

Interaction of Polynucleotides in Aqueous NaCl Solution (II): Poly(I)–Poly(C) System

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ABSTRACT: The heats of mixing for an equimolar mixture of poly(inosinic acid) (poly(I)) and poly(citidylic acid) (poly(C)) in solutions of various NaCl concentrations were measured by using the microcalorimeter of LKB batch type at 298 ± 0.002 K. In a dilute NaCl solution, an equimolar mixture of poly(I) and poly(C) forms a poly(I)·poly(C) duplex(I) with double-stranded helical structure and its heat of interaction was estimated to be about -21 kJ per mole of base pair. On the other hand, when a NaCl concentration is more than 0.1 mol dm^{-3} , an equimolar mixture of poly(I) and poly(C) also forms a poly(I)·poly(C) duplex(II) although poly(I) exists as an ordered structure such as a triple- or quadruple-stranded structure with an increase of the NaCl concentration, and its heat of interaction was estimated to be about -22 kJ per mole of base pair.

KEY WORDS Poly(inosinic acid) / Poly(citidylic acid) / Heat of Mixing /
Ultraviolet and Circular Dichroism Spectra / Poly(inosinic acid)·
Poly(citidylic acid) Duplex / Heat of Interaction /

In previous papers,^{1,2} it was reported that the heats of interaction between poly(riboadenylic acid) (poly(A)) and poly(ribouridylic acid) (poly(U)) and those between poly(guanylic acid) (poly(G)) and poly(citidylic acid) (poly(C)) in solutions containing various NaCl concentrations were estimated from the heats of mixing, and the enthalpy changes between poly(A) and poly(U) differ from those between poly(G) and poly(C) although the enthalpy changes for both systems are based on the interactions between the analogous bases: purine and pyrimidine, and also an equimolar mixture of poly(A) and poly(U) in diluted NaCl solution forms poly(A)·poly(U) duplex with double-stranded helical structure, but that of poly(G) and poly(C) forms a weak complex.

From these facts, it may be considered that the formation of double-stranded helical structure of polynucleotide depends on the characteristic properties of each polynucleotide such as single-stranded structure of poly(A)³ and poly(C),⁴ and self-association of poly(G).⁵

In order to obtain further information on the interaction between polynucleotides, the heats of mixing of poly(inosinic acid) (poly(I)) and poly(C) in solutions containing various NaCl concentrations were measured and the heat of interaction between poly(I) and poly(C) was estimated.

EXPERIMENTAL

Materials

The polynucleotides (potassium salts of poly(I) and poly(C)) were purchased from Miles Co. Ltd., USA and used without further purification. All other materials were the analytical reagent grade of commercial products. The buffer solution used to adjust pH was 0.1 mol dm^{-3} Tris-HCl solution (pH 7.60).

Apparatus and Procedure

Poly(I) and poly(C) samples were dissolved in buffer solutions of various NaCl concentrations. When the concentration of NaCl was beyond 0.5 mol dm^{-3} , in order to obtain a homogeneous poly(I) solution, poly(I) solution was placed in a

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water bath kept at 333 K and was then allowed to stand for about 15 hours at room temperature.

The concentration of nucleotide phosphate was determined by the phosphorous analysis method.⁶

The calorimeter used in this study was the same as that reported previously.² For the calorimetric measurement, equal volumes (about 1 cm³) of poly(I) and poly(C) solutions having about 4×10^{-4} mol dm⁻³ of nucleotide phosphate were mixed. However, the measurement of the heat of mixing at a concentration beyond 1.0 mol dm⁻³ NaCl was impossible because poly(I) precipitated.

Ultraviolet (UV) and circular dichroism (CD) spectra were measured with a spectrophotometer (Hitachi 124, Hitachi Ltd., Japan) and a spectropolarimeter (J-20, JASCO, Japan), respectively. The concentration of nucleotide phosphate of spectrophotometric measurement was about one-seventh of the calorimetric measurement.

