BULLETIN OF THE CHEMICAL SOCIETY OF JAPAN VOL. 39 1886—1893 (1966)

The Effects of Polyamines on the Melting of Polyriboadenylic Acid Plus Polyribouridylic Acid Complexes in a Solution

By Shigesada HIGUCHI and Masamichi TSUBOI

Faculty of Pharmaceutical Sciences, University of Tokyo, Hongo, Bunkyo-ku, Tokyo

(Received January 17, 1966)

Ultraviolet absorptions of aqueous systems of polyriboadenylic acid (poly A) plus polyribouridylic acid (poly U) have been examined with various Na^+ and Mg^{2+} concentrations and at various temperatures. The transition temperatures (T_m) among the two-stranded conformation, poly(A+U), the three-stranded conformation, poly(A+2U), and the two single-stranded conformations, poly A and poly U, were determined. The effects upon the T_m of adding NH_3^+-$(CH_2)_nNH_3^+$-type diamines (n=2, 3, 4, 5, 6, and 8), spermine, and actinomycin D to the solutions were observed. It was found that these amines stabilize three-stranded poly(A+2U) more than they stabilize the two-stranded (poly(A+U)) and the single-stranded (poly A and poly U) conformations. Among the diamines examined, those with n=2 and 3 values show a greater effect than those with n=4, 5, 6, and 8 values.

This is our second paper on the effects of polyamines on the melting of synthetic nucleic acids. In our first paper1) we dealt with an aqueous-solution system containing polyriboinosinic acid (poly I) and polyribocytidylic acid (poly C).

---

1) K. Matsuo and M. Tsuboi, This Bulletin, **39**, 347 (1966).

The word "melting" means a transition in the solution from the double-helical conformation of a 1 : 1 complex, poly(I+C), of these two polymers into random coils of poly I and poly C, i. e.:

$$\text{Poly(I+C)} \rightarrow \text{Poly I} + \text{poly C}. \qquad (1)$$

In the present paper we will deal with a system containing polyriboadenylic acid (poly A) and polyribouridylic acid (poly U). Both of these two acids, like poly I and poly C, are synthetic polymers which have the same ribose-phosphate backbone as natural ribonucleic acid (RNA).[2] The system of poly A and poly U is, however, somewhat more complicated than the system of poly I and poly C. In the former, not only a double-helical complex[3] poly(A+U), but also a triple-helical complex[4] poly(A+2U), is formed. Not only a helix-to-random-coil transition, but also transitions of double-to-triple and triple-to-double helices, take place.[5,6] The transition temperatures are affected by the salt concentration in the system, and also by the polyamines added to the solution. The effects of these polyamines are the main subject of this paper.

Experimental

Two samples of polyriboadenylic acid (poly A) were used in the present study. One was purchased from the California Biochemicals Corporation, it showed 4S in an ultracentrifugal sedimentation experiment (Cal. Bio. poly A). The other, purchased from the Miles Chemical Company, showed 8.3S in an ultracentrifugal sedimentation experiment (Miles poly A). Polyribouridylic acid (poly U) was prepared by the use of polynucleotide phosphorylase obtained from *Azotobacter vinelandii*. The product was purified as follows: first it was precipitated by cold ethanol; then it was dissolved in water, deproteinized by chloroform, dialysed against an aqueous solution of 0.01 M ethylene diamine tetraacetate (pH 8), a 1.0 M sodium chloride solution, and distilled water successively, and finally lyophilized. Seven different samples were obtained: Nos. 24, 27, 38, 40, 47, 49, and 50. The sedimentation constants of these samples were, respectively, 8S, 6S, 3.6S, 5.0S, 4.1S, 3.5S, and 1.2S. The polynucleotide concentration in each standard solution was determined by measuring the phosphorous content.[7]

The samples of ethylenediamine dihydrochloride ($NH_3^+(CH_2)_2NH_3^+\cdot 2Cl^-$), 1, 3-diaminopropane dihydrochloride ($NH_3^+(CH_2)_3NH_3^+\cdot 2Cl^-$), 1, 4-diaminobutane dihydrochloride ($NH_3^+(CH_2)_4NH_3^+\cdot 2Cl^-$), 1, 5-diaminopentane dihydrochloride ($NH_3^+(CH_2)_5NH_3^+\cdot 2Cl^-$), 1, 6-diaminohexane dihydrochloride ($NH_3^+(CH_2)_6NH_3^+\cdot 2Cl^-$), 1, 8-diaminooctane dihydrochloride ($NH_3^+(CH_2)_8NH_3^+\cdot 2Cl^-$), and spermine tetrahydrochloride ($NH_3^+(CH_2)_3NH_2^+(CH_2)_4NH_2^+(CH_2)_3NH_3^+\cdot 4Cl^-$) used in this work are all the same as those used in the previous work.[1]

For tracing the conformational changes of the polymers, the ultraviolet absorptions were observed by the use of an Ito Spectrophotometer, Model QU-3. The temperature of the sample solution was controlled as described in our previous paper.[1]

Results and Discussion

1. Evidence of the Formation of a Two-stranded Complex and a Three-stranded Complex.

1. Evidence of the Formation of a Two-stranded Complex and a Three-stranded Complex.—First, the following three preliminary examinations were made to ascertain whether our samples of poly A and poly U show evidence of forming a multi-stranded complex in solution, as previous investigators[3-6] have reported.

