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Interaction of Poly-L-ornithine with Nucleic Acids[1]

KOSUKE MORIKAWA and MASAMICHI TSUBOI

Faculty of Pharmaceutical Sciences, University of Tokyo[2]

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Ultraviolet absorbance versus temperature profile has been examined of seven helical nucleic acids in aqueous solutions with and without poly-L-ornithine. The hyperchromicity observed at the melting temperature of each nucleic acid is found to be lowered linearly as the amount of poly-L-ornithine added to the solution. This fact is taken as indicating that there is a stoichiometric complex of polyornithine plus nucleic acid formed. The ornithine/nucleotide mole ratio was determined to be 1/1 for the complexes of calf thymus DNA,[3] poly dAT, rice dwarf virus RNA, and poly rAU. The ratio was found to be 2/3 for the complexes of poly (A+U) and poly (A+2U), and 1/2 for the complex of poly (I+C).

Poly-L-lysine was found to form a stoichiometric complex with a helical nucleic acid, which is soluble in a low-salt aqueous solution.[4-9] The stoichiometric mole ratio (lysine/nucleotide) was found to be 1/1 for every complex with double-helical DNA.[6,8] In the complex with a double-helical RNA, however, the ratio was found to be smaller than 1/1.[5-8] It was 2/3 for rice dwarf virus RNA, for poly rAU,[3] and for poly (A+U), and 1/2 for poly (I+C). Such a difference in stoichiometry was explained[7] by taking a lateral association of the 1/1 complex molecule and the free nucleic acid molecule into account. If this association takes place with a stoichimetric ratio 1/1, the mole ratio of the lysine residues versus the total nucleotide residues in the aggregate should be 1/2; if the association takes place with a ratio of 2/1, the lysine/nucleotide stoichiometry should be 2/3. In this explanation, such an association should take place only for RNA but not for DNA. This difference was related to a structural difference between the DNA and RNA molecules.[7] According to what was proposed, poly-L-lysine first winds helically around the double-helical polynucleotide chain to form a 1/1 complex in both cases. The bound poly-L-lysine chain, however, is placed nearer to the helix axis in DNA than in RNA, because the DNA has a deep groove along the helix while RNA does not. In this way, the positive charge of poly-L-lysine may be buried deeply in the DNA complex, while they may be exposed on the surface in the RNA complex. This may cause a greater tendency for the polylysine–RNA complex molecule to associate with the negatively charged free molecule, than for the polylysine–DNA molecule.

1) This paper was presented at the 88th Annual Meeting of the Pharmaceutical Society of Japan, in Tokyo, April, 1968.
2) Location: *Hongo, Bunkyo-ku, Tokyo.*
3) Abbreviations used in this paper: DNA, deoxyribonucleic acid; RNA, ribonucleic acid; A, riboadenylic acid; U, ribouridylic acid; I, riboinosinic acid; C, ribocytidylic acid; poly dAT, copolymer with deoxyriboadenylic acid and deoxyribothymidylic acid in the alternating sequence (double helix); poly rAU, copolymer with riboadenylic acid and ribouridylic acid in the alternating sequence (double helix); poly (A+U), 1 mole to 1 mole complex of polyriboadenylic acid and polyribouridylic acid; poly (I+C), 1 mole to 1 mole complex of polyriboinosinic acid and polyribocytidylic acid.
4) E. Raukas, *Biokhimiya*, **30**, 1122 (1965).
5) K. Matsuo and M. Tsuboi, *Bull. Chem. Soc. Japan*, **39**, 347 (1966).
6) M. Tsuboi, K. Matsuo, and P.O.P. Ts'o, *J. Mol. Biol.*, **15**, 256 (1966).
7) S. Higuchi and M. Tsuboi, *Biopolymers*, **4**, 837 (1966).
8) M. Tsuboi, in "*Conformation of Biopolymers*," ed. by G.N. Ramachandran, Vol. 2, Academic Press, London and New York, 1967, p. 689.
9) D.E. Olins, A.L. Olins, and P.H. von Hippel, *J. Mol. Biol.*, **24**, 157 (1967).

Poly-L-ornithine is a similar basic polypeptide to poly-L-lysine, but its side-chain is shorter than that of poly-L-lysine by one C–C bond. It is condsiered, therefore, this polypeptide may form a complex with double-helical RNA, in which the positive charge is placed more deeply than that of the polylysine–RNA complex. On the basis of what was proposed, the amino-acid/nucleotide stoichiometric ratio in the polyornithine–RNA complex may be 1/1, unlike that in the polylysine–RNA complex. With this in mind, we have made an examination on the interaction of poly-L-ornithine with nucleic acids. The results will be given below.

