Dependence of pH on the Poly(A)·Poly(U) Duplex Conformation

Shigeyuki Tanaka, Kozo Tada, Yoshihiro Baba, Akihiro Kagemoto,
and Ryoichi Fujishiro

The influence of pH on an equimolar mixture of poly(A) and poly(U) in 0.1 mol dm^{-3} acetate buffer solution was studied by means of heat of mixing and CD spectra.

The heat of mixing, ΔH^M, in the region where pH is less than 5.5 is nearly zero, suggesting that in acidic solution an equimolar mixture of poly(A) and poly(U) may be due to result in the interaction between poly(A)·poly(A) duplex and poly(U) as pointed out by CD spectral measurement.

On the other hand, in less acidic solution where pH is more than 5.5 an equimolar mixture of poly(A) and poly(U) leads to poly(A)·poly(U) duplex formed by the interaction between poly(A) and poly(U) and its enthalpy change is estimated to be about -17.6 ± 2.1 kJ per mole of base pair of nucleotide.

Calorimetric studies of acidic and less acidic solutions seem to reflect exactly the results of the CD spectral measurements.

1. INTRODUCTION

In the previous paper[1], the thermal properties of the double stranded helical structure of poly(A) in acidic solution (pH 4.32~5.12) have been studied by using a differential scanning calorimeter (DSC) and it was reported that the transition temperature and the heat of helix-coil transition of poly(A)·poly(A) duplex depended both on pH and salt concentration, and that electrostatic interactions between the negatively charged phosphate group of poly(A) and the positively charged adenine group of poly(A) contributed significantly to the formation of the duplex. And the calorimetric studies of an equimolar mixture of poly(A) and poly(U) in neutral solutions (pH 7.21 and 7.60) with various concentrations of NaCl showed that poly(A)·poly(U) duplex by an equimolar mixture of poly(A) and poly(U) was formed and thermal properties depended on the environmental parameters such as ionic strength, temperature and concentration of metallic ions[2,3].

These studies have been carried out only in neutral and acidic solutions, respectively, and no

information has been obtained about the formation of the poly(A)·poly(U) duplex in acidic solutions.

In this paper, we will report the enthalpy change of poly(A)·poly(U) duplex in acidic solutions in which pH is 4.5 to 6.5.

2. EXPERIMENTAL

2.1 Materials

Polyriboadenylic acid (poly(A), No. 11-82-301) and polyribouridylic acid (poly(U), No. 103 C) samples were purchased from Miles Laboratories Inc., USA and all other materials were commercial products of analytical reagent grade.

The buffer solution used to adjust pH was 0.1 mol dm^{-3} acetate buffer solutions (pH 4.5 to 6.5).

2.2 Apparatus and procedure

The calorimeter used for measuring the heat of mixing is the same as that of LKB batch type microcalorimeter, except for that the calorimeter was placed in an air bath controlled by circulating the controlled water kept at 298.15 ± 0.005 K around the chamber.

Circular dichroism (CD) spectra used in this study were made with a JASCO J - 20 spectropolarimeter.

The concentration of nucleotide used for CD spectral measurements was 5×10^{-5} unit mol dm^{-3}.

Department of General Education, Osaka Institute of Technology, Asahi-Ku, Osaka 535, Japan.

When pH is less than 6.0, poly(A) was dissolved in a water bath kept at about 363 K because poly(A) was insoluble in this buffer solution at room temperature. And the poly(A) solution prepared at high temperature was used at room temperature.

The concentration of poly(A) and poly(U) used for the measurement of heat of mixing was 5×10^{-4} unit mol dm^{-3}.

3. RESULTS AND DISCUSSION

3.1 CD spectra

Typical CD spectra for only poly(A) solutions and for an equimolar mixture of poly(A) and poly(U) solutions of pH 4.5 to 6.5 were shown in Figs. 1(a) and (b), respectively.

