

Interaction between Polynucleotides VI. Ionic Strength Dependence of the Poly(A)·Poly(U) Duplex Formation

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ABSTRACT: The heats of mixing for an equimolar mixture of poly(A) and poly(U) in buffer solutions of various concentrations were measured at 298.15 ± 0.005 K by calorimetry. The apparent heats of mixing obtained depended on buffer concentration. The degree of formation of the poly(A)·poly(U) duplex and fraction of stacking of poly(A) in various buffer concentrations were also estimated from CD spectral measurements. By a combination of the results of calorimetric and spectral methods, the net heat of formation of poly(A)·poly(U) duplex with high ordered structure from random coil conformation of constituent polynucleotide was estimated to be about -67.2 kJ (bpm)⁻¹, using the reaction cycle.

KEY WORDS Heat of Mixing / Circular Dichroism Spectrum / Poly(A)·Poly(U) Duplex / Degree of Duplex Formation / Heat of Duplex Formation / Degree of Helix of Poly(A) / Ionic Strength /

It is well known that an equimolar mixture of poly(riboadenylic acid), poly(A), and poly(ribouridylic acid), poly(U), forms a well-defined poly(A)·poly(U) duplex by interactions between different chains, and the structure of this duplex is a double stranded helix similar to that of DNA. The stability of this structure depends on environmental conditions such as temperature, pH, and ionic strength of the solution.¹ However, it is interesting that the poly(A)·poly(U) duplex does not form from an equimolar mixture of poly(A) and poly(U) in pure water at room temperature.

In the previous paper,² the heat of formation based on the poly(A)·poly(U) duplex from an equimolar mixture of poly(A) and poly(U) in 0.1 mol dm⁻³ Tris-HCl buffer solutions (pH 7.60) containing various NaCl concentrations was estimated to be about -15 kJ (bpm)⁻¹ from calorimetric experiments.

This heat of formation showed a definite value nearly independent of NaCl concentration when was less than 0.01 mol dm⁻³. Thus, the formation of the poly(A)·poly(U) duplex may depend considerably on the concentration of the buffer solution. The hydrogen-bonding energy between adenine and uracil for Watson-Crick^{3,4} and Hoogsteen pairs⁴ was calculated by ab initio MO calculation with the STO-3G minimal basis set. The hydrogen-bonding energy was reported to be about -60.9 kJ³ and -58.8 kJ⁴ for Watson-Crick pair and about -51.4 kJ⁴ for Hoogsteen one.

Under the assumption that the heat of formation of the poly(A)·poly(U) duplex formed from an equimolar mixture of poly(A) and poly(U) may be based on the hydrogen bonding energy between poly(A) and poly(U), the heat of formation of about -15 kJ (bpm)⁻¹ determined by experiment does not agree

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with the hydrogen bonding energy estimated by calculation. The magnitude of discrepancy may be attributable to the structure of poly(A) which exists as a single stranded helical structure stabilized by base stacking,⁵ while poly(U) has a random coil conformation.⁶ To obtain the net heat of formation based on poly(A)·poly(U) duplex formation, it may be necessary to consider the states of poly(A) in solutions.

In this paper, the first aim is to obtain information on the duplex formation in buffer solutions of various concentrations and then the second is to obtain the net heat of formation of the duplex from an equimolar mixture of poly(A) having a random coil conformation and poly(U) by calorimetric and spectral experiments.

EXPERIMENTAL

Poly(riboadenylic acid), poly(A), and poly(ribouridylic acid), poly(U), were purchased from Yamasa Shoyu Co., Ltd., Japan. All other materials were analytical reagent grade of commercial product.

The solvents used to adjust pH (7.60) in this study were Tris-HCl {Tris(hydroxymethyl)-aminomethane-hydrochloric acid} buffer solutions of various concentrations.