(*i*) *Ultracentrifugal Sedimentation.*—It has been shown that a 1 mole-to-1 mole mixture solution (in a 0.1 M sodium chloride + 0.01 M sodium citrate buffer, pH 7.0) of our poly A and poly U shows only one peak in an untracentrifugal sedimentation pattern (with Schlielen optics). This indicates that a 1 : 1 complex is formed. The sedimentation constant of the complex is found to be appreciably greater than those of the components. For example, the complex of poly A from the California Biochemicals Corp. with 4S and No. 38 poly U with 3.6S gives a sedimentation constant of 6.8S. The complex of Miles poly A with 8.3S and No. 49 poly U with 3.5S gives a sedimentation constant of 11.6S.

(*ii*) *Ultraviolet Absorptions.*—Poly A in a 0.15 M sodium chloride + 0.015 M sodium citrate buffer of pH 7.0 gives a spectrum with an absorption maximum at 257 $m\mu$, and poly U in the same solvent gives a spectrum with an absorption maximum at 261 $m\mu$, while a 1 : 1 mixture shows a spectrum with a maximum at 257 $m\mu$. This spectrum was exactly the same as that reported by Warner.[8] On alkali hydrolysis a gerat hyperchromicity is observed in this absorption band.

(*iii*) *Mixing Curve.*—The absorbance was measured at 259 $m\mu$ for several mixtures (at 18±2°C) containing varying proportions of poly A and poly U, while the total nucleotide concentration was kept constant. The solvents examined were as follows:

a) 0.15 M NaCl plus a 0.015 M Na-citrate buffer (pH 7.0).

b) 0.025 M NaCl plus a 0.0005 M Na-citrate buffer (pH 7).

c) 0.015 M NaCl plus a 0.045 M glycylglycine buffer (pH 7.0).

d) 0.1 M NaCl plus a 0.01 M glycylglycine buffer (pH 7.1).

---

[2] M. Grunberg-Manago, P. J. Ortiz and S. Ochoa, *Science*, **122**, 907 (1955).

[3] A. Rich and D. R. Davies, *J. Am. Chem. Soc.*, **78**, 3548 (1956).

[4] G. Felsenfeld, D. R. Davies and A. Rich, ibid., **79**, 2023 (1957).

[5] J. R. Fresco, "Informational Macromolecules," Ed. by H. J. Vogel, V. Bryson and J. Q. Lampen, Academic Press, New York (1963), p. 121.

[6] C. L. Stevens and G. Felsenfeld, *Biopolymers*, **2**, 293 (1964).

[7] G. R. Bartlett, *J. Biol. Chem.*, **234**, 466 (1959).

[8] R. C. Warner, ibid., **229**, 771 (1957).

Table I. A summary of what Stevens and Felsenfeld[6] found of the melting process in mixted solutions of poly A and poly U.

A : U mole ratio in the solution	Salt concn.	Melting process	At 259 mμ	At 280 mμ
1 : 1	Low	(A) Poly(A + U) $\rightarrow$ poly A + poly U	hyper	no change
	High or + Mg^{2+}	(B) 2 poly(A + U) $\rightarrow$ poly(A + 2U) + poly A	no change	hypo
		$\rightarrow$ 2 poly A + 2 poly U	hyper	hyper
1 : 2	Low	(C) Poly(A + 2U) $\rightarrow$ poly(A + U) + poly U	hyper	hyper
		$\rightarrow$ poly A + 2 poly U	hyper	no change
	High or + Mg^{2+}	(D) Poly(A + 2U) $\rightarrow$ poly A + 2 poly U	hyper	hyper

(This is what Felsenfeld, Davies, and Rich[4] used.)

e) d) plus 0.00025 M ethylenediaminetetraacetic acid (EDTA).

f) d) plus 0.0016 M EDTA.

g) 3 ml. of d) plus 1 ml. of a 0.04 M EDTA solution.

h) d) plus 0.01 M EDTA.