RESULTS AND DISCUSSION

Heat of Mixing

The heats of mixing of an equimolar mixture of poly(I) and poly(C) solutions containing various NaCl concentrations were measured with a microcalorimeter at 298 ± 0.002 K. This system was found to be exothermic, indicating that the interaction takes place between poly(I) and poly(C). The results obtained are shown in Figure 1, where the heats of mixing per mole of base pair (bpm) of nucleotide, ΔH^M , are plotted against the NaCl concentration. As seen in Figure 1, ΔH^M has a definite value of

about -21 kJ which is nearly independent of concentration in the region where that of NaCl is below 0.1 mol dm⁻³. When the concentration of NaCl exceeds 0.1 mol dm⁻³, ΔH^M increases greatly and then approaches a definite value with an increase in NaCl concentration.

CD and UV Spectra

In order to confirm the data regarding ΔH^M , the CD spectra of poly(I), poly(C) and an equimolar mixture of poly(I) and poly(C) in solutions containing 0.01, 0.1, and 0.5 mol dm⁻³ NaCl were measured at room temperature and the results obtained are shown in Figure 2. It can be seen from Figure 2 that the CD spectra of an equimolar mixture of poly(I) and poly(C) are different from those synthesized by adding the CD spectra of only poly(I) to those of only poly(C), and the CD spectra of an equimolar mixture of poly(I) and poly(C) obtained are in good agreement with those reported by Haynes *et al.*⁷ and Thile *et al.*,⁸ indicating that in an equimolar mixture of poly(I) and poly(C) may cause an interaction between poly(I) and poly(C).

In order to obtain further information on the interaction between poly(I) and poly(C), the absorbance at 249 nm was also measured for various mole fractions of poly(I), X_1 , as shown in Figure 3. It is seen from Figure 3 that the absorbance mixing curves for poly(I) and poly(C) in solutions containing 0.01 and 0.5 mol dm⁻³ NaCl show discontinuous points in the vicinity of 0.5 of X_1 , demonstrating that the complex formed by mixing of poly(I) and poly(C) has a double-stranded

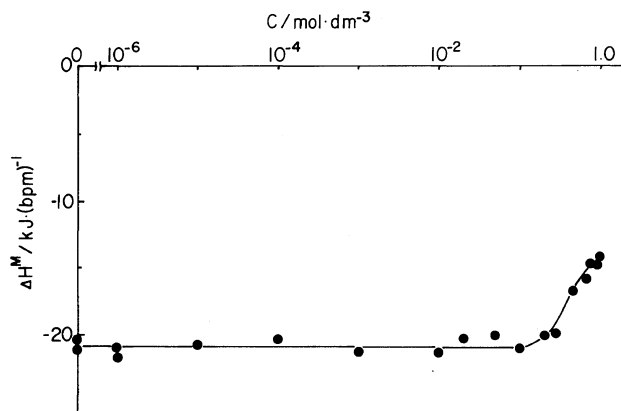


Figure 1. The heats of mixing for an equimolar mixture of poly(I) and poly(C) in 0.1 mol dm⁻³ Tris-HCl buffer solution (pH 7.60) at various NaCl concentrations at 298 K.

Interaction of Poly(I)-Poly(C) System

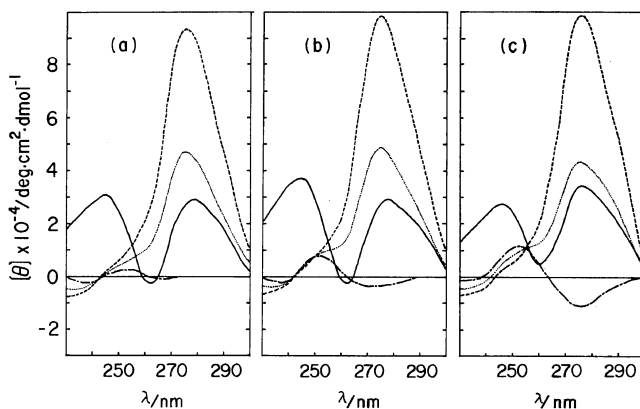


Figure 2. CD spectra of poly(I) (—), poly(C) (---), and an equimolar mixture of poly(I) and poly(C) (—) in solution at various NaCl concentrations: (a), $10^{-2} \text{ mol dm}^{-3}$; (b), $10^{-1} \text{ mol dm}^{-3}$; (c), 0.5 mol dm^{-3} . A dotted line (-----) is the curve synthesized by adding the intensity of CD spectra of poly(I) to that of poly(C).

