prepared. The ultraviolet absorption measurements were carried out with aqueous solutions of mixtures of poly I,U and polytribocytidylic acid (poly C) of various mole ratios. It has been shown that a double-helical structure is formed with an I···C type inter-base binding and with the U residue looping out of the helix. The structure was found to break on heating the solution. The process of break down was followed by means of ultraviolet absorption measurements.

Polyriboinosinic acid (poly I) and polyribocytidylic acid (poly C) are known to form a double-helical complex poly I·poly C in aqueous solution.[2,3] The structure involves specific hydrogen bonds (I···C) between inosine and cytidine residues. It can be schematically expressed as in Fig. 1(a). Here, inosine (I) and cytidine (C) are complementary nucleosides. The question now arises: what is the effect of introducting uridine (U) residue (which is complementary to neither I nor C) into this system. In a previous paper[4] we examined the effect of introducing the U residue into the poly C moiety. In this paper we describe the effect of its introduction into the poly I moiety.

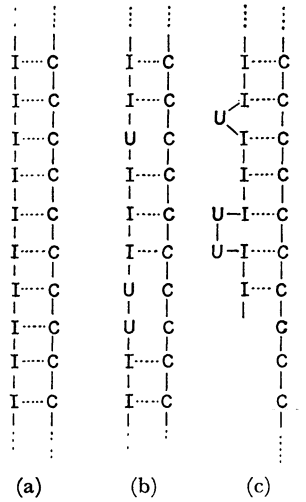

Fig. 1. Schematic drawings of (a) the double-helical structure of poly I·poly C complex, (b) a double-helical structure with the U residues inside, and (c) a double-helical structure with the U residues rotating out.

Preparation of Polynucleotide Samples

The polynucleotide samples were prepared by use of polynucleotide phosphorylase obtained from *Azotobacter vinelandii*. Compositions of the reaction mixtures and incubation times are given in Tables 1 and 2. The incubation temperature was 30°C. After the completion of enzymatic reaction, the product was precipitated with ethanol and then purified by phenol extraction.

TABLE 1. AN EXAMPLE OF REACTION MIXTURES IN ENZYMATIC PREPARATION OF POLYNUCLEOTIDES

Inosine-5′-diphosphate	(27 mg/m*l*)	0.8 m*l*
Uridine-5′-diphosphate	(26 mg/m*l*)	0.8
Tris buffer	(0.5M, pH 8.1)	1.5
EDTA[a]	(1 mM in β-mercaptoethanol)	0.05
$MgCl_2$	(50 mM)	0.6
Enzyme		0.7[b]
H_2O		0.55
	Total	5.0

a) Ethylenediamine tetraacetic acid
b) This amount contains 12 units of enzyme. 1 unit=amount of enzyme which can liberate 1μmol of orthophosphate in the enzymatic reaction (15 min)

TABLE 2. ENZYMATIC PREPARATION OF HOMOPOLYMERS OF CYTIDYLIC ACID AND COPOLYMERS OF INOSINIC AND URIDYLIC ACIDS

Polymer	Substrate[a]		Amount of enzyme	Incubation time	Product				
					Yield		I	U	Sedimentation coeff.
	IDP mg	UDP mg	unit	min	mg	%	%	%	S_{20}
Poly I,U (1)	32.4	10.4	12	90	10.6	33	80	20	15.7
Poly I,U (2)	48.6	26.0	24	90	19.5	35	68	32	12.5
Poly I,U (3)	21.6	20.8	12	90	7.2	23	56	44	13.8
Poly C	CDP 40 mg		10	90	12.4	31			8.6

a) IDP: inosine-5′-diphosphate, UDP: uridine-5′-diphosphate, CDP: cytidine-5′-diphosphate

1) Paper I in this series, H. Akutsu and M. Tsuboi, This Bulletin, **43**, 3391 (1970).
2) D. R. Davies and A. Rich, *J. Amer. Chem. Soc.*, **80**, 1003 (1958).
3) D. R. Davies, *Nature*, **186**, 1030 (1960).
4) M. Tsuboi, K. Matsuo, and M. Nakanishi, *Biopolymers*, **6**, 123 (1968).