In most cases the absorbance was observed immediately after the mixing of poly A and poly U. In the solvent d) however, the absorbance was observed not only immediately after the mixing, but also 25 min., 2 hr. and 54 hr. after the mixing. In the solvent g) the absorbance was observed 15 min. and 40 min. after the mixing, and also after the mixed solution had been kept overnight. The resulting mixing curve was always similar to what Felsenfeld, Davies, and Rich[4] observed in a solvent with 1.2×10^{-2} M magnesium chloride. It is composed of two straight lines which intersect sharply at a poly A : poly U mole ratio of 1 : 2, as is illustrated in Fig. 1. This indicates that, in a mixture of 1 mole of poly A and 2 moles of poly U, a three-stranded complex, poly(A+2U), is formed under any of the above sets of conditions. What we observed in solvent d) is, however, different from what Felsenfeld, Davies, and Rich[4] observed. In this solvent, they observed a mixing curve composed of two straight lines which intersect at a mole ratio of 1 : 1. As will be shown below, this difference may be partly due to the difference in the temperature of the solution. They observed the mixing curve at 25°C, while we observed it at 18°C. On the basis of our rather detailed examination (see above), we may conclude that, at 18°C, a 1 mole-to-2 mole mixture of our poly A and poly U forms a three-stranded complex, poly(A+2U), even in a solvent with a low salt concentration.

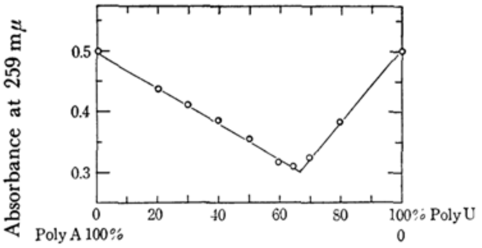

Fig. 1. A mixing curve for poly A and poly U. Solvent: 0.1 M NaCl plus 0.01 M glycylglycine buffer, pH 7.1. Temperature : 18±2°C. The absorbance at 259 mμ was observed at 54 hr. after every mixing of poly A and poly U.

2. The Melting Process of Poly(A+U) and Poly(A+2U).—A series of examinations were made to find out the conditions under which poly(A+U) and poly(A+2U) are stable and the process through which these complexes melt into random coils of poly A and poly U. As we were conducting our examinations, a paper of Stevens and Felsenfeld[6] reporting a similar attemt appeared. They showed that the melting process can be followed by tracing the absorbance at 260 mμ and that at 280 mμ. What they found is summarized in Table I. We found, as is illustrated in Fig. 2, that our samples of poly A and poly U show the same behavior as they found (compare Fig. 2 with Table I). On the basis of the results of other similar experiments of ours, as well as of a few previous works by others,[5,6] we have constructed diagrams shown in Figs. 3 and 4. These diagrams illustrate how the melting process in question depends upon the Na$^+$ concentration in the solvent. Because of an unknown factor, different batches of samples show some small but appreciable differences in melting temperatures. For example, poly(A+U) with No. 49 poly U shows a slightly but still appreciably higher T_m value than poly(A+U) with No. 47 poly U. The sedimentation constants of these two poly U's (No. 49 and 47) are 3.5S and 4.1S respectively, almost equal to each other. The transition temperatures of poly(A+2U) to poly(A+U) in the 1 mole-to-2 moles mixture of our samples are appreciably higher than those observed by Fresco[5] in a region with a lower salt concentration (see Fig. 4). As has been described in the preceding section, our sample does not form poly(A+U) at 18°C, even in a very low-salt environment. In general, the equilibrium of poly(A+U) and poly(A+2U) is a rather delicate one, as will be illustrated in the next section.

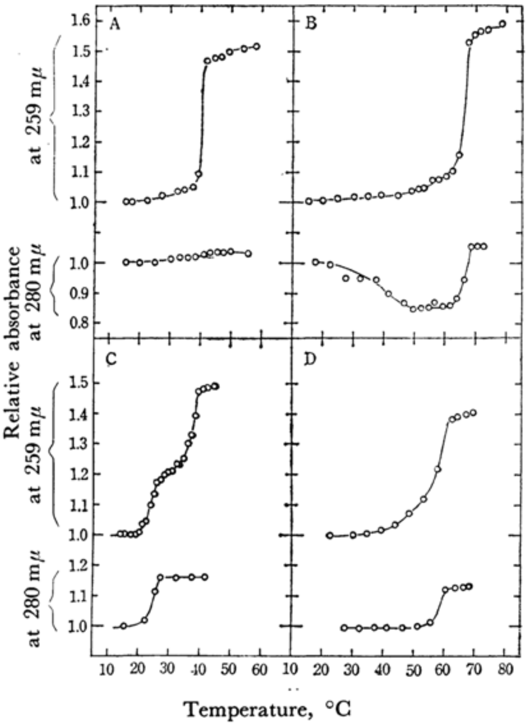

Fig. 2. Four types of melting process A, B, C, and D (See Table I) of helical complexes in mixed solution of poly A and poly U, as followed by observing the absorbance at 259 mμ and 280 mμ.