The calorimeter used was similar to that reported previously.⁷

For the calorimetric measurements, the concentrations of poly(A) and poly(U) were about 5.0×10^{-4} nucleotide unit mol dm^{-3} . Polynucleotide concentration was determined by the phosphorus analysis method.⁸

CD spectra were taken by a spectropolarimetry JASCO J-20A. The polynucleotide concentration used for the CD spectral measurement was identical to that for the calorimetric measurements and light-path length of cell was 1 mm.

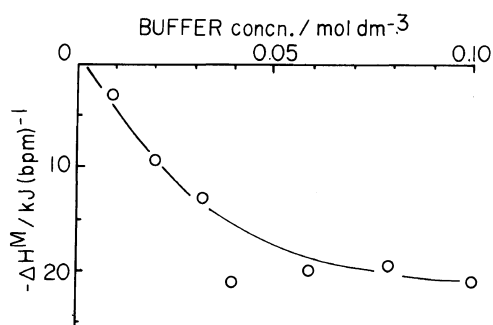


Figure 1. The plots of the heats of mixing per mole of a base pair, ΔH^M against buffer concentration for an equimolar mixture of poly(A) and poly(U).

RESULTS AND DISCUSSION

Heat of Mixing

The heats of mixing of an equimolar mixture of poly(A) and poly(U) in Tris-HCl buffer solutions (pH 7.60) of various concentrations were measured by LKB batch type micro-calorimeter at 298.15 ± 0.005 K. The heats of mixing proved to be exothermic, suggesting that enthalpy changes may correspond to the formation of the poly(A)·poly(U) duplex, under the assumption that the heat of dilution of polynucleotide solution may be negligible. The results obtained are summarized in the second column of Table I and shown in Figure 1, where the heat of mixing per mole of base pair (bpm) of poly(A)·poly(U) duplex, ΔH^M is plotted against the concentration of buffer solution. As seen in Figure 1, the absolute value of ΔH^M increases at first, and then levels off with increasing concentration of buffer solution. This buffer concentration dependence of ΔH^M seems to be due to the degree of duplex formation.

CD Spectrum

To confirm information on the results obtained from the heat of mixing, CD spectra for an equimolar mixture of poly(A) and poly(U) solutions containing various concentrations of buffer solution were measured at room temperature.

Interaction between Polynucleotides

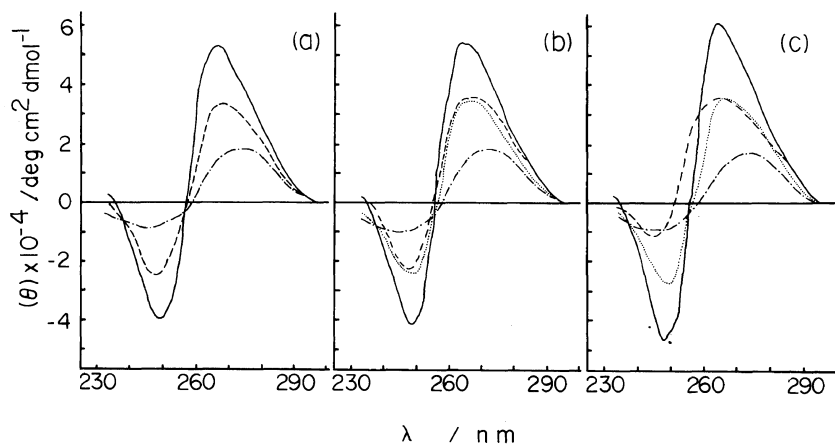


Figure 2. The typical CD spectrum, (—); poly(A), (---); poly(U), (....); an equimolar mixture of poly(A) and poly(U), and (— · — · —); synthesized by adding the CD spectrum of poly(A) to that of poly(U) at various concentrations of the buffer solutions; (a): 0.001, (b): 0.01, and (c): 0.1 mol dm⁻³.

