

Influence of pH and Salt Concentration on the Helix—Coil Transition of Poly A Determined by a Modified DSC

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ABSTRACT: The thermal effects of pH and salt concentration on the helix—coil transition of Poly A were studied calorimetrically by a modified differential scanning calorimeter (DSC). The transition temperature (T_m) and enthalpy (ΔH) of Poly A in acid solution depend both on pH and on the concentration of NaCl. T_m and ΔH increase with decreasing pH at infinite dilution of salt concentration, and decrease with increasing salt concentration at a definite pH. These results indicate that electrostatic interactions between the negatively charged phosphate group and the positively charged adenine group contribute significantly with decreasing pH.

KEY WORDS DSC / Poly A / Helix—Coil Transition / Transition Temperature / Transition Enthalpy / Dependence on pH and Concentration of NaCl /

INTRODUCTION

Poly(riboadenylic acid) (Poly A) forms a double-strand helical structure in acid solutions,¹ and this structure undergoes a transition to a random coil with increasing temperature.² It has been reported from circular dichroism³ and hypochromism⁴ that double-strand structure of Poly A is not stable, but a single-strand helical structure stabilized by base stacking exists at neutral pH.

The helix—coil transition temperature of Poly A in acid solution is sensitive to the salt concentration^{2,5} and to the pH.⁶ It increases with decreasing pH, and its dependence on salt concentration is the reverse of that in the DNA system,^{7,8} since it decreases with increasing salt concentration. It is also reported that the transition enthalpy of Poly A, as well as the transition temperature, depends on pH.⁹ But the dependence of the transition enthalpy of Poly A on salt concentration has not yet been measured using calorimetry.

In order to study the effect of pH and salt concentration upon the transition, the thermal stability of the double-strand helical structure of Poly A in acid solutions with various concentrations of NaCl has been studied by calorimetry; the enthalpy and the characteristic

temperature of the helix—coil transition of Poly A are the important parameters in understanding the stability of such molecules with double-strand helical structures.

EXPERIMENTAL

Materials

Poly(riboadenylic acid) (Poly A) was purchased from Miles Co Ltd. (control No. 11-76-301) and was used without further purification. All other materials were commercial preparations of analytical reagent grade. 0.1-mol acetate buffer solution was prepared to obtain the definite pH values for the experiment.

The Poly A sample was dissolved into the buffer solution with different NaCl concentrations by raising the temperature and then the Poly A solution obtained was allowed to stand overnight at about 278 K.

For the calorimetric measurements the Poly A concentration was about 0.3%, as determined by the measurement of absorption spectra at 252 nm taking $E(P)=10100$ at this wavelength,¹⁰ and the quantity of the Poly A solution used was about 30 μ l.

Apparatus

The modified differential scanning calorimeter (DSC) was the same as that in a previous paper.¹¹

The optical rotation measurement was made using a spectropolarimeter (Japan Spectroscopic Co. Ltd., J-20). Water from a thermostat was circulated through the cell holder. The relation between both temperature and equilibrium cell temperature was established by a series of calibration experiments.

RESULTS AND DISCUSSION

The helix—coil transition of Poly A in acid solution with different concentrations of NaCl was studied by using a modified DSC. The heating rate was about 10 K/min. A typical DSC curve obtained for the concentration of Poly A of about 0.3% (the quantity of Poly A was about 50 μg and the concentration of NaCl at pH 4.73 was 10^{-3} mol) is shown in Figure 1. The temperature width of the endothermic peak of the DSC curve, which ranges from about 343 to 353 K, is about 10 K. The maximum peak temperature of the thermal absorption is about 347 K.

In order to confirm the results of the DSC method, we have measured the optical rotatory dispersion (ORD) in the same system. In Figure 1, the optical rotations per mean nucleotide residue ($[\text{R}]$) at wavelength 282 nm are plotted against the temperature for the Poly A solution with 10^{-3} -mol NaCl at pH 4.73. The helix—coil transition temperature (T_m) of Poly A is about 345K. The agreement between the transition temperature estimated from the

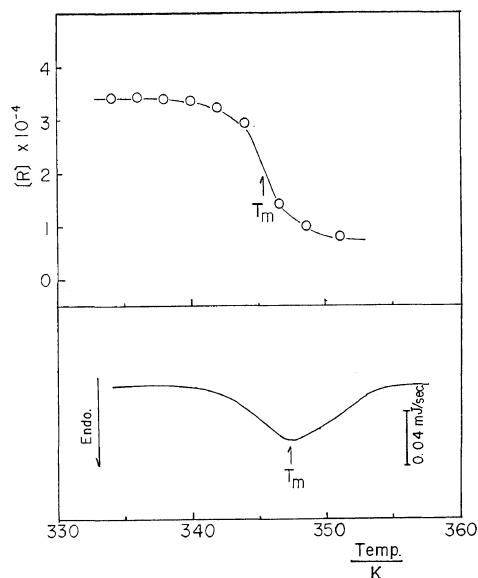


Figure 1. DSC curve and the temperature dependence of the optical rotation per mean nucleotides residue ($[\text{R}]$) at 282 nm for Poly A solution with 10^{-3} mol NaCl in PH 4.32 (0.1 M acetate buffer solution): T_m , transition temperature.

