NOTE

Interaction of Polynucleotide in Aqueous NaCl Solution. III. Poly(riboadenylic acid)–Poly(riboinosinic acid) System

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It is well known that deoxyribonucleic acid (DNA) forms the double helical structure by the interaction between the bases: purine and pyrimidine.

In order to obtain thermodynamic information about the formation of this double helix, we measured the enthalpy change in the complex formation between synthetic polynucleotides such as poly(riboadenylic acid) (poly(A)) and poly(ribouridylic acid) (poly(U)),^{1,2} poly(riboguanilyc acid) (poly(G)) and poly(ribocytidylic acid) (poly(C)),³ and poly(riboinosinic acid) (poly(I)) and poly(C),⁴ and showed that the enthalpy change in this formation depends on the chemical structure of the base of a polynucleotide and the microscopic medium surrounding the polynucleotide, such as an NaCl concentration. Hitherto, there has been no information on the behavior of poly(A) and poly(I) in solution.

In this paper, in order to obtain further information about the effect of chemical structure on the enthalpy change of base pairing in nucleotides, we measured the heat of mixing of poly(A)–poly(I) system.

EXPERIMENTAL

Materials

Poly(riboadenylic acid), poly(A), and poly(riboinosinic acid), poly(I), were purchased from

Miles Lab., USA and used without further purification. The NaCl used in this study was the analytical reagent grade of commercial products. All measurements were made in 0.1 mol dm⁻³ Tris-HCl buffer solution (pH 7.60). The concentration of nucleotide phosphate was determined by the phosphorous analysis method.⁵

Apparatus and Procedure

The calorimeter used was the same as that described in a previous paper.³ For measuring the heat of mixing of poly(A) and poly(I) solutions containing various concentrations of NaCl, the concentration of nucleotide was made to be about 6 × 10⁻⁴ mol dm⁻³ and equal volumes (1 cm³) of those solution were mixed.

Ultraviolet (UV) and circular dichroism (CD) spectra were measured by a spectrophotometer (Hitachi 124, Hitachi Ltd., Japan) and a spectropolarimeter (J-20, JASCO, Japan). In this case, the UV and CD spectra of a mixture of poly(A) and poly(I) solutions were measured within 10 min after mixing. The poly(A) and poly(I) concentrations for the spectrophotometric measurements were about one-sixth those of the calorimetric ones.

RESULTS AND DISCUSSION

The heats of mixing of an equimolar mixture of poly(A) and poly(I) in solution at various NaCl concentrations were measured by a microcalorimeter at 298 ± 0.002 K. The results obtained are

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Figure 1. The heats of mixing of an equimolar mixture of poly(A) and Poly(I) in 0.1 mol dm^{-3} Tris-HCl solution (pH 7.60) at various NaCl concentrations at 298 K.

shown in Figure 1, where the heats of mixing per mole of base pair (bpm) of poly(A) and poly(I), ΔH^M , is plotted against the NaCl concentration of

the solution. As seen in Figure 1, ΔH^M is nearly zero when the NaCl concentration is less than about $0.005 \text{ mol dm}^{-3}$, demonstrating that no interaction between poly(A) and poly(I) exists under this condition.

With a NaCl concentration greater than 0.1 mol dm^{-3} , ΔH^M was exothermic and showed a definite value in the vicinity of 0.1 mol dm^{-3} NaCl and increased sharply as the concentration of NaCl increased. Therefore, ΔH^M may correspond to the heat of interaction, going on the assumption that the heat of dilution of the polymer is negligibly small.

In addition, ΔH^M , in a NaCl concentration range from 0.01 to 0.05 mol dm^{-3} , could not be estimated because the exothermic curve areas of the reaction runs for mixing of poly(A) and poly(I) were negligibly small, compared with the sharp exothermic curves obtained when the concentration of

Figure 2. CD spectra of poly(A) (-----), poly(I) (----), and an equimolar mixture of poly(A) and poly(I) (—) in solution at various NaCl concentration: (a), 0.01 mol dm^{-3} ; (b), 0.1 mol dm^{-3} ; (c), 0.3 mol dm^{-3} . The dotted line (·····) is the CD curve synthesized by adding the CD spectra of only poly(A) to those of only poly(I). The broken line (—) represents the CD spectra of an equimolar mixture of poly(A) and poly(I) at 353 K.

Figure 3. The plots of absorbance at 249 nm against the mole fraction of poly(A), X_A , for mixtures of poly(A) and poly(I) in solutions at various NaCl concentrations: (a), 0.01 mol dm^{-3} ; (b), 0.1 mol dm^{-3} ; (c), 0.3 mol dm^{-3} .

NaCl was in excess of 0.1 mol dm^{-3} .

