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Hybridization of Polymers of Antibiotic C-Nucleoside Phosphates, Poly(formycin phosphate) and Poly(laurusin phosphate)†

S. Uesugi, T. Tezuka, and M. Ikehara*

ABSTRACT: The ability of complex formation of poly(formycin phosphate), poly(F), and poly(laurusin phosphate), poly(L), with the polymers of natural polynucleotides was examined mainly by mixing experiments in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0) at 2°. Poly(F) formed complexes with poly(U) and poly(I) in the ratio of 1:1 and 1:2, respectively. Poly(L) formed complexes with poly(A) in 2:1 ratio and poly(C) in 1:2 and 2:1 ratios in addition to a self-complex. Poly(F) and poly(L) also formed a 1:2 complex between them. Some of these complexes were assumed to contain novel types of base pairings using the 7-NH group. Thus it was concluded that poly(L) could form complexes with both, the oligomer of cycloadenylic acid (ϕ_{cn} −120°) and polymers of natural nucleotides (ϕ_{cn} 0°), showing flexibility of the torsion angle of the laurusin residue.

We have been investigating the effect of the torsion angle on the properties of oligo- and polynucleotides, mainly using cyclonucleoside derivatives, in which the torsion angle is fixed (Uesugi et al., 1972; Ikehara and Uesugi, 1972; Ikehara et al., 1974; Ikehara and Tezuka, 1973, 1974a,b). In this course, it is found that the oligomers of 8,2′-cycloadenylic acid form a left-handed helical structure (Uesugi et al., 1972; Ikehara and Uesugi, 1972, Ikehara et al., 1974), contrary to natural polynucleotides. The reason for this unusual conformation was ascribed to the value of the fixed torsion angle ϕ_{cn} (Donohue and Trueblood, 1960), about −120° (Tomita et al., 1972), which is in a syn-anti boundary region and is different from those of natural nucleosides possessing an anti conformation. Although these oligomers of 8,2′-cycloadenylic acid do not form complexes with the homopolynucleotides having natural nucleoside residues, the octamer of 8,2′-*S*-cycloadenosine 5′-monophosphate, $(pA^s)_8$,[1] is shown to form a left-handed helical complex

† From the Faculty of Pharmaceutical Sciences, Osaka University, Toyonaka, Osaka, Japan. *Received December 2, 1974.* This work was supported by a Grant-in-aid for Scientific Research from the Ministry of Education. This paper is Part XXVII in a series entitled Polynucleotides. Part XXVI: M. Ikehara and T. Tezuka (1974).

[1] Abbreviations used are: $(pA^s)_8$, octamer of 8,2′-*S*-cycloadenosine 5′-monophosphate; poly(F), poly(formycin phosphate); poly(L), poly(laurusin phosphate).

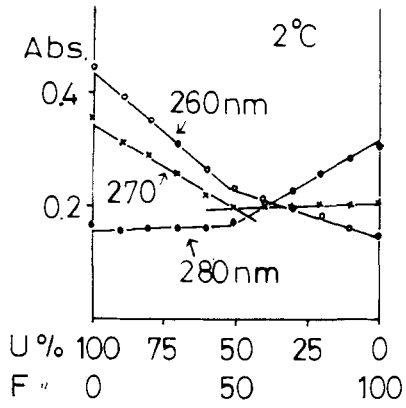
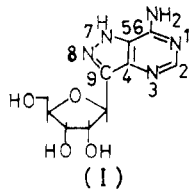


FIGURE 1: Mixing curves for poly(F) and poly(U) at 260 nm (○), 270 nm (×), and 280 nm (●) in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0) at 2°.

with the octamer of 6,2′-*O*-cyclouridine 5′-monophosphate which is thought to have the same torsion angle[2] as the former nucleotide (Ikehara and Tezuka, 1973).

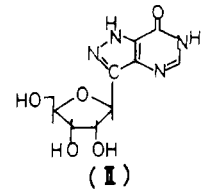
On the other hand, an antibiotic C-nucleoside, formycin (I), was shown to have a ϕ_{cn} of about −110°, very close to that of 8,2′-cycloadenosine, from X-ray analysis of formycin dihydrate (Prusiner et al., 1973), though a ϕ_{cn} of about −210° was reported for a crystal of formycin hydrobromide (Koyama et al., 1966). So it was supposed that poly(formycin phosphate), poly(F), might form a complex, presumably a left-handed helical complex, with an oligomer of cyclouridylic acid. Although the complex formation between them was not proved under the conditions examined, the polymer of laurusin (II) (Ward et al., 1968) phosphate, poly(L),

Formycin



(I)

Laurusin
(Formycin B)



(II)

which is a deamination product of formycin phosphate, was found to form a complex with $(pA^s)_8$ (Ikehara and Tezuka, 1974b). It has also been shown that poly(F) can form a complex with poly(U) and the formycin residues in it might be in anti conformation (Ward et al., 1968).