The typical CD spectra obtained are shown in Figure 2. As seen in Figure 2, the CD spectrum for an equimolar mixture of poly(A) and poly(U) in 0.001 mol dm⁻³ buffer solutions is the same as that synthesized by adding the CD spectrum of poly(A) to that of poly(U) in the same buffer solution. An equimolar mixture of poly(A) and poly(U) in buffer solutions with 0.01 and 0.1 mol dm⁻³ showed characteristic CD spectra different from those synthesized by adding the CD spectrum of poly(A) to that of poly(U), demonstrating that considerable interaction occurs between poly(A) and poly(U) in these buffer solutions. The CD spectrum for an equimolar mixture of poly(A) and poly(U) in 0.1 mol dm⁻³ buffer solution, comparable to that reported by Brahms,⁹ is different from that in the 0.01 mol dm⁻³ one. This suggests that the degree of formation of the duplex depends on the concentration of buffer solution.

The wavelength, λ_{cross} for the crossover of CD spectrum obtained for an equimolar mixture of poly(A) and poly(U) is plotted against the concentration of buffer solution in Figure 3(a). In this figure, λ_{cross} , when the buffer concentration is zero, shows a wavelength on the crossover of CD spectrum synthesized by

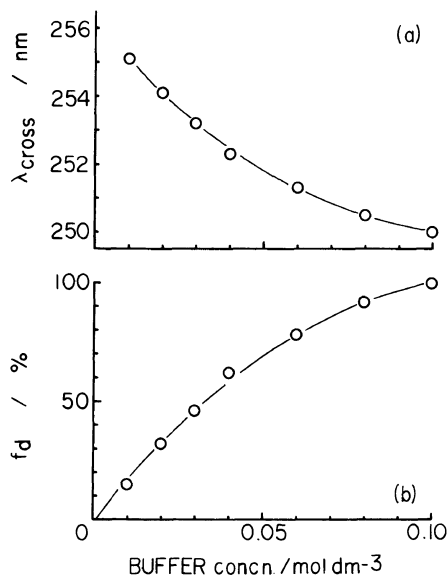


Figure 3. Dependence of (a) the wavelength, λ_{cross} on the crossover of the CD spectrum and (b) the degree of the duplex formation, f_d on buffer concentration for an equimolar mixture of poly(A) and poly(U).

adding the CD spectrum of poly(A) to that of poly(U) in 0.001 mol dm⁻³ Tris-HCl buffer solution. This wavelength of the crossover means that no interaction between poly(A) and poly(U) takes place. In the figure, λ_{cross} decrease at first and then levels off with in-

creasing concentration of buffer solution, demonstrating that the degree of formation of the poly(A)·poly(U) duplex depends on the concentration of the buffer solution.

Assuming that the degree of formation, f_d of the poly(A)·poly(U) duplex is 100% at 0.1 mol dm⁻³ buffer solution and 0% at the 0.001 mol dm⁻³ one, we can calculate f_d for an equimolar mixture of poly(A) and poly(U) in the buffer solutions of various concentrations from the concentration dependence of λ_{cross} . The results estimated are also listed in the third column of Table I and shown in Figure 3(b), where f_d is plotted against the concentration of the buffer solution.

As seen in Figure 3(b), f_d increases with an increase of the buffer concentration, and then levels off when the buffer concentration is higher than about 0.05 mol dm⁻³. This behavior is comparable to the buffer concentration dependence of ΔH^M as shown in Figure 1.

Table I. The heat of mixing, ΔH^M , the degree of duplex formation, f_d , and the heat of formation, ΔH_{form} for an equimolar mixture of poly(A) and poly(U) in Tris-HCl buffer solutions of various concentrations

Buffer concn mol dm ⁻³	$-\Delta H^M$ kJ (bpm) ^{-1 a}	f_d %	$-\Delta H_{\text{form}}^b$ kJ (bpm) ^{-1 a}
0.001	$\cong 0^c$	0 ^c	30.0 ^d
0.01	3.3	15	29.1
0.02	9.6	32	28.1
0.03	13.2	46	27.1
0.04	21.7	62	26.1
0.06	20.1	78	24.1
0.08	18.0	98	22.1
0.10	20.7	100	20.7

^a bpm here refers to a mole of an adenine-uracil pair.
^b Calculated from the least square treatment of ΔH^M versus buffer concentration.