A: 1 mol. to 1 mol. mixture of Miles poly A and No. 49 poly U in 0.01 M NaCl+0.001 M Na-citrate buffer, pH 7

B: The same with A except +0.001 M $MgCl_2$

C: 1 mol. to 2 mol. mixture of Miles poly A and No. 50 poly U in 0.01 M NaCl+0.001 M Na-citrate buffer, pH 7

D: 1 mol. to 2 mol. mixture of Miles poly A and No. 50 poly U in 0.001 M $MgCl_2$+0.001 M Na-citrate buffer, pH 7

3. A Metastable Poly(A+2U).—In connection with the equilibrium between the two- and three-stranded complexes, we also noticed the following phenomenon.

Miles poly A plus No. 50 (or No. 47) poly U were dissolved, in a 1 : 1 mole ratio, into 0.01 M sodium chloride plus a 0.001 M sodium citrate buffer (pH 7), the solution was then heated. The absorbance at 259 mμ shows one-step melting at 39°C, while the absorbance at 280 mμ shows almost no change throughout the temperature range (15—45°C) examined. The solution of the melted polynucleotides was slowly cooled to 15°C (2 hr. for 45→15°C) and was then examined again as to its melting profile. The absorbance at 259 mμ shows two-step transitions at 26°C and 39°C, while the absorbance at 280 mμ shows a transition at 26°C. This melting profile is similar to

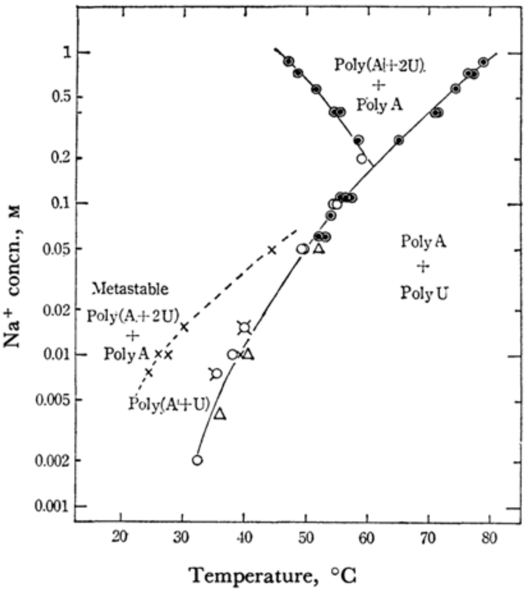

Fig. 3. Melting temperatures of poly(A+U) and poly(A+2U) in solutions with poly A and poly U in 1 : 1 mole ratio and with various Na^+ concentrations.

○: Miles poly A+No. 47 poly U
△: Miles poly A+No. 49 poly U
⊙: Observed by Stevens and Felsenfeld[6)]
×: Observed for metastable poly(A+2U) (See section 3)

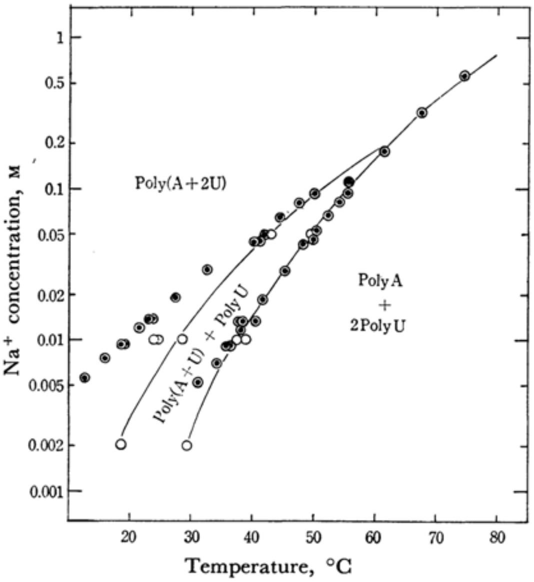

Fig. 4. Melting temperatures of poly(A+2U) and poly(A+U) in solutions with poly A and poly U in 1 : 2 mole ratio and with various Na^+ concentrations.

○: Miles poly A+No. 50 poly U
⊙: Observed by Fresco[5)]

that of poly(A+2U) (the type C profile given in Table I or in Fig. 2).