Experimental

Poly-L-ornithine hydrochloride used in this work was prepared and kindly supplied by Dr. Koichi Morita, Chugai Pharmaceutical Co. Its sedimentation constant s_0 (extrapolated to polymer concentration=0) in H_2O+1M NaCl was found to be 1.22S. The degree of polymerization (z) should be about 600, if the s_0–z relation is the same for polyornithine with that for polylysine.[10] The concentration of poly-L-ornithine was determined by hydrolysis with 6N HCl followed by the reaction with ninhydrin.[11]

Calf thymus DNA with a high molecular weight was purchased from the Sigma Chemical Co. Poly dAT was prepared by the use of DNA polymerase obtained from *E. coli*.[12] The sample of rice dwarf virus RNA[13] was a gift from Dr. Kin-ichiro Miura, Institute of Molecular Biology, Nagoya University. Poly rAU was prepared by the use of RNA polymerase from *E. coli*. and with poly dAT as the template.[14] The poly A and poly U samples are the same with those used in the previous work of Higuchi and Tsuboi,[15] and the poly I and poly C samples are the same as those used in the work of Matsuo and Tsuboi.[5] The polynucleotide concentration in each standard solution was determined by measuring the phosphorus content.[16]

The ultraviolet absorption measurements were made by the use of an Ito Model QU-3 Spectrophotometer. The temperature of the sample solution was controlled as described in our previous papers.[5,6]

Results

Calf Thymus DNA and Poly dAT

Calf thymus DNA shows at 73° a sharp rise in the absorbance at 260 mμ in the 0.015M Na^+ solution (Fig. 1, A). On adding a small amount of poly-L-ornithine to this solution, the amount of the hyperchromicity at 73° decreases and another rise in absorbance appears at about 95° (Fig. 1, B—F). The latter rise is considered to correspond to the helix–coil transition (or the melting) of the polyornithine–DNA complex molecule and the rise at 73° to the melting of the double-helical DNA molecule wihch is not bound with of polyornithine. Exactly the same type of experimental result was obtained for poly dAT (Fig. 2) as that for calf thymus DNA (Fig. 1). The melting temperatures (T_m's) of poly dAT (47°) and polyornithine–poly dAT complex (82°), however, are both much lower than the corresponding T_m's in the system with calf thymus DNA.

The stoichiometric ratio of ornithine residue to nucleotide residue in these polyornithine–DNA complexes can be determined by examining the relation between the amount of poly-L-ornithine added to the solution and the amount of DNA which is not bound with poly-L-ornithine. The amount of free DNA may be estimated from the amount of hyperchromicity remaining at the melting temperature T_m of free DNA. In Fig. 3, this is plotted against the ornithine/nucleotide mole ratio in the solution. The resulting straight line intersects the abscissa at 1. Therefore, the ornithine/nucleotide ratio in the polyornithine plus DNA complex is estimated to be 1/1.

10) E. Daniel and Z. Alexandrowicz, *Biopolymers*, **1**, 473 (1963).
11) E.C. Cocking and E.W. Yemm, *Analyst*, **80**, 209 (1955); *Biochem. J.*, **58**, xii (1954).
12) H.K. Schachman, J. Adler, C.M. Radding, I.R. Lehman, and A. Kornberg, *J. Biol. Chem.*, **235**, 3242 (1960).
13) K. Miura, I. Kimura, and N. Suzuki, *Virology*, **28**, 571 (1966).
14) M. Chamberlin, R.L. Baldwin, and P. Berg, *J. Mol. Biol.*, **7**, 334 (1963).
15) S. Higuchi and M. Tsuboi, *Bull. Chem. Soc. Japan*, **39**, 1886 (1966).
16) B.N. Ames and D.T. Dubin, *J. Biol. Chem.*, **235**, 769 (1960).

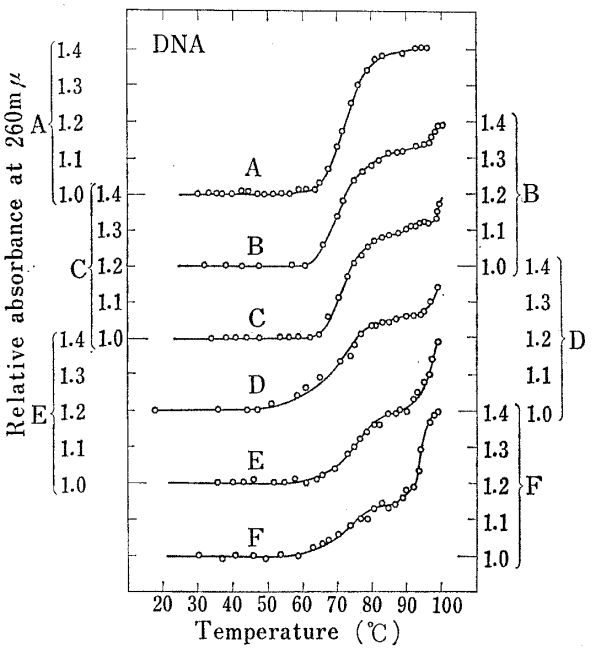
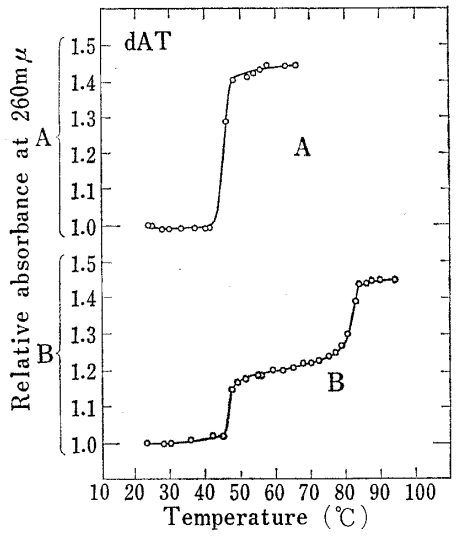


