

The Heats of Mixing of Poly(guanylic acid) and Poly(cytidylic acid)

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(Received June 20, 1977)

ABSTRACT: The heats of mixing of an equimolar mixture of poly G and poly C in solutions of various pH values were measured using a LKB batch type of microcalorimeter at 298 ± 0.002 K. The interaction between poly G and poly C in neutral medium seems to lead to a complex such as poly(G+C). In order to obtain further information about the interaction between poly G and poly C, the CD spectra for poly G, poly C, and an equimolar mixture of poly G and poly C in solutions with various pH were also measured. From the results of the heats of mixing and the spectra measurements, it was concluded that poly G and poly C in neutral medium leads to a weak complex formation and the enthalpy of complex formation was stoichiometrically estimated to be about -9.3 kJ per mol of base pair.

KEY WORDS Heats of Mixing / CD Spectrum / Poly G / Poly C / Weak Interaction of Poly G and Poly C /

In a previous paper,¹ we measured the heats of mixing of an equimolar mixture of poly A and poly U using a twin microcalorimeter at 298 K, and reported that an equimolar mixture of poly A and poly U forms the poly(A+U) complex between different chains and also the stability of this structure depends on the concentration of salt; also, the enthalpy of complex formation is estimated to be approximately about -15.5 kJ at pH 7.60.

In addition, the poly(guanylic acid) (poly G)—poly(citidylic acid) (poly C) base pair is generally the most common base pair as well as that of poly A and poly U found in DNA molecule.

The spectrophotometric studies on the interaction between poly G and poly C have been reported by many investigators.²⁻⁵ In 1971, Thiele, *et al.*,² reported that an equimolar mixture of poly G and poly C forms the poly(G+C) complex in acid solution (pH 2—4) from the measurement of the optical method.

In this paper, in order to obtain further information about the stability of the DNA structure, the heats of mixing of an equimolar mixture of poly G and poly C were measured

using a LKB microcalorimeter at 298 ± 0.002 K.

EXPERIMENTAL

Apparatus

The calorimeter used in this study was the LKB batch type of the microcalorimeter; it was placed in an air bath kept at 298 ± 0.002 K. The air bath was designed as follows. Around the outside surface of the air bath, a copper pipe (10 mm diameter) was wound and the water maintained at 298 ± 0.01 K in the water bath was circulated around the outside surface of chamber through the copper pipe using a thermoelectric apparatus (Model DE 18 Sharp Electronic Co. Ltd., Japan). The temperature change in the air bath was detected by a thermister which has 34.0 k Ω at 298 K.

The circular dichroism of the poly G, poly C, and an equimolar mixture of poly G and poly C solutions were measured by a spectropolarimeter (Japan Spectroscopic Co. Ltd., J-20).

Materials

Poly G and poly C samples were purchased from Miles Co. Ltd. All other materials were

commercial preparations of analytical reagent grade. The buffer solution used to adjust pH in this study was 0.01-mol/l tris-HCl buffer* solution (pH 7.60) and the McIlvaine buffer** solution (pH 3.00–8.00).

RESULTS AND DISCUSSION

The Heats of Mixing of Poly G and Poly C

The heats of mixing of an equimolar mixture of poly G and poly C in the solutions with various pH were measured. The results obtained are shown in Figure 1, showing the heats of mixing per mol of base pair, ΔH^M , plotted against pH.

As seen in Figure 1, ΔH^M in the McIlvaine buffer solution without NaCl is zero. In the case of buffer solution containing 0.01-mol/l NaCl, ΔH^M is zero in the pH ranges from 3 to 4, and proves to be exothermic when the pH is higher than 4, and reaches a definite value of -3.9 kJ which is nearly independent of pH when pH is higher than 5; this indicates that the interaction between poly G and poly C in neutral medium exists.

Assuming that the interaction between poly G and poly C in neutral medium may form the poly(G+C) complex, the heats of the mixing of an equimolar mixture of poly G and poly C were measured over a concentration range from

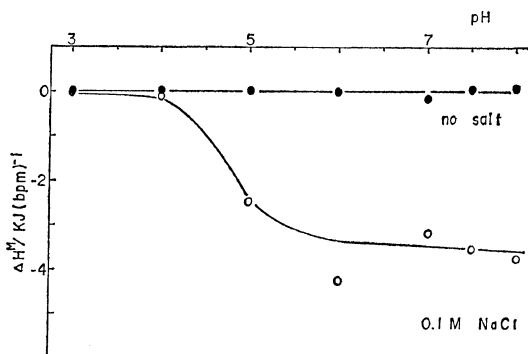


Figure 1. The heats of mixing of an equimolar mixture of poly G and poly C in solutions with 0.1-mol/l NaCl (○), and without NaCl (●) at various pH.