This fact may be interpreted as indicating that the mixing of poly A and poly U (1 : 1) at room temperature causes this reaction:

$$\text{poly A} + \text{poly U} \rightarrow \text{poly(A + U)}, \qquad (2)$$

However, the cooling of the melted poly A and poly U (1 : 1) mixture causes another reaction:

$$\text{poly A} + \text{poly U} \rightarrow {}^{1}/_{2}\,\text{poly(A + 2U)} + {}^{1}/_{2}\,\text{poly A}. \qquad (3)$$

Support for this interpretations was obtained by another experiment. First, the three-stranded complex poly(A+2U) was formed in a solution, and then poly A was added to the solution so that the resulting total amounts of poly A and poly U became equivalent to each other (1 : 1 mole ratio). The resultant solution shows a melting profile similar to that of poly(A+2U) in a solution with a 1 : 2 mole ratio. Thus, the added poly A does not react with poly(A+2U); in other words, the reaction:

$${}^{1}/_{2}\,\text{poly(A + 2U)} + {}^{1}/_{2}\,\text{poly A} \rightarrow \text{poly(A + U)} \qquad (4)$$

does not proceed in a low-salt-content environment.

Poly A does not form any highly-ordered conformation by itself at a neutral pH value. Therefore, at lower temperatures, poly(A+U) may be considered to be more stable than the partiallymelted form, 1/2 poly(A+2U) + 1/2 poly A. Thus, the poly(A+2U) resulting from reaction (3) may be considered to be in a metastable state.

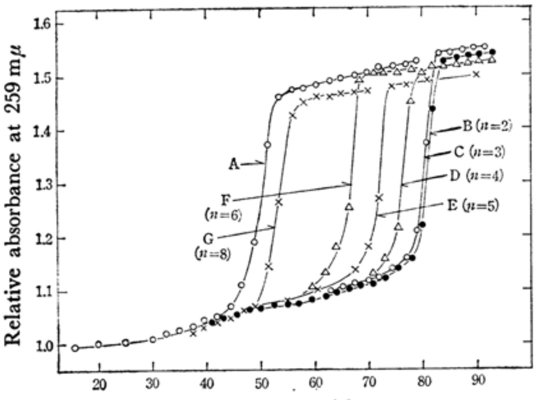

Fig. 5. The variation of the absorbance of Miles poly A plus No. 47 poly U (1 : 1) plus diamine ($NH_3^+(CH_2)_nNH_3^+$) solutions at 259 mμ with temperature.

Solvent: 0.05 M NaCl plus 0.001 M Na-citrate buffer, pH 6.5—7.0

A: No. amine, B: 0.01 M $NH_3^+(CH_2)_2NH_3^+$, C: 0.01 M $NH_3^+(CH_2)_3NH_3^+$, D: 0.01 M $NH_3^+(CH_2)_4NH_3^+$, E: 0.01 M $NH_3^+(CH_2)_5NH_3^+$, F: 0.01 M $NH_3^+(CH_2)_6NH_3^+$, G: 0.005 M $NH_3^+(CH_2)_8NH_3^+$

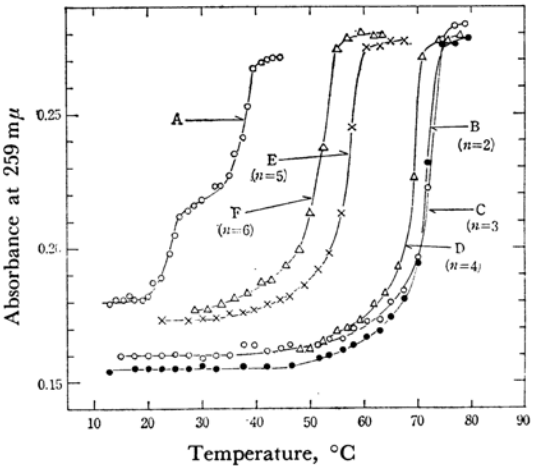

Fig. 6. The variation of the absorbance of Miles poly A plus No. 50 poly U (1 : 2) plus diamine ($NH_3^+(CH_2)_nNH_3^+$) solutions at 259 mμ with temperature.

Solvent: 0.01 M NaCl plus 0.001 M Na-citrate buffer, pH 7

A: No amine, B: 0.001 M $NH_3^+(CH_2)_2NH_3^+$, C: 0.001 M $NH_3^+(CH_2)_3NH_3^+$, D: 0.001 M $NH_3^+(CH_2)_4NH_3^+$, E: 0.001 M $NH_3^+(CH_2)_5NH_3^+$, F: 0.001 M $NH_3^+(CH_2)_6NH_3^+$

4. The Effects of Diamines.

—When a $NH_3^+(CH_2)_nNH_3^+$-type diamine is added to a solution of poly(A+U) or poly(A+2U), an effect on the melting profile similar to that of an elevation of the salt concentration of a solution