ORD method and peak temperature of the DSC curve is fairly close. Hence, the endothermic peak of the DSC curve may be regarded as corresponding to the helix—coil transition of Poly A.

The absorbed heat estimated from the peak area of the DSC curve seen in Figure 1 is about

Table I. Transition temperature (T_m) and enthalpy (ΔH) estimated from DSC curves of Poly A solution with different concentrations of NaCl in acid region

NaCl, mol	pH 4.32		pH 4.73		pH 5.12	
	T_m , K	ΔH , ^a kJ mol ⁻¹	T_m , K	ΔH , ^a kJ mol ⁻¹	T_m , K	ΔH , ^a kJ mol ⁻¹
10^{-5}	364	19 \pm 2	344	18 \pm 2	330	12 \pm 3
10^{-4}	364	18 \pm 2	345	18 \pm 3	330	12 \pm 2
10^{-3}	363	19 \pm 2	345	18 \pm 2	330	11 \pm 2
5×10^{-3}	360	17 \pm 4	345	15 \pm 3	329	10 \pm 2
10^{-2}	360	12 \pm 3	344	13 \pm 2	327	10 \pm 2
5×10^{-2}	359	11 \pm 2	341	13 \pm 3	326	9 \pm 3
10^{-1}	358	11 \pm 2	338	11 \pm 2	326	9 \pm 3
5×10^{-1}	357	11 \pm 3	339	12 \pm 2	318	8 \pm 2
1.0	—	—	—	—	317	8 \pm 3

^a The "mol" is a mol of base pair.

1.53 mJ, and the transition enthalpy per mol of base pair of nucleotides is about 19 kJ.

The peak temperature (T_m) and the transition enthalpy (ΔH) per mol of base pair estimated from the DSC curve of Poly A solution with different NaCl concentrations at a definite pH are summarized in Table I.

Transition Temperature

Plots of T_m of Poly A against the logarithm of salt concentration at a definite pH show a contrary sigmoidal curve, as in Figure 2, and approach a definite value in the lower concentration ranges of salt (10^{-5} to 10^{-3} mol/l). Values of T_m of Poly A at infinite dilution of salt concentration at a given pH are shown in Table II. This T_m depends on pH only, and increases considerably with decreasing pH; similar results have been reported by other authors.^{5,6}

In the moderate concentration ranges of salt,

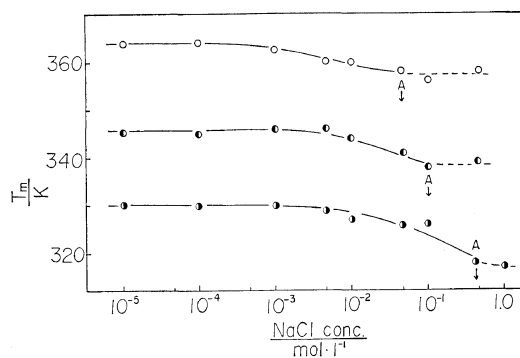


Figure 2. The plots of transition temperature (T_m) against the logarithm of NaCl concentration.

pH: ○—4.73; ●—4.73; ●—5.12.

A point shows the NaCl concentration required for aggregates of Poly A solution at room temperature, and the broken lines show T_m of this solution.

Table II. Thermodynamic parameters of Poly A at infinite dilution of NaCl concentration at various pH values

pH	T_m , K	ΔH , ^a kJ mol ⁻¹	ΔS , ^a J deg. ⁻¹ mol ⁻¹
4.32	364	19	5.2
4.73	345	18	5.2
5.12	330	12	3.6

^a The "mol" is a mol of base pair.

from 10^{-3} to about 5×10^{-2} mol/l, T_m decreases with increasing salt concentration, in contrast to the result obtained from DNA of double-strand helical structure. This difference may be due to the fact that the electrostatic interstrand interaction between the negatively charged phosphate group and the positively charged adenine contributes significantly to the stabilization of the helix of Poly A, as pointed out by Gabbay.¹²

Poly A solutions with higher concentrations of salt form aggregations at room temperature, and become homogeneous upon raising the temperature. T_m of this Poly A show a definite value in spite of increasing salt concentration, as shown by the broken line in Figure 2. The salt concentration required for formation of aggregates of Poly A solution decreases with decreasing pH, as shown by A point in Figure 2. Further study is needed to clarify these results.