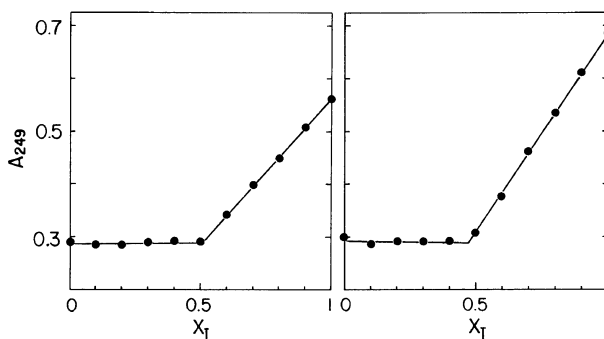


Figure 3. The plots of absorbance at 249 nm against the mole fraction of poly(I), X_1 , for a mixture of poly(I) and poly(C) in solutions varying in NaCl concentration: (a), $10^{-2} \text{ mol dm}^{-3}$; (b), 0.5 mol dm^{-3}

structure, the poly(I)·poly(C) duplex.

Accordingly, it is considered that the poly(I)·poly(C) duplex is formed by poly(I) and poly(C), from the results of spectra measurements mentioned above.

In Figure 2, CD spectra of poly(I)·poly(C) duplex in the solution where the NaCl concentration is below 0.1 mol dm^{-3} differ from those in the solution where the NaCl concentration is above 0.5 mol dm^{-3} . This indicates that the conformation of poly(I)·poly(C) duplex in 0.5 mol dm^{-3} solution can be distinguished from that in the solution where the NaCl concentration is less than 0.1 mol dm^{-3} . The intensity at 277 nm in the CD spectra, which reflects the formation of a triple- or quadruple-stranded structure of poly(I),^{9,10} is nearly zero when the NaCl concentration is below 0.1 mol dm^{-3} and

begins to increase when the NaCl concentration exceeds 0.1 mol dm^{-3} as shown in Figure 4, indicating that poly(I) starts to take on a structure when the NaCl concentration is over 0.1 mol dm^{-3} .

From the above results, it is reasonable to

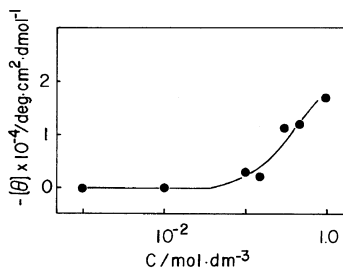
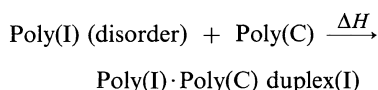


Figure 4. Dependence of molar ellipticity at 277 nm of poly(I) solution on NaCl concentration.

consider that poly(I) also forms poly(I)·poly(C) duplex with poly(C) in a concentrated NaCl solution. The formation of the poly(I)·poly(C) duplex in a concentrated NaCl solution seems to differ from poly(I)·poly(C) duplex formed in dilute NaCl solutions.

Heat of Interaction of Poly(I)·Poly(C) Duplex

From the results of UV, and CD spectra measurements, and calorimetric works, the following two processes, depending on NaCl concentration, may be considered: The first process occurs when NaCl concentration is less than 0.1 mol dm^{-3} and results from a following reaction between poly(C) and poly(I) having a disordered structure such as that of a random-coil conformation.