As was discussed by Prusiner et al. (1973), the increased exocyclic angles of C^4–C^9–$C^{1'}$, N^8–C^9–$C^{1'}$, and N^3–C^4–C^9[3] and the increase in the bond distances $C^{1'}$–C^9 and C^9–C^4 in formycin would reduce the barrier to rotation about the C-glycosyl bond. Therefore, formycin may be able to take syn, syn-anti boundary, and anti conformations depending on the conditions. The same situation can be assumed in the case of laurusin. Although some properties of poly(F) and poly(L) have been briefly reported by Ward et al. (1968, 1969) no mixing experiments with normal polynucleotides are described so far. In this paper, the interactions of poly(F) and poly(L) with homopolyribonucleotides possessing natural nucleoside residues were investigated. Various complex formations between them were assumed to contain novel types of base pairings using the 7-NH group.

Materials and Methods

Polynucleotides were prepared by polymerization of the corresponding nucleoside 5′-diphosphates with polynucleotide phosphorylase from *Escherichia coli*. The preparation of poly(F) and poly(L) is described previously (Ikehara and Tezuka, 1974b).

Uv absorption spectra were obtained on a Hitachi EPS-3T or Hitachi 124 spectrophotometer and circular dichroism (CD) spectra were taken with a JASCO ORD/UV-5 spectropolarimeter equipped with a CD attachment. For T_m measurements and mixing experiments, a Hitachi 124 spectrophotometer equipped with a Komatsu Solidate SPD-H-124 thermostated cell was used. CD spectra at low temperature were measured using a JASCO low-temperature device. The temperature within the cell was measured by a Cu–constantan thermocouple. All measurements were carried out in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0). Molecular extinction coefficient (ϵ) and molecular ellipticity ($[\theta]$) are presented as per residue values. For mixing experiments, the mixture of the two components was heated to 50–60°, cooled down slowly to room temperature, and allowed to stand at room temperature overnight. Then it was kept in a refrigerator for 1 day before measurement.

Results and Discussion

Complexes Containing Poly(F). (1) POLY(F)–POLY(U). T_m and ORD curves of a 1:1 complex of poly(F) with poly(U) have been reported by Ward et al. (1968), but there is no report on the mixing experiment. To confirm the stoichiometry of the complex, a mixing experiment was carried out in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0) at 2°. As shown in Figure 1, ultraviolet mixing curves at three wavelengths exhibit discontinuities at a mole fraction of poly(F) of 50%, showing the stoichiometry to be 1:1.

It is known that poly(A) forms 1:1 and 1:2 complexes with poly(U) depending on the composition of the mixture under similar condition (Stevens and Felsenfeld, 1964). From X-ray analysis, it is shown that in the 1:2 complex poly(A) forms a Watson-Crick type base pair (Watson and Crick, 1953), with one strand of poly(U) and a Hoogsteen type base pair (Hoogsteen, 1959) with another strand of poly(U) (Arnott and Bond, 1973a). Because poly(F) does not have a proton acceptor site N-7, it cannot form a Hoogsteen type complex with poly(U). The most possible structure is the Watson-Crick type.

(2) POLY(F)–POLY(I). As shown in Figure 2, the mixing experiment between poly(F) and poly(I) shows the formation of a 1:2 complex at 2°. The 1:2 mixture of poly(F) and poly(I) gives a CD curve different from the calculated summation curve (Figure 3), thus confirming the formation of the complex. Poly(A) should form a 1:2 complex with poly(I) under present conditions (Rich, 1958). From X-ray analysis, it is shown that in the 1:2 complex poly(A) forms a base pair equivalent to the Watson-Crick type with one strand of poly(I) and a base pair equivalent to the Hoogsteen type with the other strand of poly(I) (Arnott and Bond, 1973b). Poly(F)·2 poly(I) could contain a base pair equivalent to the Watson-Crick type and a base pair involving N^6-H and 7-NH of poly(F).