* Tris(hydroxymethyl)aminomethane—hydrochloric acid.

** Mixture of 0.02-mol/l disodium hydrogenphosphate and 0.01-mol/l citric acid.

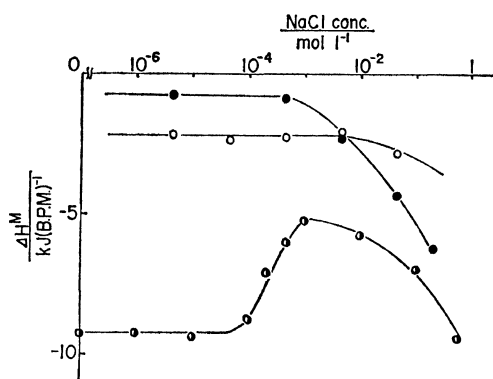


Figure 2. The heats of mixing of an equimolar mixture of poly G and poly C (●), poly G—NaCl (●), and poly C—NaCl (○) systems at pH 7.60 (tris-HCl buffer solution).

0 to 0.5-mol/l NaCl solution, using the same calorimeter at 298 ± 0.002 K.

This system proved to be exothermic, and the results obtained are shown in Figure 2, in which the heats of mixing per mol of base pair, ΔH^M , are plotted against the concentration of NaCl.

As seen in Figure 2, ΔH^M has a definite value of -9.3 kJ at NaCl concentration below 10^{-4} molar. However, ΔH^M shows a drastic decrease in the 10^{-4} to 10^{-3} molar concentration region of NaCl, and again increases with an increasing concentration of NaCl.

To obtain more information about the interesting behavior of ΔH^M , the heats of mixing of poly G- and poly C-NaCl systems at various concentrations of NaCl under the same experimental conditions, as in an equimolar mixture of poly G and poly C, were also measured, and the results obtained are included in Figure 2. As seen in Figure 2, the heats of mixing of poly C and NaCl solutions have a definite value of -2.3 kJ which is nearly independent of the concentration of NaCl. On the other hand, the heats of mixing of poly G and NaCl solutions have a definite value of -0.8 kJ when the concentration of NaCl is 10^{-6} to 10^{-3} molar, but there is a great increase when the concentration of NaCl is higher than 10^{-3} molar. This great increase of ΔH^M may arise from the fact that the poly G plays an important role for the interaction of an equimolar mixture of poly G and poly C in salt solution.

The Heats of Mixing of Poly G and Poly C

CD Spectra for Poly G, Poly C, and Poly(G+C)

In order to obtain further information about the interaction between poly G and poly C in neutral medium, the CD spectra for poly G, poly C and an equimolar mixture of poly G