Base Composition and Degree of Polymerization of the Products

The base composition of each poly I,U sample was determined by its hydrolysis with 1N HCl, paper chromatographic separation of the resulting hypoxanthine and uridylic acid and ultraviolet absorption measurements. The composition thus determined is given in Table 2.

The degrees of polymerization of the polymers were not determined, but they are considered to be sufficiently high from the sedimentation constants given in the last column of Table 2. The constants were determined in a solvent with 0.1M NaCl and 0.01M Na-cacodylate, pH 7.0.

Experimental

Ultraviolet absorption measurements were carried out with an Ito spectrophotometer Model QU-3. The temperature of the samples was controlled as previously descrived.[5] The polynucleotide concentration (in M) was determined by measuring phosphorus content.[6] The moles of polynucleotide means here moles of phosphorus (or the moles of nucleotide residue).

Mixing Curves

Solutions of poly I,U and poly C of various mole ratios were prepared, and their ultraviolet absorbances at a few points in the 240—250 mμ region were recorded. The results are shown in Fig. 2. From the absorbance *versus* mole-ratio profile, we can judge whether the two polymers in question form a complex or have no interaction. When a complex is formed, its stoichiometric ratio is determined from the observed profile of the mixing curve.

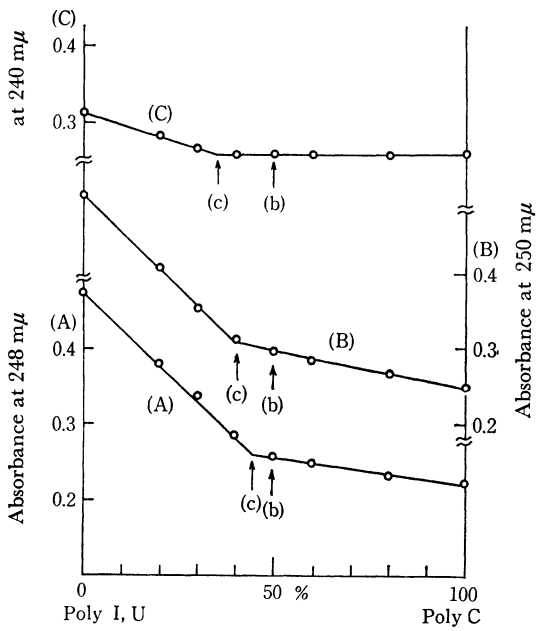

Fig. 2. Mixing curves of poly I,U and poly C.
Abscissa: mol% of poly C (determined by measuring phosphorus contents), ordinate: absorbance at 248, 250, or 240 mμ. Total nucleotide concentration in the solution was kept at 4.8×10^{-5}M. Solvent: 0.1M NaCl+0.001M $MgCl_2$+0.01M Na-cacodylate buffer, pH 7.0. Temperature: 25°C.
(A) Poly I,U(1) and poly C, (B) poly I,U(2) and poly C, (C) poly I,U(3) and poly C.

5) K. Matsuo and M. Tsuboi, This Bulletin, **39**, 347 (1966).
6) B. N. Ames and D. T. Dubin, *J. Biol. Chem.*, **235**, 769 (1960).

As may be seen in Fig. 2, each poly I,U, shows evidence of interaction with poly C. At room temperature, each of the mixing curves consists of two straight lines which intersect at a proper mole ratio. This should correspond to the stoichiometric mole ratio in which the two polynucleotides form a complex. The stoichiometric mole ratio, poly I,U/poly C, should be 50/50, if the complex has a structure, in which the non-complementary U residues are incorporated in the double-helix as shown schematically in Fig. 1(b). On the other hand, if the U residues rotate out of the helix and thus no C residue fails to form an I···C base-pair (see Fig. 1(c)), then the intersect (or the maximum hypochromicity) should take place at poly I,U/poly C=100/x. Here, x=mol% of I in the copolymer of I and U. The stoichiometric mole ratios expected for structures (b) and (c) in Fig. 1 are indicated by arrows in Fig. 2.