Fig. 1. Absorbance–temperature Profile of Calf Thymus DNA Plus Poly-L-ornithhine at 260 mμ

solvent: For A—E, 0.015M NaCl+0.0015M Tris buffer, pH 7.5; and for F, 0.0049M NaCl+0.00049M tris buffer, pH 7.5
DNA concentration: 5×10^{-5}M
poly-L-ornithine concentration: A, zero; B, 1×10^{-5}M ($NH_2/P=1/5$); C, $1.2_5\times10^{-5}$M ($NH_2/P=1/4$); D, $1.6_7\times10^{-5}$M ($NH_2/P=1/3$); E, 2.5×10^{-5}M ($NH_2/P=1/2$); and F, $3.3_3\times10^{-5}$M ($NH_2/P=2/3$)



Fib. 2. Absorbance–temperature Profile of Poly dAT Plus Poly-L-ornithine at 260 mμ

solvent: 0.015M NaCl+0.0015M Tris buffer, pH 7.5
A, poly dAT 5×10^{-5}M; B, poly dAT 5×10^{-5}M +poly-L-ornithine 2.5×10^{-5}M ($NH_2/P=1/2$)

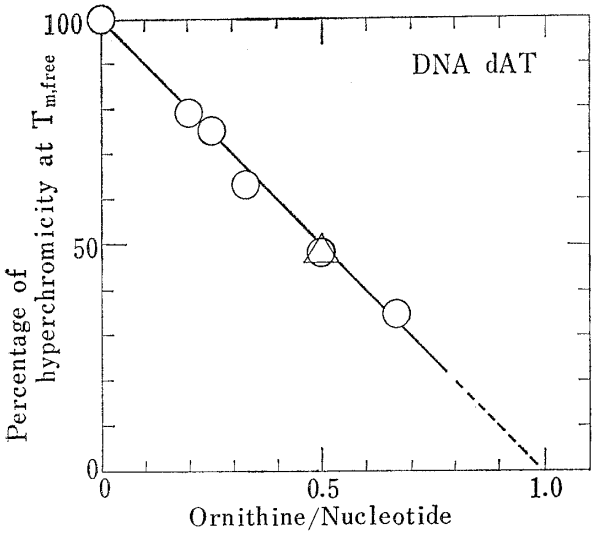


Fig. 3. Effect of the Addition of Poly-L-ornithine on the Percentage of the Hyperchromicity Remaining at the T_m of Calf Thymus DNA or Poly dAT which is not bound with Polyornithine

○: calf thymus DNA △: poly dAT

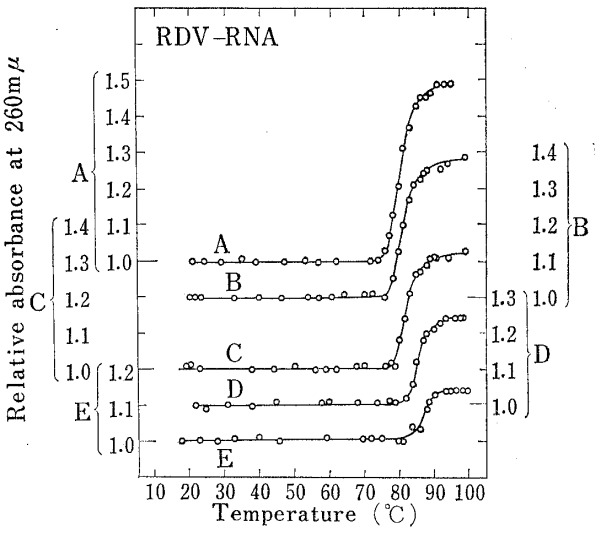


Fig. 4. Absorbance–temperature Profile of Rice Dwarf Virus RNA Plus Poly-L-ornithine at 260 mμ

solvent: 0.0015M NaCl+0.00015M Na-citrate buffer, pH 7.0
RNA concentration: 2.5×10^{-5}M
poly-L-ornithine concentration: A, zero; B, 0.5×10^{-5}M ($NH_2/P=1/5$); C, $0.8_3\times10^{-5}$M ($NH_2/P=1/3$); D, $1.2_5\times10^{-5}$M ($NH_2/P=1/2$); and E, 1.6_7M ($NH_2/P=2/3$)

Rice Dwarf Virus RNA.