[2] Recently, the torsion angle of 6,2′-*O*-cyclouridine was determined to be −104° by X-ray crystallography (M. Nishikawa, personal communication).

[3] A numbering system analogous to that of the purine nucleoside is used in this paper in order to facilitate comparison with the purine nucleoside as indicated in I and II.

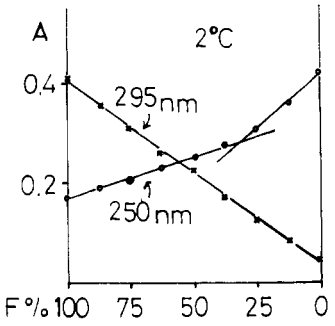


FIGURE 2: Mixing curves for poly(F) and poly(I) at 250 nm (O) and 295 nm (×) in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0) at 2°.

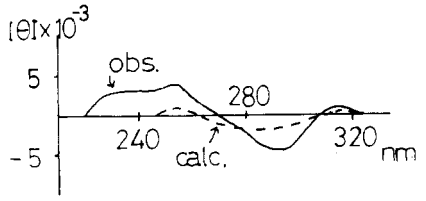


FIGURE 3: Circular dichroism spectrum of the 1:2 mixture of poly(F) and poly(I) under the same condition as the mixing experiment. A summation curve of both components is also presented as a broken line.

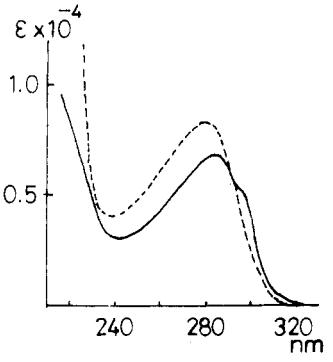


FIGURE 4: Ultraviolet absorption spectra of poly(L) (—) and laurusin 5′-diphosphate (- - -) in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0) at room temperature.

Complexes Containing Poly(L). (I) POLY(L)–POLY(L). The uv absorption spectrum of poly(L) (Figure 4) at 0.15 *M* Na^+ concentration and pH 7.0 shows a different shape, hypochromism, and unusual bathochromism in comparison with that of monomer, laurusin 5′-diphosphate. Measurement of the uv absorption spectra at various temperatures gives fairly sharp melting curves (Figure 5, temperature range 20°) and T_m around 37°. Since a single-stranded homopolynucleotide generally gives a very broad melting curve, this relatively sharp melting suggests the formation of a self-complex. CD spectrum of this self-complex at 13° is entirely different from that of the melted polymer at 63° as shown in Figure 6. It shows a large Cotton effect around 270 nm.

Poly(I) is known to form a triple-stranded complex at high salt concentration (Rich, 1958), although it is in a single-stranded state and gives a broad temperature absorbance profile at low salt concentration (Hinz et al., 1970). Considering that poly(I) shows a fairly sharp melting curve and a marked difference in CD spectrum, it may be assumed that poly(L) forms a multistranded self-complex at moderate salt concentration.

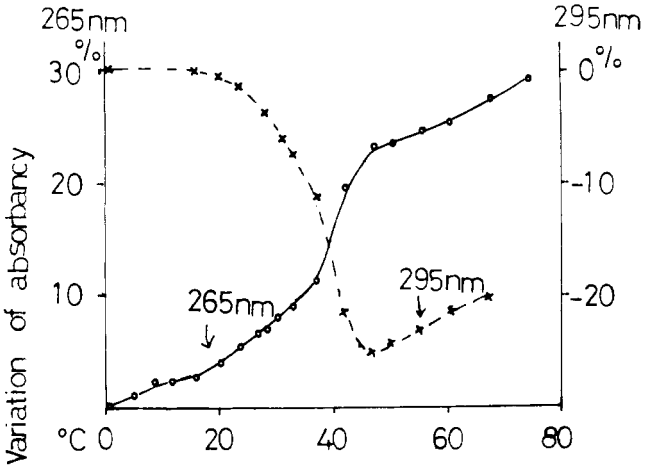


FIGURE 5: Absorbance–temperature profiles of poly(L) in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0) at 265 nm (O) and 295 nm (×).