ΔH^M is negligibly small in a NaCl concentration range from 0.01 to 0.05 mol dm^{-3} , and this seems to be due to the following reason. Rich⁶ reported that the formation of poly(A)·poly(I) or poly(A)·poly(2I) complexes is controlled by the ionic strength of medium, and at a low ionic strength, only the 1:1 complex forms after 18 h. From the results by Rich and those obtained in our present work, we suggest that poly(I) having a random-coil state may need additional time to form a complex with poly(A) in a dilute NaCl solution.

To clarify the formation of the 1:1 complex at very low ionic strength, further study will be necessary.

In order to confirm the interaction between poly(A) and poly(I), the CD spectra of various mixture of poly(A) and poly(I) in solution were measured. The results obtained are shown in Figure 2. As seen in Figure 2, the CD spectra of an equimolar mixture of poly(A) and poly(I) in a solution containing 0.1 and 0.3 mol dm^{-3} NaCl differ from the spectrum in a 0.01 mol dm^{-3} NaCl solution which is the same as that synthesized by adding the CD spectrum of only poly(I) to that of only poly(A) at room temperature, and also differ from the CD spectra of an equimolar mixture of poly(A) and poly(I) at 353 K. This suggests that in an equimolar mixture of poly(A) and poly(I) in a 0.1 and 0.3 mol dm^{-3} NaCl solution there is some interaction between poly(A) and poly(I).

In order to confirm the interaction in an equimolar mixture of poly(A) and poly(I) in a concentrated NaCl solution exceeding 0.1 mol dm^{-3} , the absorbances at 249 nm for various mole fractions of poly(A), X_A , were measured and the results obtained are plotted against X_A , as shown in Figure 3.

As shown in Figure 3, the absorbance mixing curves for NaCl solutions of 0.1 and 0.3 mol dm^{-3} show discontinuous points which do not appear in the absorbance mixing curve for 0.01 mol dm^{-3} NaCl solution. These discontinuous points appearing in the vicinity of 0.3 of X_A correspond to the some interaction between poly(A) and poly(I), demonstrating that this interaction is based on the formation of a triple stranded helical structure of poly(A)·poly(2I).

To obtain information about the poly(A)·poly(2I) triplex, plots were made of molar ellipticity at 277 nm of CD spectra against NaCl concentration. And these seem to reflect the extent of the ordered structure of poly(I), as shown in Figure 4.

Figure 4. Dependence of molar ellipticity at 277 nm of poly(I) solution on NaCl concentration.

city at 277 nm of CD spectra against NaCl concentration. And these seem to reflect the extent of the ordered structure of poly(I), as shown in Figure 4.

Figure 4 shows that the intensity is nearly zero when the NaCl concentration is below 0.1 mol dm^{-3} and that this intensity begins to increase when the NaCl concentration exceeds 0.1 mol dm^{-3} . This increase in intensity indicates that poly(I) can form an ordered structure such as a triple or quadruple stranded structure^{7,8} at a NaCl concentration above 0.1 mol dm^{-3} . On the other hand, CD spectra of poly(A) are independent of NaCl concentration, as seen in Figure 2.

These facts are interpreted as follows: at a NaCl concentration below 0.1 mol dm^{-3} , no complex will form between poly(A) and poly(I) having a random-coil state under our experimental conditions. On the other hand, in the concentrated NaCl solution, a complex, poly(A)·poly(2I) triplex will be formed, indicating that the interaction between poly(A) and poly(I) is stronger than that between poly(I) and poly(I). The formation of poly(A)·poly(2I) triplex by mixing of poly(A) and poly(I) may be due to the following reactions,

poly(A) + poly(I) (order)

where poly(I) (order) and poly(I) (disorder) refer to the triple or quadruple stranded structure and random-coil state, respectively. Thus, ΔH^M can be represented as the sum of the heats of the order-disorder transition of poly(I), ΔH_i , and the heat of formation of the poly(A)·poly(2I) triplex, ΔH ,

$$\Delta H^M = \Delta H_t + \Delta H$$

ΔH_t is equal to zero since the triple or quadruple stranded structure of poly(I) does not exist in 0.1 mol dm⁻³ NaCl solution, according to CD spectra results obtained, and so ΔH becomes equal to ΔH^M . The ΔH calculated as the value per mole of base pair must be converted to the ΔH value per mole of base triplet according to the reaction 1, and consequently, ΔH is obtained as about -42 kJ per mole of base triplet in aqueous 0.1 mol dm⁻³ NaCl solution.

Furthermore, ΔH^M increases when the NaCl concentration is more than 0.1 mol dm⁻³, as seen in Figure 1. This increase of ΔH^M corresponds to an increase in the apparent heat of transition of poly(I) (order) because the extent of structure of poly(I) depends on the NaCl concentration. This extent,

however, was not estimated since the dependence of the heat of transition of poly(I) on the NaCl concentration was not estimated in this work.

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