This RNA is known to have a complete double-helical structure.[17] On heating the solution (0.0015 M Na^+) to 80°, this RNA shows a sharp hyperchromicity at 260 mμ (Fig. 4, A), which is considered to be due to the melting of the double helix into random coils of single RNA chains. On adding poly-L-ornithine, the hyperchromicity at 80° decreases (Fig. 4, B—E). This fact is interpreted as due to the formation of a polyornithine–RNA complex whose T_m is higher than 80°. The T_m of this complex is considered to be higher than 100°, because no new transition is observed for the polyornithine plus RNA solution up to 100°.

The percentage of hyperchromicity remaining at 80° was plotted against the ornithine/nucleotide ratio in the solution (Fig. 5). These are found to be in a linear relation, and from the inclination of the straight line obtained, the ornithine/nucleotide ratio on the polyornithine plus rice dwarf virus RNA is estimated to be 1/1.

Poly rAU.

The T_m of this double-helical polynucleotide[14] is found to be 48° in 0.005 M phosphate buffer (Fig. 6, A). When an amount of poly-L-ornithine is added to the solution, a new transition appears at 95° (Fig. 6, B—E). By a similar method to that for rice dwarf virus RNA, the ornithine/nucleotide mole ratio in the polyornithine–poly rAU complex is estimated to be 1/1 (Fig. 7).

Poly (A+U)

An equimolar mixture of poly A and poly U is considered to form a double-helical complex in 0.01 M NaCl containing 0.001 M Na citrate buffer (pH 7.0) at 20°.[15,18] On heating the solu-

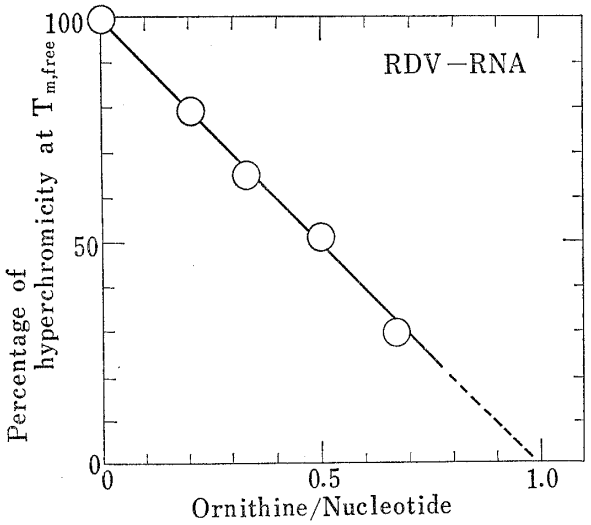


Fig. 5. Effect of the Addition of Poly-L-ornithine on the Percentage of the Hyperchromicity Remaining at the T_m of Rice Dwarf Virus RNA which in not bound with Polyornithine

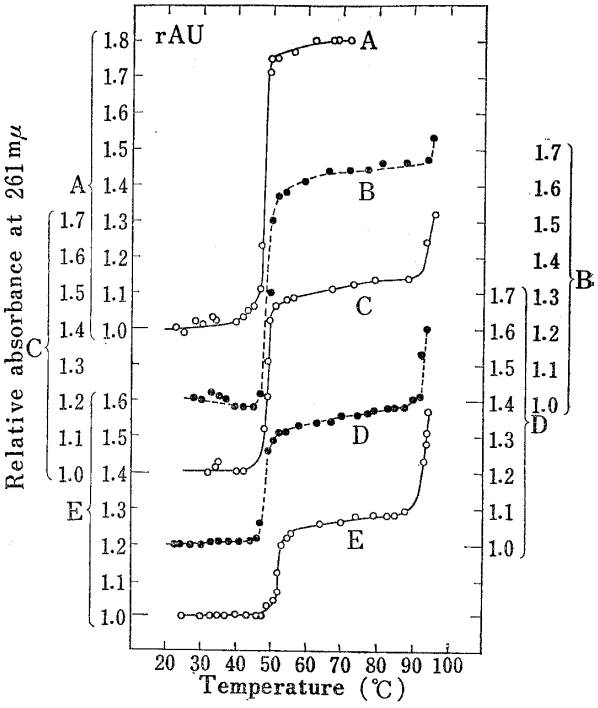


Fig. 6. Absorbance–temperature Profile of Poly rAU Plus Poly-L-ornithine at 261 mμ

solvent: 0.005 M phosphate buffer+0.0001 M NaCl, pH 7.0
poly rAU concentration: 2.5×10^{-5} M
poly-L-ornithine concentration: A, zero; B, 0.5×10^{-5} M (NH_2/P=1/5); C, $0.8_3\times10^{-5}$ M (NH_2/P=1/3); D, $1.2_5\times10^{-5}$ M (NH_2/P=1/2); and E, $1.6_7\times10^{-5}$ M (NH_2/P=2/3)

17) T. Sato, Y. Kyogoku, S. Higuchi, Y. Mitsui, Y. Iitaka, M. Tsuboi, and K. Miura, *J. Mol. Biol.*, **16**, 180 (1966).