Fig. 1(a) shows the typical CD spectra for poly(A)·poly(A) duplex (pH 4.9, curve - I)[4] and for a single stranded poly(A) (pH 7.4, curve - II)[4], respectively. As seen in Fig. 1(a), intensity curves of poly(A) over the wavelengths of 250 to 300 nm are in agreement with those of single stranded poly(A) in the pH region of 5.9 to 6.5. These
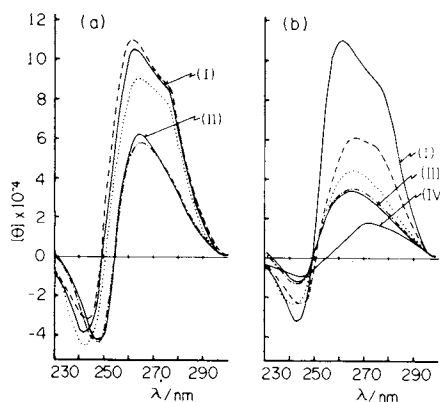

Fig. 1 Typical CD spectra of poly(A) and equimolar mixture of poly(A) and poly(U) in pH ranges of 4.5 to 6.5, at 298 K.
The concentration of polynucleotides was 5×10^{-5} unit mol dm^{-3}.
(a) poly(A) system; -----: pH 4.5 to 5.5, ···········: pH 5.7, ——·——: pH 5.9 to 6.5, curve - I: poly(A)·poly(A) duplex[4], and curve - II: single stranded poly(A)[5]
(b) equimolar mixture of poly(A) and poly(U); -----: pH 4.5 to 5.5, ···········: pH 5.7, ——·——: pH 5.9 to 6.5, curve - I: poly(A)·poly(A) duplex[4] at pH 4.9, curve - III: poly(A)·poly(U) duplex[4], and curve - IV: poly(U) in the pH range of 4.5 to 6.5

curves, however, become higher with a decrease in pH and reach the curve - I[4] of poly(A)·poly(A) duplex at the pH value of 4.5 to 5.5. This will suggest that poly(A) in acidic solutions of pH 4.5 to 5.5 exists as poly(A)·poly(A) duplex and transforms to the single stranded helical structure with an increase in pH. And this behavior will be explained by considering that in acidic solutions strong interactions between negatively charged phosphate groups and positively charged adenine groups produced from poly(A) overcome those between bases of adenine of poly(A) which are responsible for the formation of the single stranded helical structure in less acidic or neutral solutions, leading to the formation of poly(A)·poly(A) duplex.

Fig. 1(b) shows also the typical CD spectra for poly(A)·poly(A) duplex (pH 4.9, curve-I)[4], for poly(A)·poly(U) duplex (pH 7.4, curve - III)[4] and for a random coil state of poly(U) (pH 4.5 to 6.5, curve - IV), respectively.

It is seen from Fig. 1(b) that CD spectra of an equimolar mixture of poly(A) and poly(U) in the pH region of 4.5 to 5.5 do not agree with both curve - I and III. On the other hand, as the solution becomes less acidic, an equimolar mixture of poly(A) and poly(U) converges on the curve - III in Fig. 1(b) and begins to form poly(A)·poly(U) duplex.

In order to examine CD spectra in further detail, the molar ellipticity of positive maximum, $[\theta]_{max}$, at wavelengths of 261 to 272 nm is plotted against pH and shown in Fig. 2, together with those of only poly(A)·poly(U) duplex in pH 7.4 determined by the other investigators[4,5]. As seen in Fig. 2, $[\theta]_{max}$ for poly(U) assumes a definite value through the pH region of 4.5 to 6.5. However, $[\theta]_{max}$ of poly(A) changes from a definite and higher value in acidic solutions (pH 4.5 to 5.5) to another definite value in the solution of pH 5.9 to 6.5, indicating that, as shown in Fig. 1(a), poly(A) exists as poly(A)·poly(A) duplex in acidic solutions of pH 4.5 to 5.5 and as a single stranded poly(A) in the solutions of pH 6.0 to 6.5.