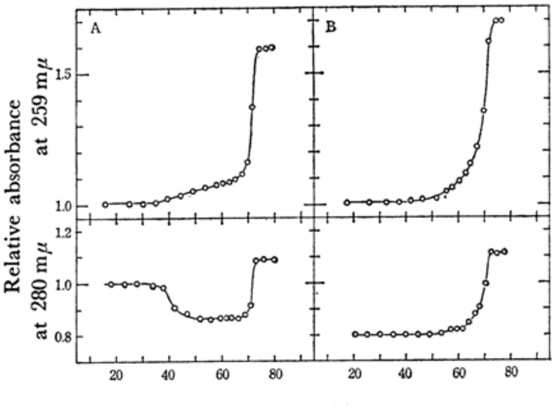

Fig. 7. The variation of the absorbance at 259 mμ and at 280 mμ of poly A plus poly U plus ethylenediamine dihydrochloride solutions with temperature.

Solvent: 0.001 M NaCl plus 0.001 M Na-citrate buffer, pH 7

A: Miles poly A plus No. 49 poly U in 1 : 1 mole ratio plus 0.001 M $NH_3^+(CH_2)_2NH_3^+$

B: Miles poly A plus No. 50 poly U in 1 : 2 mole ratio plus 0.001 M $NH_3^+(CH_2)_2NH_3^+$

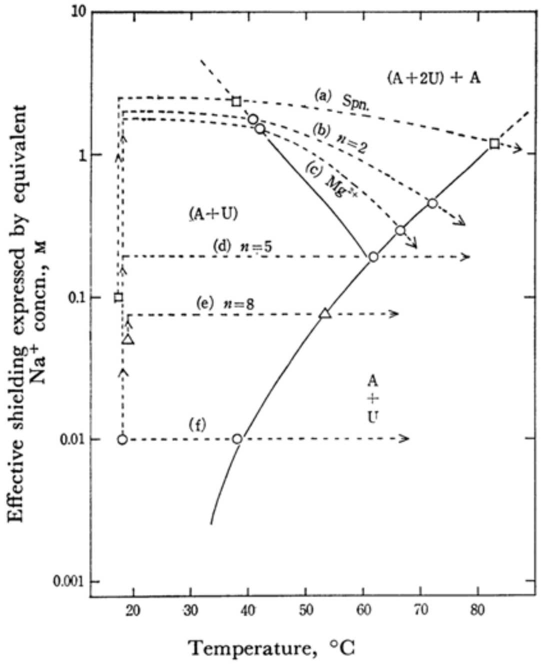

Fig. 8. Route of melting of poly(A+U) drawn on a phase diagram similar to Fig. 3, in a solution (a) with 0.001 M $NH_3^+(CH_2)_3NH_2^+(CH_2)_4$-$NH_2^+(CH_2)_3NH_3^+$, (b) with 0.001 M NH_3^+-$(CH_2)_2NH_3^+$, (c) with 0.001 M $MgCl_2$, (d) with 0.001 M $NH_3^+(CH_2)_5NH_3^+$, (e) with 0.005 M $NH_3^+(CH_2)_8NH_3^+$, or (f) without any divalent cation. The vertical dotted lines indicate the effect of the addition of divalent cations. The starting points of these lines indicate the Na^+ concentration of the solution before the addition of the divalent cations.

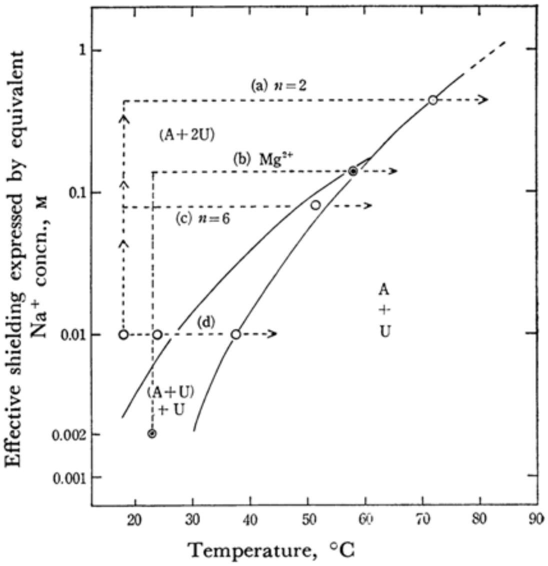

Fig. 9. Route of melting of poly(A+2U) drawn on a phase diagram similar to Fig. 4.
In a solution (a) with 0.001 M $NH_3^+(CH_2)_2NH_3^+$, (b) with 0.001 M $MgCl_2$, (c) with 0.001 M $NH_3^+(CH_2)_6NH_3^+$, or (d) without any divalent cation