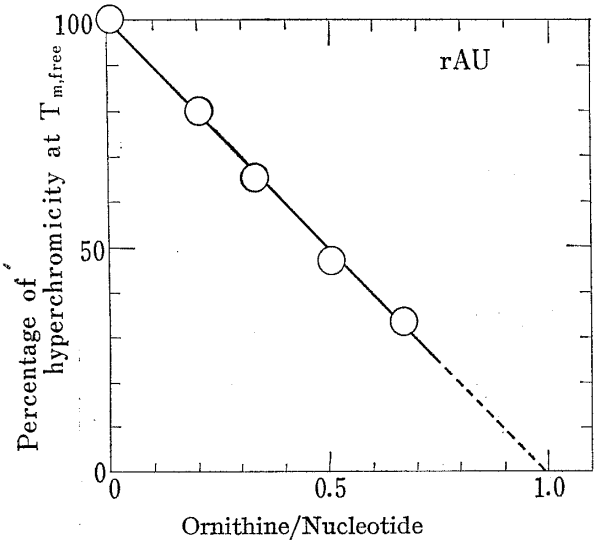


Fig. 7. Effect of the Addition of Poly-L-ornithine on the Percentage of the Hyperchromicity Remaining at the T_m of Poly rAU which is not bound Polyornithine

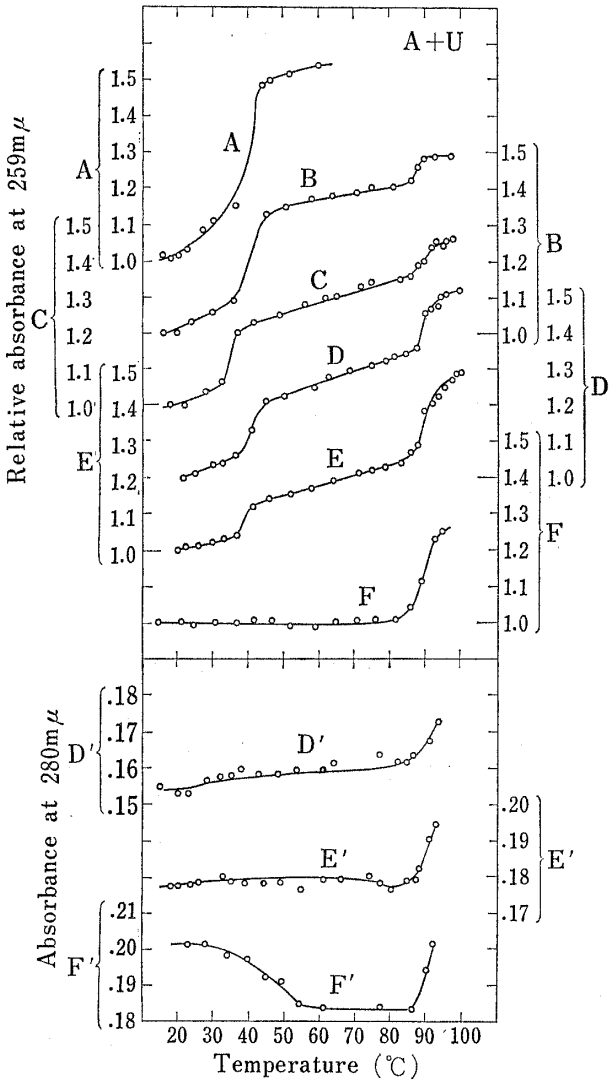


Fig. 8. Absorbance–temperature Profile of Poly (A+U) Plus Poly-L-ornithine at 259 and 280 mμ

solvent: 0.01M NaCl+0.001M Na-citrate buffer, pH 7.0
polynucleotide concentration: poly A, 2.5×10^{-5}M; and poly U, 2.5×10^{-5}M
poly-L-ornithine concentration: A, zero; B, $0.8_3\times10^{-5}$ (NH_2/P=1/6); C, $1.2_5\times10^{-5}$ (NH_2/P=1/4); D and D′, $1.6_7\times10^{-5}$ (NH_2/P=1/3); E and E′, 2.5×10^{-5} (NH_2/P=1/2); F and F′, 3.3×10^{-5} (NH_2/P=2/3)