We see that each mixing curve shows its minimum at the mole ratio expected for structure (c) but not for structure (b) in Fig. 1.

Heating Curves

Absorbance-temperature profile of each of the poly

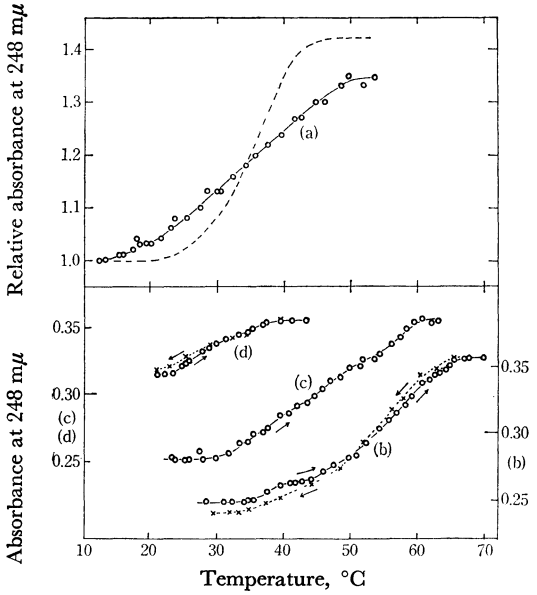

Fig. 3. Variation of the absorbance at 248 mμ with temperature of mixture solutions of poly I,U and poly C.
The mole ratio in each mixture solutions was that for the maximum hypochromicity (see Fig. 2). (a) Poly I,U(2) 2.9×10^{-5}M and poly C 1.9×10^{-5}M. Solvent: 0.1M NaCl+0.01M Na-cacodylate buffer, pH 7.0. (b) Poly I,U(1) 2.6×10^{-5}M and poly C 2.1×10^{-5}M. Solvent: 0.1M NaCl+0.001M $MgCl_2$ +0.01M Na-cacodylate buffer, pH 7.0. (c) Poly I,U(2) 2.9×10^{-5}M and poly C 1.9×10^{-5}M. Solvent: the same as that for (b). (d) Poly I,U(3) 3.1×10^{-5}M and Poly C 1.7×10^{-5}M. Solvent: the same as that for (b). The dotted curve in the upper portion of this figure is the absorbance (at 248 mμ)-temperature profile of poly C,U (with 33% U) and poly I observed in the same solvent as that for (a).

I,U-poly C mixtures at the mole ratio with maximum hypochromicity are shown in Fig. 3. In general, such observed profiles do not always represent the melting profiles of the secondary structures of the complex molecules. As Fresco and Alberts[7] indicated, the absorbance of not only the complex but also the non-interacted constituent may depend upon the temperature. In the present case, however, the absorbance of poly I,U and of poly C would not depend too much on the temperature. Therefore, the observed profiles given in Fig. 3 would not greatly differ from the "true melting profile" of the complex molecule.

As may be seen in Fig. 3, the increase in absorbance takes place over a wide range of temperature for every complex even in a solvent with Mg^{2+}. This is in contrast with the case of the complex of poly C,U and poly I,[4] where a sharp rise in absorbance takes place. The midpoint (T_m) of the transition, however, is almost the same for poly C,U·poly I complex and poly I,U·poly C complex when the U contents in these two complexes are almost equal (32—33% in each copolymer). The T_m becomes lower as the U content in the copolymer increases. As in the case of the poly C,U·poly I complex, the cooling curve of each of these complexes was found to be almost equal to the heating curve.

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7) J. R. Fresco and B. M. Alberts, *Proc. Nat. Acad. Sci. U. S.*, **46**, 311 (1960).