On the other hand, $[\theta]_{max}$ for an equimolar mixture of poly(A) and poly(U) changes from a definite value in acidic solutions to another definite value in less acidic solutions which coincides with that for poly(A)·poly(U) duplex. The former

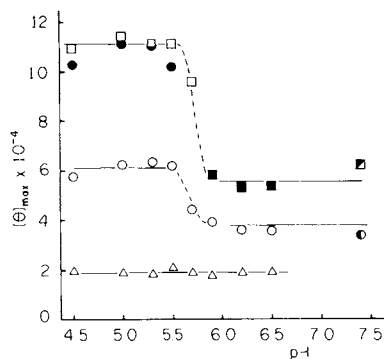Fig. 2 The plots of molar ellipticity of positive maximum, $[\theta]_{max}$, at 298 K against pH; ⊓ : poly(A)·poly(A) duplex, ■ : single stranded poly(A), ○ : poly(A)·poly(U) duplex, ● : calculated values of equimolar mixture of poly(A) and poly(U) converted into molar ellipticity of poly(A)·poly(A) duplex, and △ : poly(U), ◖ : reference 4) and ◤ : reference 5)
The concentration of polynucleotides was 5×10^{-5} unit mol dm^{-3}.

definite value for $[\theta]_{max}$ in acidic solutions is nearly equal to the average of those for poly(A)· poly(A) duplex and for poly(U). Thus, it is shown that in acidic solutions, an equimolar mixture of poly(A) and poly(U) consists of poly(A)·poly(A) duplex and poly(U), rather than poly(A)·poly(U) duplex, whereas in less acidic solutions, it is made up of poly(A)·poly(U) duplex.

3.2　Heat of mixing

In order to obtain information about enthalpy change of the interaction between poly(A) and poly(U) over all pH regions, the heats of mixing of an equimolar mixture of poly(A) and poly(U) were measured by using the microcalorimeter at 298.15 ± 0.005 K.

All systems proved to be exothermic.

The results obtained are listed in Table 1 and shown in Fig. 3, where the heat of mixing, ΔH^M, per mole of base pair of polynucleotide is plotted against pH. As seen in Fig. 3, ΔH^M in the region where pH is below 5.5 is nearly zero and when pH exceeds 5.5, ΔH^M shows a drastic decrease, and reaches a definite value which is nearly independent of pH when pH is more than 6.0.

As in acidic solutions, poly(A) forms poly(A)· poly(A) duplex and poly(U) exists as a single molecule, mixing of two solutions results in the

Table 1 The dependence of heat of mixing of poly(A) and poly(U), ΔH^M, on pH at 298.15 ± 0.005 K. The concentration of polynucleotides was 5×10^{-4} unit mole dm^{-3}.

pH	ΔH^M/kJ (bpm)$^{-1}$
4.5	0
5.0	0
5.3	0
5.4	0
5.5	-1.4 ± 0.7
5.6	-4.6 ± 0.3
5.7	-7.7 ± 0.8
5.8	-8.8 ± 2.0
5.9	-15.2 ± 1.5
6.0	-15.9 ± 0.5
6.1	-17.6 ± 1.9
6.2	-18.0 ± 2.1
6.3	-15.5 ± 2.0
6.4	-18.5 ± 2.1
6.5	-18.4 ± 2.2

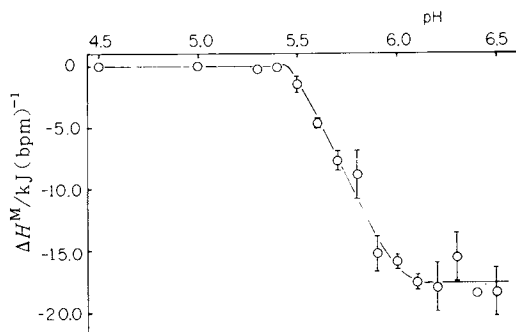bpm here is mole of base pair of polynucleotide

Fig. 3 The dependence of heat of mixing, ΔH^M, of poly(A) and poly(U) on pH at 298.15 ± 0.005 K. The concentration of polynucleotides was 5×10^{-4} unit mol dm^{-3}.

interaction between poly(A)·poly(A) duplex and poly(U).