tion, a sharp increase of the absorbance at 259 mμ takes place at 40°(Fig. 8, A). This is considered to correspond to the melting of the double-helical poly (A+U) into random coils of poly A and poly U. When a small amount of poly-L-ornithine is added to the solution, the amount of increase in the absorbance at 259 mμ is lowered at 40°, and a new transition is observed at 90° (Fig. 8, B—F). This fact is interpreted as indicating that a part of poly (A+U) forms a complex with the poly-L-ornithine added and the rest of the poly (A+U) remains free. The melting process of the complex is considered to be essentially the same with that of the polylysine–poly (A+U) complex[7] or with that of poly (A+U) in hte high salt medium (*e.g.*, 0.001 M Mg^{2+} [15,18] or 1 M Na^{+} [18]). As may be judged from the absorbance–temperature curves F and F′ in Fig. 8, the melting takes place in two steps: first at about 40°, where no change in the absorbance at 259 mμ and a small but appreciable decrease in the absorbance at 280 mμ are observed; secondly at 90°, where an increase in absorbance is observed at 259 mμ as well as at 280 mμ. As was detailed in previous papers,[7,15,18] the first step corresponds to the transition from double- to triple-helices, and the second step to a complete dissociation into single chains of poly A and poly U.

The stoichiometric ratio of ornithine to nucleotide residues in the complex is know to be 2/3, by examining the relation between the amount of poly-L-ornithine added and the amount of poly (A+U) remaining free from polyornithine (Fig. 9).

18) C.L. Stevens and G. Felsenfeld, *Biopolymers*, **2**, 293 (1964).

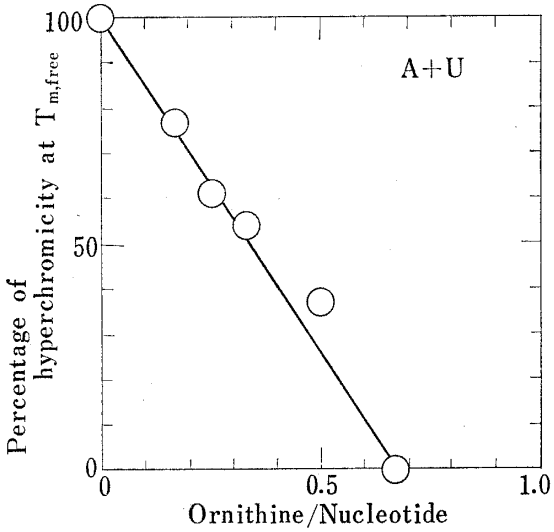


Fig. 9. Effect of the Addition of Poly-L-ornithine on the Percentage of the Hyperchromicity Remaining at the T_m of Poly (A+U) which is not bound with Poly-ornithine

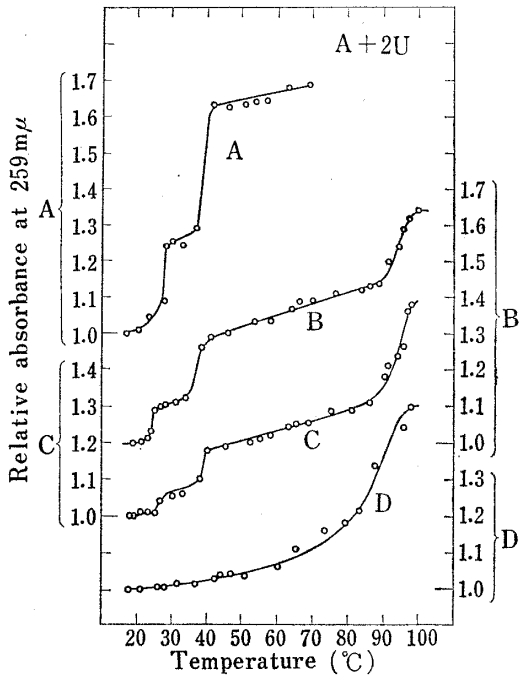


Fig. 10. Absorbance–temperature Profile of Poly(A+2U) Plus Poly-L-ornithine at 259 mμ

solvent: 0.01M NaCl+0.001M Na-citrate buffer, pH 7.0
polynucleotide concentration: poly A, $1.2_5\times10^{-5}$M; polyU, 2.5×10^{-5}M
poly-L-ornithine concentration: A, zero; B, $1.2_5\times10^{-5}$ M ($NH_2/P=1/3$); C, $1.8_8\times10^{-5}$M ($NH_2/P=1/2$); D, 2.5×10^{-5}M ($NH_2/P=2/3$)

Poly (A+2U).