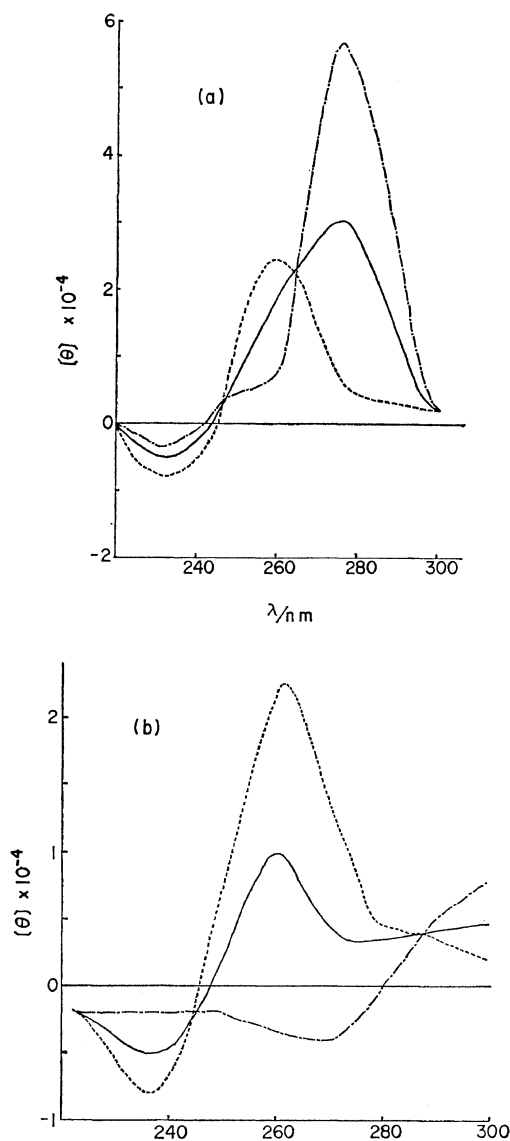


Figure 3. CD spectra of poly G (—), poly C (---), and an equimolar mixture of poly G and poly C (····) which is equal to the sum of the CD spectra of poly G and poly C (—) under various experimental conditions: (a) 0-mol/l NaCl at pH 6.50 (McIlvaine buffer solution); (b) 0.1-mol/l NaCl at pH 3.00 (McIlvaine buffer solution).

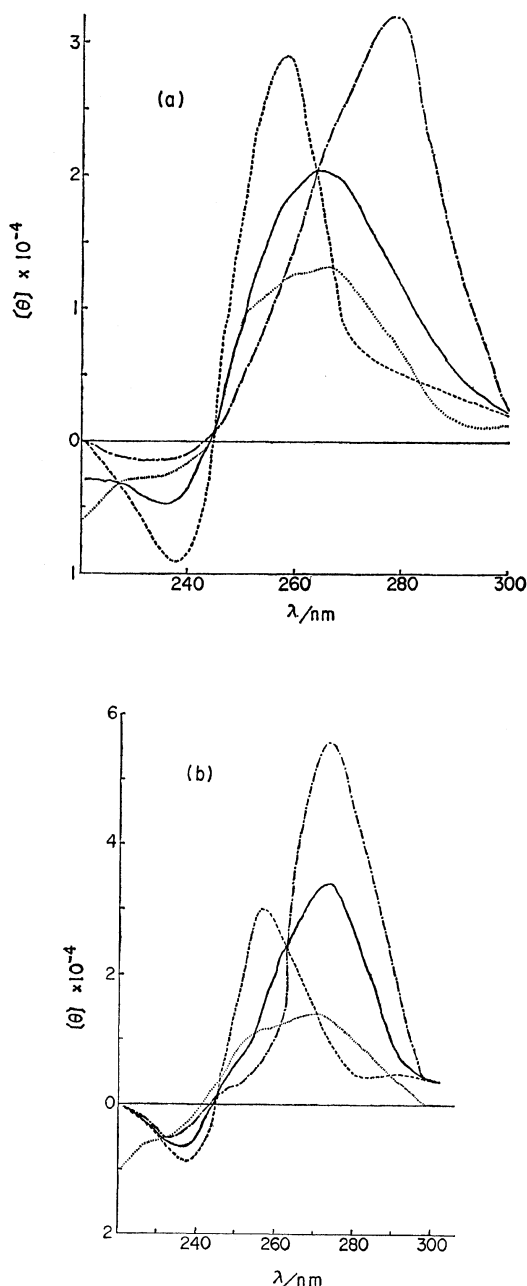
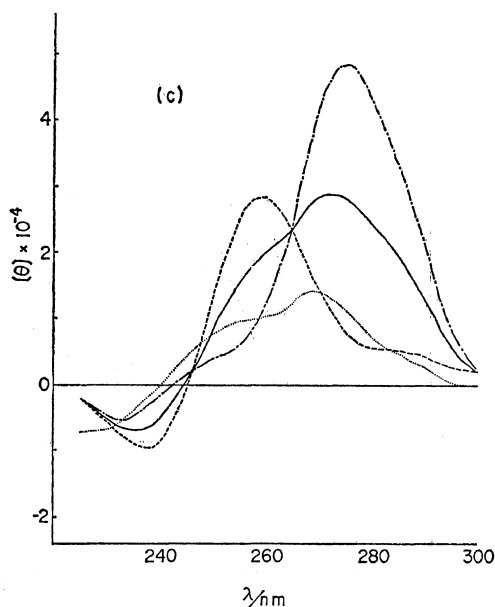