is observed (Figs. 5 and 6). The melting temperature of poly(A+U) observed by the absorbance at 259 mμ is elevated (Fig. 5). The melting profile is changed from Type A into Type B (see Table I, Fig. 2, and Fig. 7, A). For poly(A+2U), the two-step melting profile traced by the absorbance at 259 mμ disappears; D-type melting profile is now observed (Fig. 6 and Fig. 7, B). This fact may be interpreted by considering that the effect of the diamine cation is essentially the same as the effect of Na^+, except that the former is much greater than the latter. In general, cations in the solution are considered to be bound by the PO_2^- group of the polynucleotide molecules and to shield the negative charge, so that the interstrand repulsion is reduced in the two- or three-stranded complex. Therefore, it is probable that phase diagrams similar to those given in Figs. 3 and 4 are valid for every $NH_3^+(CH_2)_nNH_3^+$-type cation, if the ordinate, instead of the actual Na^+ concentration, is assumed to be a measure of the effectiveness of a certain amount of each cation in shielding the negative charge of the polynucleotide chains. On the basis of this interpretation, the effects of a few diamines are shown in Figs. 8 and 9, where the same phase diagrams as are given in Figs. 3 and 4 are used.

As may be seen in Fig. 8, the shielding effect of the $NH_3^+(CH_2)_2NH_3^+$ cation is slightly greater than that of Mg^{2+} for poly(A+U). For poly (A+2U), the shielding effect of $NH_3^+(CH_2)_2$·NH_3^+ is also somewhat greater than that of Mg^{2+} (see Fig. 9). These facts may be explained by considering that, while the shielding effect of the Mg^{2+} bound to the PO_2^- group may be greater than that of the $NH_3^+(CH_2)_2NH_3^+$, the binding constant of the $NH_3^+(CH_2)_2NH_3^+$ bound to polynucleotide is greater than that of the Mg^{2+}. The binding constant of a $NH_3^+(CH_2)_nNH_3^+$-type cation can be great if the distance of the two amino groups in the cation is nearly equal to the distance between two PO_2^- groups (or between two O^- atoms) in the secondary structure of the polynucleotide molecule in question. In a comparison of diamines with n values of 2, 3, 4, 5, 6, and 8 (cf. Fig. 5), the melting temperatures observed with the $n=2$ and $n=3$ diamines are almost equal to each other and are much higher than the melting temperatures observed with other diamines (see also Fig. 10, (b)). The melting temperature higher than 61°C observed in the system where poly A: poly U=1 : 1 (see Fig. 8) should correspond to the melting reaction:

$$\text{poly(A + 2U) + poly A} \rightarrow \text{2 poly A + 2 poly U} \qquad (5)$$

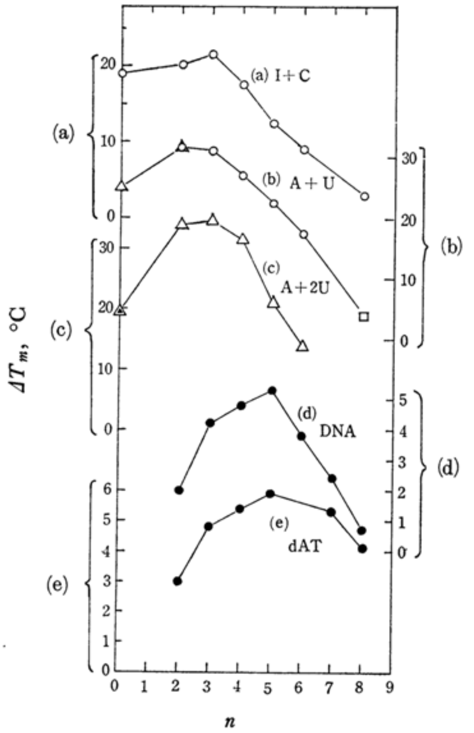

Fig. 10. A relative efficiency of diamines NH_3^+-$(CH_2)_nNH_3^+$ in stabilizing helical nucleic acids plotted against n, the number of the methylene groups placed between the two NH_3^+ groups. The stabilizing efficiency is expressed by an increment in the melting temperature, ΔT_m, of the nucleic acid solution caused by adding a certain amount of the diamine. For $n=0$, ΔT_m caused by Mg^{2+} was plotted. (a) poly(I+C),[1] (b) poly(A+U), (c) poly(A+2U), (d) calf thymus deoxyribonucleic acid,[9] and (e) alternating copolymer of deoxyadenylic acid and deoxythymidylic acid.[9]

○: ΔT_m observed with 0.01 M diamine or Mg^{2+} in 0.05 M NaCl plus 0.01 M Na-citrate buffer, pH 7.0.

△: ΔT_m observed with 0.001 M diamine or Mg^{2+} in 0.01 M NaCl plus 0.001 M Na-citrate buffer, pH 7.