A 1 mole to 2 mole mixture of poly A and poly U is known to form a triple-helix poly (A+2U) in 0.01M Na^++0.001M Na-citrate buffer (pH=7.0) at 20°.[15,19] On heating, it shows a two-step melting at 26° and 38° (Fig. 10, A). As was shown by previous investigators[15,19], the first step corresponds to a partial dissociation of the triple-stranded molecule into double- and single-stranded molecules (poly (A+U) and polyU), and the second step to the complete dissociation into single-stranded poly A and poly U chains. When a certain amount of poly-L-ornithine is added to the solution, a new transition appears at about 95°, and the total hyperchromicity in the 20—40° region is lowered. The transition at 95° is considered to correspond to the melting of a polyornithine–poly (A+2U) complex, and it appears to be a single-step melting.

The amount of the total hyperchromicity in the two-step melting region (20—40°), which corresponds to the amount of poly (A+2U) free of polyornithine, is plotted against the amount of polyornithine added to the solution. As may be seen in Fig. 11, all the points fall nearly on a straight line. From the inclination of this straight line, the ornithine/nucleotide mole ratio in the complex is known to be 2/3.

Poly (I+C)

A 1:1 mixture of poly I and poly C is known to form a double-helical complex poly (I+C) in solution.[5,20] On heating, it shows a sharp hyperchromicity (248 mμ) at 50° in 0.05M NaCl

19) J.R. Fresco, in "*Informational Macromolecules*," H.J. Vogel, V. Bryson, and J.O. Lampen, Eds., Academic Press, New York, 1963, p. 121.
20) D.R. Davies and A. Rich, *J. Am. Chem. Soc.*, **80**, 1003 (1958).

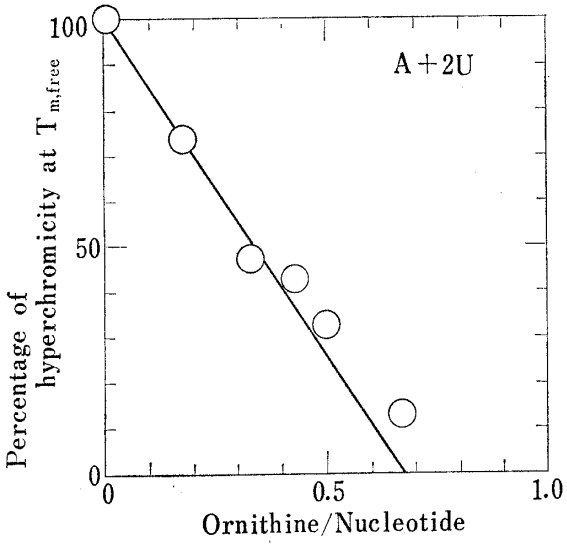


Fig. 11. Effect of the Addition of Poly-L-ornithine on the Percentage of the Hyperchromicity Remaining at the T_m of Poly(A+2U) which is not bound with Polyornithine

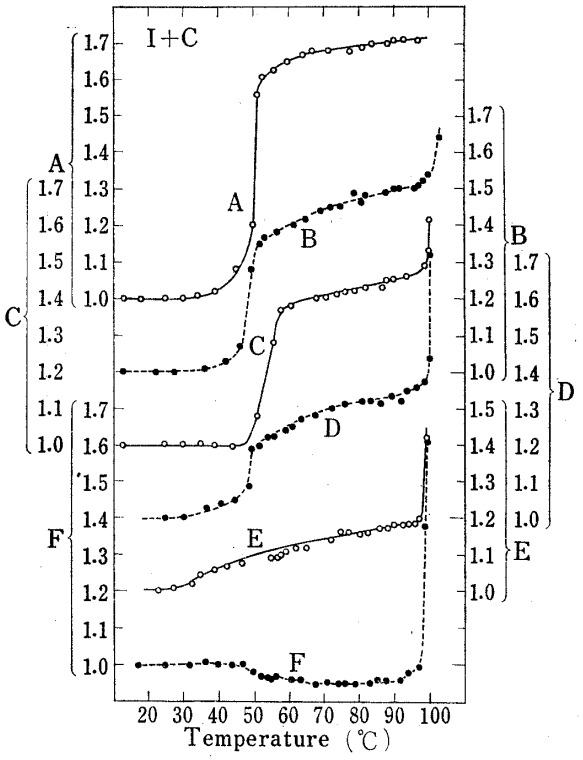


Fig. 12. Absorbance–temperature Profile of Poly(I+C) Plus Poly-L-ornithine at 248 mμ

solvent: 0.05M NaCl+0.001M tris buffer, pH 7.5
polynucleotide concentration: poly I, 2.5×10^{-5}M, poly C, 2.5×10^{-5}M
poly-L-ornithine concentration: A, zero; B, $0.6_8\times10^{-5}$M ($NH_2/P=1/8$); C, $0.8_3\times10^{-5}$M ($NH_2/P=1/6$); D, $1.6_7\times10^{-5}$M ($NH_2/P=1/3$); E, 2.5×10^{-5}M ($NH_2/P=1/2$); F, $3.7_5\times10^{-5}$M ($NH_2/P=3/4$).