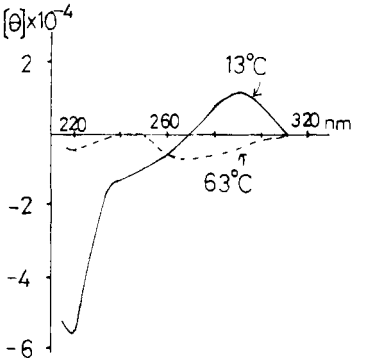


FIGURE 6: Circular dichroism spectra of poly(L) in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0) at 13° (—) and 63° (- - -)

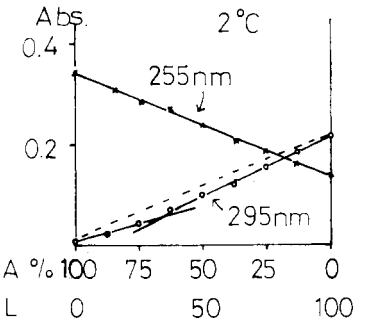


FIGURE 7: Mixing curves for poly(L) and poly(A) at 255 nm (×) and 295 nm (O) in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0) at 2°.

(2) POLY(L)–POLY(A). A mixing experiment at 20° (Figure 7) suggests the formation of a triple-stranded complex, poly(L)·2 poly(A) between poly(L) and poly(A), though 2 poly(I)·poly(A) is formed between poly(I) and poly(A). The mode of base pairing may be the same as that proposed by Davies for a complex, poly(L)·2A, between poly(L) and monomeric adenosine (Davies, 1973).

(3) POLY(L)–POLY(C). As shown in Figure 8, mixing curves at three different wavelengths show discontinuities at mole fractions of poly(L) of 33 and 67%. At least two different complexes, poly(L)·2 poly(C) and 2 poly(L)·poly(C) may be formed. The same C–L base pair could be involved in both complexes and the third component may be interchangeable.

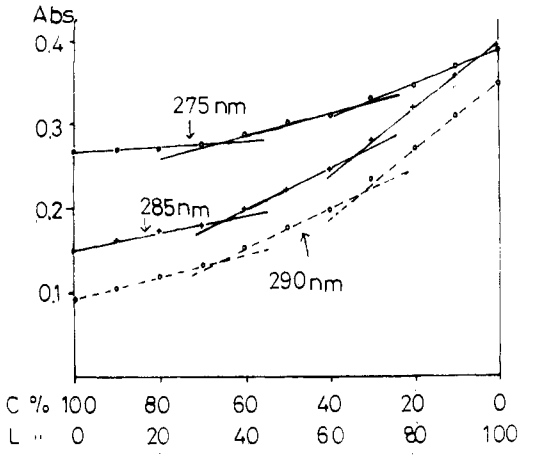


FIGURE 8: Mixing curves for poly(L) and poly(C) at 275 nm (O), 285 nm (X), and 290 nm (O, broken line) in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer (pH 7.0) at 2°.

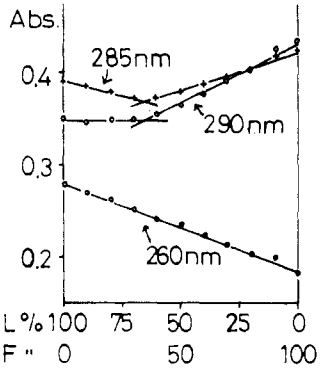


FIGURE 9: Mixing curves for poly(L) and poly(F) at 260 nm (O, lower), 285 nm (X) and 290 nm (O, upper) in 0.1 *M* NaCl–0.05 *M* sodium cacodylate buffer at 2°.

Complex between Poly(F) and Poly(L). The result of a mixing experiment (Figure 9) indicates the formation of poly(F)·2 poly(L) at 2°, though poly(A) and poly(L) form poly(A)·poly(L). In comparison with the 2 poly(A)·poly(L) complex, the reason why 2 poly(F)·poly(L) is not formed in this case may be that poly(F) does not have the ability to pair with poly(L) so strong as poly(A). The melting temperature of poly(A)·poly(U) (57°) is much higher than that of poly(F)·poly(U) (22°) at 0.1 *M* K^+ concentration (Ward et al., 1968). In conclusion, poly(F) and poly(L) do form various complexes with homopolynucleotides possessing natural nucleoside residues, in which the base torsion angle around the glycosidic linkage is assumed to be in the anti region though poly(L) also forms a complex with $(pA^s)_8$, in which the torsion angle is in a syn-anti boundary region. The handedness of the helical complex is assumed to be right in the former case and left in the latter case. Because poly(F) and poly(L) have 7-NH as hydrogen donor instead of 7-N hydrogen acceptor in other natural base residues, they are assumed to form new types of complexes in addition to the usual types of base pairing.

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