Figure 4. CD spectra of poly G (—), poly C (---), an equimolar mixture of poly G and poly C (····), and (—) is the sum of the CD spectra of poly G and poly C under various experimental conditions: (a) 0.1-mol/l NaCl at pH 5.00 (McIlvaine buffer solution); (b) 0.1-mol/l NaCl at pH 6.50 (McIlvaine buffer solution); (c) 10^{-4} -mol/l NaCl at pH 7.60 (tris-HCl buffer solution).



and poly C solutions with various concentrations of NaCl were measured and the results obtained are shown in Figure 3(a), (b), and 4(a), (b), (c), respectively.

As seen in Figure 3(a), (b), the CD spectra for an equimolar mixture of poly G and poly C in solution without NaCl at pH 6.50 and in 0.1-mol/l NaCl solution at pH 3.00 are consistent with those obtained by adding the CD spectrum of poly G and poly C, suggesting that poly G and poly C may not lead to a complex formation under these experimental conditions. On the other hand, those in the McIlvaine buffer solution with 0.1-mol/l NaCl at pH 5.00 and 6.50 and in the tris-HCl buffer solution with 10^{-4} -mol/l NaCl at pH 7.60 differ from the spectra obtained by adding the CD spectrum of poly G and poly C as shown in Figure 4(a), (b), (c).

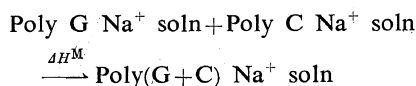
Thus, the intensity of the positive maximum for an equimolar mixture of poly G and poly C is weaker than that obtained by adding the CD spectrum of poly G and poly C, and this decrease of positive maximum corresponds to the formation of a double stranded helical structure of poly(A+U) from an equimolar mixture of poly A and poly U,^{6,7} suggesting that poly G and poly C leads to a complex formation between their bases in the McIlvaine buffer

solutions with 0.1-mol/l NaCl at pH 5.00 and 6.50 and in the tris-HCl buffer solution with 10^{-4} -mol/l NaCl at pH 7.60. The interaction at pH 5.00, 6.50, and 7.60 may lead to a weak complex from the results of CD spectra measurements.

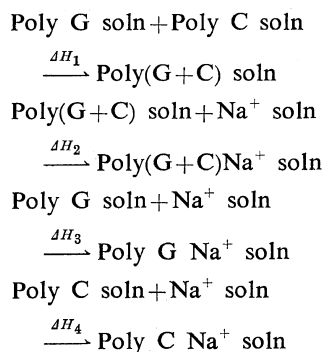
The Enthalpy of Interaction of Poly(G+C) Complex

It is difficult to analyze the behavior of ΔH^M of an equimolar mixture of poly G and poly C in a salt solution. But these results may be interpreted as follows.

The mixing process of poly G and poly C in a salt solution such as,



may be divided into four hypothetical processes as follows.



Assuming that the heats of dilution in these processes may be negligibly small, the heats of mixing, ΔH^M , will be written as a combination of the heats in the processes mentioned above as follows.

$$\Delta H^M = \Delta H_1 + \Delta H_2 - (\Delta H_3 + \Delta H_4)$$

Therefore, the net enthalpy change to form the complex, ΔH_1 , may be estimated as $\Delta H^M - \Delta H_2 + (\Delta H_3 + \Delta H_4)$. The values of ΔH^M , ΔH_3 , and ΔH_4 are measured, but the estimation of ΔH_2 is very difficult. It may be considered that there are the following two approaches for determining the value of ΔH_2 .

First, the simplest assumption that ΔH_2 may be equal to $\Delta H_3 + \Delta H_4$, will give ΔH_1 , which is equal to ΔH^M with -9.3 kJ. Second, if ΔH_2 is lower than $\Delta H_3 + \Delta H_4$, ΔH_1 will be lower than ΔH^M , and *vice versa*.

The Heats of Mixing of Poly G and Poly C

The complex formation heat of -9.3 kJ seems small in comparison with that of the poly(A+U) complex, indicating that a weak complex is formed between poly G and poly C in a neutral medium. But an exact determination of ΔH_2 will give the exact value of the net enthalpy, ΔH_1 .

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