The heat of interaction, ΔH , in this process may correspond to the heat of mixing which is nearly independent of concentration in a solution in which the NaCl concentration is below 0.1 mol dm^{-3} , assuming that the heat of dilution of polymer is negligibly small, being -21 kJ as seen in Figure 1. The ΔH value obtained is in good agreement with that estimated by Ross *et al.*¹¹

ΔH of poly(I)·poly(C) duplex is larger than that of the poly(A)·poly(U) duplex in dilute NaCl solutions as shown in Table I. However, poly(I)·poly(C) and poly(A)·poly(U) duplexes are based on the analogous hydrogen bonds between purine and pyrimidine bases of nucleotide as shown in Figure 5. The difference in ΔH -values may possibly depend on the nature of the force involved in base stacking between base pairs such as in the adenine–uracil and inosine–cytosine base pairs.

Although the chemical structure of inosine is

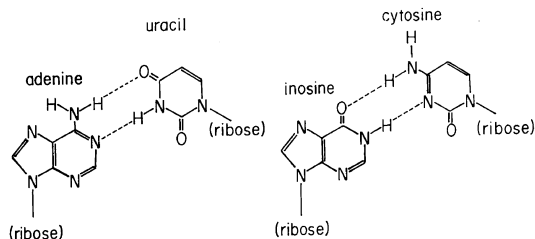
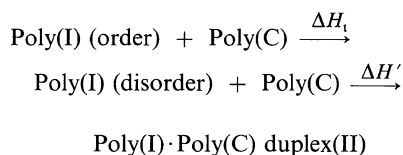


Figure 5. Hydrogen bonds between nucleotide base pairs: adenine–uracil and inosine–cytosine pairs.

similar to that of guanine, the enthalpy change of -21 kJ for poly(I)·poly(C) duplex in dilute NaCl solution estimated from the present work is large in comparison with -9 kJ for the poly(G)·poly(C) complex reported in a previous paper.² This difference is due perhaps to the fact that poly(I) in a dilute NaCl solution exists as a random-coil conformation, while poly(G) makes a considerably strong self-association in a dilute NaCl solution as pointed out previously.^{5,12} This self-association of poly(G) may weaken the formation of the poly(G)·poly(C) complex.

When the NaCl concentration is more than 0.1 mol dm^{-3} , the following reaction takes place between poly(C) and poly(I) with the ordered structure.



where ΔH_i is the heat of order–disorder transition of poly(I), $\Delta H'$ the heat of interaction between poly(C) and poly(I) having the disordered structure. The conformation of the poly(I)·poly(C) duplex(II) differs from that of the poly(I)·poly(C) duplex(I) in dilute NaCl solutions as indicated by spectra measurements.

ΔH^M , when the NaCl concentration is above 0.1 mol dm^{-3} , increases with increase in the NaCl concentration as seen in Figure 1. This increase may be considered to correspond to the increase in the fraction of the ordered structure of poly(I).

When the NaCl concentration is beyond 1.0 mol dm^{-3} , no ΔH^M -value can be obtained because of the precipitation of poly(I) mentioned in the experimental section. Thus, ΔH^M -value for a perfectly ordered structure of poly(I) is more than or at least equal to

Table I. Heat of interaction between polynucleotide in dilute NaCl solution at 298 K

System	$\Delta H/\text{kJ (bpm)}^{-1a}$
Poly(I)–Poly(C)	-21
Poly(A)–Poly(U) ¹	-15
Poly(G)–Poly(C) ²	-9

^a bpm refers here to mole of base pair.

-14 kJ when the NaCl concentration is 1.0 mol dm⁻³.

ΔH^M is expressed as the sum of ΔH_i and $\Delta H'$.

ΔH_i was determined to be about 8 kJ by Hinz *et al.*¹³ and $\Delta H^M \geq -14$ kJ. Thus $\Delta H' \geq -22$ kJ. $\Delta H'$ of poly(I)·poly(C) duplex(II) is expected to be equal to or somewhat larger than ΔH of the poly(I)·poly(C) duplex(I) since ΔH of poly(I)·poly(C) duplex(I) is -21 kJ. Assuming that ΔH^M is equal to -14 kJ, $\Delta H'$ of poly(I)·poly(C) duplex(II) is estimated to be about -22 kJ.

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