▲: ΔT_m observed with 0.001 M Mg^{2+} plus 0.001 M Na-citrate buffer, pH 7.

□: ΔT_m observed with 0.005 M diamine in 0.05 M NaCl plus 0.001 M Na-citrate buffer, pH 7.

●: ΔT_m observed with 0.04 M diamine in 0.15 M NaCl plus 0.015 M Na-citrate buffer, pH 7.

Therefore, the $NH_3^+ \cdots NH_3^+$ distance in the $n=2$ or $n=3$ diamine is considered to reflect a $PO_2^- \cdots PO_2^-$ distance (or $O^- \cdots O^-$ distance) in the three-stranded poly(A+2U) molecule. In the system where poly A : poly U=1 : 2, a melting temperature higher than 61°C should correspond to the melting reaction:

$$\text{poly (A + 2U)} \rightarrow \text{poly A + 2 poly U} \qquad (6)$$

In the comparison regarding this melting temperature shown in Fig. 6, the maximum is also observed around $n=2$ and $n=3$ (see Fig. 10, (c)). As has been described in a previous paper,[1] the maximum ΔT_m value was observed at $n=3$ for poly(I+C) (Fig. 10, (a)). For double-stranded deoxyribonucleic acids, on the other hand, the maximum ΔT_m value was observed at $n=5$[9] (Fig. 10, (d) and (e)). On the basis of what has been described above, these facts may be interpreted as indicating that the nearest $PO_2^- \cdots PO_2^-$ distance (or $O^- \cdots O^-$ distance) in two- or three-stranded-polyribonucleotide is smaller than that in double-stranded polydeoxyribonucleotide.

5. The Effect of Spermine.—The change in the melting profile of poly(A+U) caused by spermine is illustrated in Fig. 11. The melting profile observed with 0.001 M spermine belongs to the Type B in Table I; it is similar to the melting profile observed with 0.001 M Mg^{2+}. The first transition, however, takes place at a lower temperature (at 38°C) with spermine than with Mg^{2+}, while the second transition takes place at a higher temperature (at 83°C) with spermine than with Mg^{2+}. These facts indicate: (1) that spermine (like other cations) stabilizes the three-stranded poly(A+2U) more than it stabilizes the two-stranded poly(A+U) or single-chain poly A and poly U, and (2) that this function of spermine is stronger than that of Mg^{2+}. As may be seen

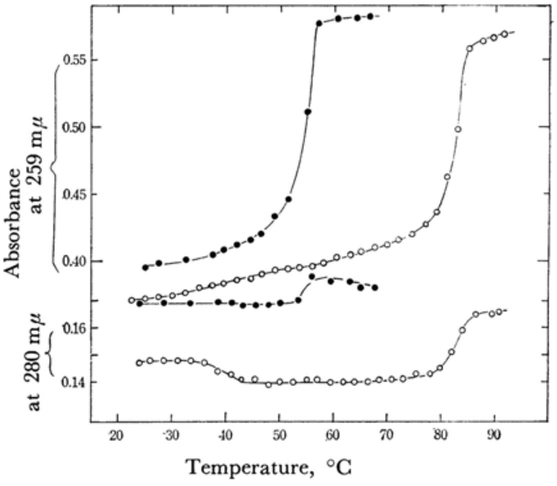

Fig. 11. The variation of the absorbance at 259 mμ and at 280 mμ of Miles poly A plus No. 48 poly U (1 : 1 mol. ratio) solutions with (○) and without (●) spermine (0.001 M).
Solvent: 0.1 M NaCl plus 0.001 M Na-citrate buffer, pH 7

---

[9] H. R. Mahler and B. D. Mehrotra, *Biochim. Biophys. Acta*, **68**, 211 (1963).

in Fig. 8, the effect of spermine (0.001 M) is not only greater than that of Mg^{2+}, but also greater than that of ethylenediamine (0.001 M). This is understandable, because the spermine cation is tetra-valent and so its binding constant to the polynucleotide would be greater and its shielding effect would be greater than those of Mg^{2+} and $NH_3^+(CH_2)_2NH_3^+$. The effect of spermine on the poly A + poly U system may thus be explained qualitatively by considering only the ordinary effect of a cation. So far, it is not necessary to assume a so-called clamping function,[1,10] although it is still probable that spermine has this function also.

6. The Effect of Actinomycin D.—It has been found that 7γ/ml. of actinomycin D has no effect on the melting profile of a 1 : 1 mixture of poly A and poly U.

This work was supported by a grant from the United States Public Health Service (No. GM 10024-02) and a grant from the Ministry of Education of Japan.

---

[10] M. Tsuboi, This Bulletin, **37**, 1514 (1964). (It should be pointed out here that there is a mistake in the DNA model shown in Fig. 11 of this paper.)