$\Delta H^M \simeq 0$ in acidic solution, however, means that the interaction energy between poly(A)· poly(A) duplex and poly(U) is nearly equal to that between duplexes and between poly(U). As the poly(A)·poly(A) duplex in acidic solutions is considered to be formed by the strong electrostatic interaction between the negatively charged phosphate group of poly(A) and positively charged adenine group of poly(A) and the interactions

between poly(A)·poly(A) duplex and poly(U) may consist of hydrogen bonds between bases of adenine and uracil, their energies will be nearly equal each other or to that between poly(U).

On the other hand, in less acidic solutions, poly(A) exists as single stranded helical structure and poly(U) exists as a random coil state, mixing of two solutions leads to poly(A)·poly(U) duplex formed by the interaction between poly(A) and poly(U). Large negative value of ΔH^M, -17.6 ± 2.1 kJ, shows that the strong poly(A)·poly(U) duplex is formed and its value corresponds to that of formation of this duplex, under the assumption

that the heat of dilution of polynucleotide is negligibly small.

REFERENCES

1) Y. Baba, S. Tanaka, A. Kagemoto, *Polym. J.* **7**, 641 (1975)
2) S. Tanaka, Y. Baba, A. Kagemoto, *Polym. J.* **8**, 491 (1976)
3) S. Tanaka, Y. Baba, A. Kagemoto, R. Fujishiro, *Netsu Sokutei*, submitted
4) K. E. Van Holde, J. Brahms, A. M. Michelson, *J. Mol. Biol.* **12**, 726 (1965)
5) J. Brahms, *J. Mol. Biol.* **11**, 785 (1965)

〈書 評〉

「無機材料関係の熱力学データ」

V. I. Babshkin, G. M. Matveev,
O. P. Mchedlov-Petrosyan 著,
Prof. Mchedlov-Petrosyan 監修による
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無機材料関係の熱力学データは, 酸化還元や水和等の化学反応の研究や, 単結晶や化合物の合成及び材料の安定性の検討などに有効であることは, 述べるまでもない。一般に無機材料の熱力学の研究は, ソビエトで多くなされているが, 特に本書は, 従来までまとめられていなかった無機結晶, 非晶体, ガラス, 稀薄水溶液, 液体, 気体などのデータを数多く集めてあることが特徴である。

本書は, 次の8章から成っている。

1章　熱力学の基礎概念と法則
　(§1.)熱力学の基礎概念　(§2.)熱力学, 熱化学の第1法則　(§3.)熱力学第2法則　(§4.)熱力学の方法　(§5.)熱力学第3法則　(§6.)化学平衡とその計算原理　(§7.)相平衡　(§8.)状態図　(§9.)最初の熱力学的データを得る方法　(§10.)熱力学データに基づくシリケート系反応における $\Delta G = f(T)$ の計算実行の概要

2章　熱力学の不可逆過程

3章　化学熱力学的方法の特殊な応用
　(§1.)3相反応　(§2.)シリケートの融解　(§3.)水和反応　(§4.)複合反応　(§5.)標準的熱力学導出の可能性の限界と出発データの正確性の問題　(§6.)3相反応における熱力学と速度論の関係

4章　反応の熱力学

5章　融解

6章　水和反応

7章　侵食反応

8章　地質化学的過程の熱力学

特に3章は, 化学熱力学的方法の特殊な応用例をいくつか挙げてあり, データの理論的取扱いといった観点から興味深い。なお, これらの無機材料のデータは, 古いものと新しいものとで著しい相違があり, 注意して用いる必要があると思われる。 (京大工　平尾一之)