^c ΔH^M cannot be observed from calorimetric and spectral methods, because of poly(A)·poly(U) duplex may not form at this buffer concentration.

^d This value was obtained by extrapolating to the 0.001 mol dm⁻³ buffer concentration.

Heat of Formation of the Duplex

ΔH^M obtained in the buffer solutions of various concentrations as shown in Figure 1 may correspond to the apparent heat of formation of duplex since the degree of formation of duplex depends on the buffer concentration as mentioned above.

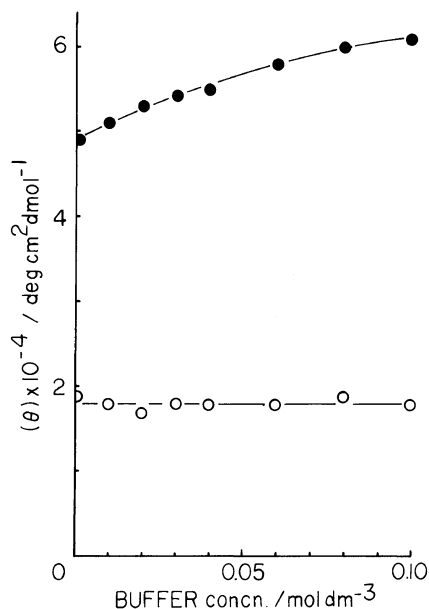


Figure 4. The dependence of molar ellipticity $[\theta]$ on buffer concentration for (●) poly(A) at a wavelength of 264 nm and (○) poly(U) at 257 nm.

Table II. Molar ellipticity, $[\theta]$ degree of helix, f_h and stacking energy, ΔH_s for poly(A) in Tris-HCl buffer solutions of various concentrations at room temperature.

Buffer concn mol dm ⁻³	$[\theta] \times 10^{-4}$ deg cm ² dmol ⁻¹	f_h %	$-\Delta H_s$ kJ (bpm) ⁻¹
0.001	4.9	78	38.9
0.01	5.1	81	42.4
0.02	5.3	85	47.1
0.03	5.4	87	46.6
0.04	5.5	89	46.0
0.06	5.8	94	51.1
0.08	6.0	98	53.3
0.10	6.1	100	—
Average			46.5

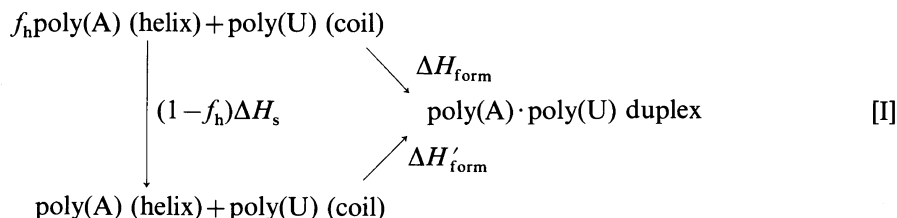
The heat of formation, ΔH_{form} was calculated by taking the degree of formation of duplex into consideration, and the results obtained are listed in the last column of Table I. ΔH_{form} has different values in the buffer solutions of various concentrations: ΔH_{form} at lower buffer concentrations is less than that at higher ones, suggesting that the states of both poly(A) and poly(U) before mixing depend on the formation of the duplex.

To obtain information on the states of poly(A) and poly(U), CD spectra were also measured. The results obtained are shown in Figure 4, where molar ellipticity, $[\theta]$ at wavelengths 264 nm for poly(A) and the 257 one for poly(U) is plotted against buffer concentration. $[\theta]$ for poly(U) has about $1.9 \times 10^4 \text{ deg cm}^2 \text{ dmol}^{-1}$ which is nearly independent of buffer concentration, demonstrating that the random coil conformation of poly(U) is maintained at various buffer concentrations. This may correspond to a random coil conformation of poly(U) in neutral solution.⁶ $[\theta]$ for poly(A) increases with an increase in buffer concentration as seen in Figure 4, demonstrating that the formation of the helix of poly(A)

based on base stacking may differ according to buffer concentration. This tendency corresponds to a single stranded helical structure stabilized by base stacking in neutral solutions.⁵