+0.001M tris buffer, pH 7.5 (Fig. 12, A). When a proper amount of poly-L-ornithine is added to the solution, a new hyperchromicity assignable to the melting of a polyornithine–poly (I+C) complex appears at about 99° (Fig. 12, B—F). The ornithine/nucleotide mole ratio in the complex is determined by the same method as that used for other helical complexes. As may be seen in Fig. 13, the ratio is 1/2, when the total amount of polyornithine is smaller in the solution. When the amount increases, the ratio is somewhat ambiguous. It is, however, certainly less than 2/3.

Discussion

On the basis of the results of our experiment, a complex of poly-L-orinithine plus nucleic acid is now considered to be quite similar to that of poly-L-lysine plus nucleic acid.[4-9] It is a soluble complex with a definite stoichiometric ratio and a definite melting temperature (T_m). The stoichiometric ratio in a polyornithine–polynucleotide complex, however, is not always equal to that in the corresponding polylysine–nucleotide complex.

As shown in Table I, amino-acid/nucleotide mole ratio is always 1/1 in the complexes with DNA's. The ratio is 2/3 for polyornithine–poly (A+2U) complex as well as for polylysine–poly (A+2U) complex. The possible and probable molecular structures of these complexes with poly-L-lysine have been described in our previous papers,[6-8] and those with poly-L-ornithine are considered to be quite similar to them. The amino-acid/nucleotide mole ratio is 1/1 in the polyornithine complexes with rice dwarf virus RNA and with poly rAU, whereas the ratio is 2/3 in the corresponding polylysine complexes (Table I). This is just what was

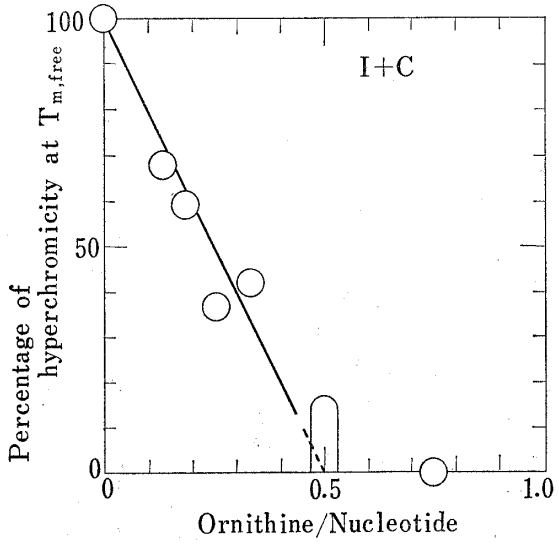


Fig. 13. Effect of the Addition of Poly-L-ornithine on the Percentage of the Hyperchromicity Remaining at the T_m of Poly (I+C) which is not bound with Polyornithine

Table 1. Amino-Acid/Nucleotide Mole Ratios in the Complexes

Polypeptide / Nucleic Acid	Poly-L-ornithine[a)]	Poly-L-lysine[b)]
Calf thymus DNA	1/1	1/1
Poly dAT	1/1	1/1
Rice dwarf virus RNA	1/1	2/3
Poly rAU	1/1	2/3
Poly(A+U)	2/3	2/3
Poly(I+C)	1/2	1/2
Poly(A+2U)	2/3	2/3

a) present work
b) previous works[4-9]

expected on the basis of the scheme of the binding of polylysine and RNA given at the begining of this paper. Thus, the polyornithine-RNA complex (unlike the polylysine–RNA complex) is considered to be free from a lateral association with negatively charged double-helical RNA. It should be pointed out, however, that the polyornithine–poly (A+U) complex has the amino-acid/nucleotide mole ratio 2/3 and the polyornithine–poly (I+C) complex 1/2, and that these ratios are equal to those of the corresponding polylysine complexes (Table I). It is clear that each of these double-helices of synthetic polynucleotides has a slightly different conformation from that of rice dwarf virus RNA, and also from that of poly rAU, in aqueous solution.

The T_m of a polyornithine–polynucleotide complex, in general, is appreciably higher than that of the corresponding polylysine–polynucleotide complex. The T_m of polyornithine–poly (I+C) complex, for example, is 99° in 0.05M Na^+, in contrast with the T_m (=91°) of polylysine–poly (I+C) complex in 0.05M Na^+.[21] The T_m of polyornithine–poly rAU complex in 0.005M phosphate buffer and that of polyornithine–poly (A+2U) in 0.01M Na^+ are both about 95°, while those of the corresponding polylysine complexes are 81° in the same solvents. In other words, a polyornithine complex with a helical nucleic acid is more stable than the corresponding polylysine complex in the same solvent.

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21) K. Matsuo and M. Tsuboi, *Biopolymers*, in press.