The single stranded helical structure of poly(A) stabilized by base stacking gradually collapses with temperature.⁹ $[\theta]$ of poly(A) obtained at high temperature (above 353 K) shows a definite value of about $8.0 \times 10^3 \text{ deg cm}^2 \text{ dmol}^{-1}$ which is nearly independent of buffer concentration. Assuming that $[\theta]$ obtained at the high temperature and at room temperature (298 K) in 0.1 mol dm^{-3} buffer solution may correspond to a random coil structure and single stranded helical structure of poly(A), respectively, the degree of helix, f_h , a measure of the fraction of single stranded helical of poly(A), was calculated, and the results are listed in the third column of Table II.

From the results as mentioned above, the stacking energy of poly(A), ΔH_s may be estimated according to the following reaction cycle [I].



In this scheme, $\Delta H'_{\text{form}}$ is the heat of formation at $f_h = 1.0$ of the degree of helix of poly(A) and equal to -20.7 kJ , corresponding to ΔH^M at 0.1 mol dm^{-3} buffer solution.

From reaction cycle [I], ΔH_s can be expressed as,

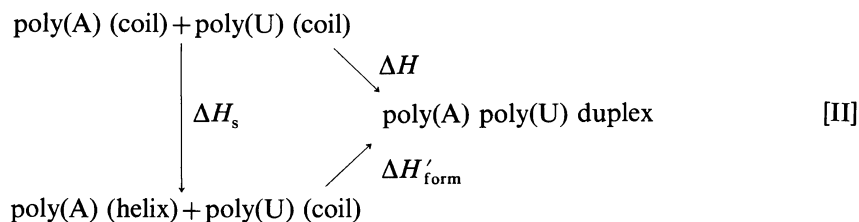
$$\Delta H_s = \frac{1}{(1-f_h)} (\Delta H_{\text{form}} - \Delta H'_{\text{form}}) \quad (1)$$

Using ΔH_{form} and f_h estimated at various buffer concentrations as shown in Tables I and

II, we can calculate ΔH_s of poly(A) according to eq 1. ΔH_s obtained at various buffer concentrations are listed in the last column of Table II. In Table II, ΔH_s seem to be scattered by the error of ΔH_{form} and f_h estimated. However, even allowing such uncertainty, the average value of ΔH_s is about -46.5 kJ , which seems to be comparable to -38 kJ for poly(A)¹¹ estimated by the calorimetric measurement and -43 kJ for poly(C)¹² estimated by the spectroscopic one.

The Net Heat of Formation of the Duplex

The enthalpy change accompanying the formation of the duplex from a random coil conformation of a constituent polynucleotide may correspond to the net heat of formation,



From this scheme as described above, ΔH is the sum of ΔH_s and $\Delta H'_{\text{form}}$. Since $\Delta H_s = -46.5 \text{ kJ}$ and $\Delta H'_{\text{form}} = -20.7 \text{ kJ}$, ΔH can be estimated to be about -67.2 kJ . The estimated ΔH is smaller than hydrogen-bonding energy ($-58.8 \text{ kJ bpm}^{-1}$ for Watson-Crick pair and $-51.4 \text{ kJ bpm}^{-1}$ for the Hoogsteen one) between adenine and uracil calculated by ab initio MO method.⁴ This seems to be due to energy contributions such as stacking energy between adenine-uracil pairs, the energy of backborn winding of the duplex accompanying the helix state from the coil one, and the energy based on the solvent effect as dehydration accompanying the duplex formation and so on.

These energies as mentioned above, however, have not yet been quantitatively estimated. To understand exactly the mechanism of the duplex formation, further study now is in progress.

ΔH of the poly(A)·poly(U) duplex from an equimolar mixture of poly(A) and poly(U).

ΔH can be obtained from the following reaction cycle [II],

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