Transition Enthalpy

The plots of transition enthalpy per mol of base pair (ΔH) against the logarithm of salt concentration, seen in Figure 3, show similar contrary sigmoidal curves to that of T_m . ΔH at infinite dilution of salt concentration decreases with increasing pH, although ΔH at pH 4.32 takes a slightly larger value than that at pH 4.73; the results obtained are summarized in Table II.

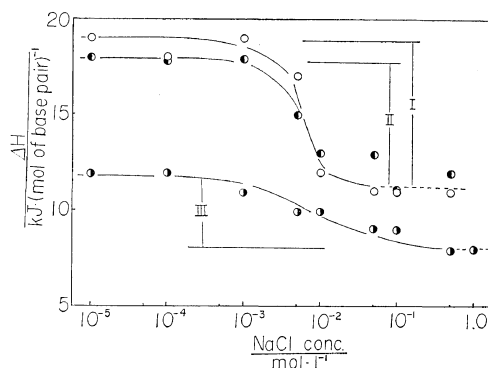


Figure 3. The plots of the transition enthalpy per mol of base pair (ΔH) against the logarithm of NaCl concentration.

pH: ○—4.32; ●—4.73; ●—5.12.

The difference of enthalpy between lower and higher salt concentrations, I (pH 4.32), II (pH 4.73) and III (pH 5.12), are about 8, 7, and 4 kJ/mol of base pair, respectively.

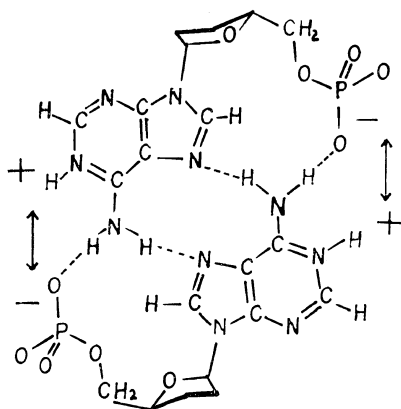
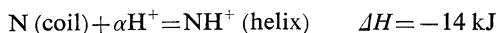


Figure 4. Double-strand molecule of Poly A in acid solution (from ref 15).

This dependence may be judged by comparison with results reported by other authors.¹³⁻¹⁵

The calorimetric study of helix formation in Poly A¹³ has been estimated by means of titration enthalpy; the following enthalpy of reaction was obtained:



where N represents the nonprotonated nucleotides' residue and α is the fraction of adenine groups protonated at pH 4 in the helical form. The helical form of Poly A is considered to involve the presence of electrostatic interactions between the negatively charged phosphate group and the positively charged adenine group,¹⁴ using the X-ray diffraction studies of Fresco¹⁴ and Rich, *et al.*,¹⁵ shown in Figure 4.

In our study, α may differ at a given pH; also, the proportion of NH^+ may be increased with decreasing pH, from the results of Sturtevant, *et al.*,¹³ since the number of protons bound per AMP unit decreases with increasing pH from the titration curve of Poly A.¹⁶ Accordingly, the important factor for the increase of ΔH with decreasing pH may be considered to be that the contribution of the electrostatic interaction energy increases by increasing the fraction of adenine groups protonated with decreasing pH.

In the moderate concentration ranges of salt (10^{-3} to 5×10^{-2} mol), ΔH decreases with increasing salt concentration; this dependence is

similar to that of T_m described above, and it may be considered that the energy of electrostatic interaction between the negatively charged phosphate group and the positively charged adenine group decreases, since the contribution of electrostatic interaction decreases significantly with increasing salt concentration. The difference of enthalpy shown in Figure 3 may be considered to reflect these contributions of electrostatic interaction and this contribution increases with decreasing pH. But this electrostatic energy cannot be estimated from our study, since we cannot know what fraction of protonated adenine groups is present at a given pH.

According to Table I, the plots of ΔH against T_m of Poly A with a given salt concentration show a curve in which ΔH approaches a definite value with increasing T_m , as shown in Figure 5. These plots of Poly A with NaCl concentration of 10^{-2} mol have a similar tendency to those with salt concentration of 10^{-5} mol, but the slopes are smaller than is the case with lower salt concentrations. If those curves are roughly regarded as linear, shown by the broken lines in Figure 5, the slopes of the straight lines decrease with increasing salt concentration, corresponding to the heat capacity of the helix-coil transition of Poly A. The slopes of the straight lines at concentration ranges larger than

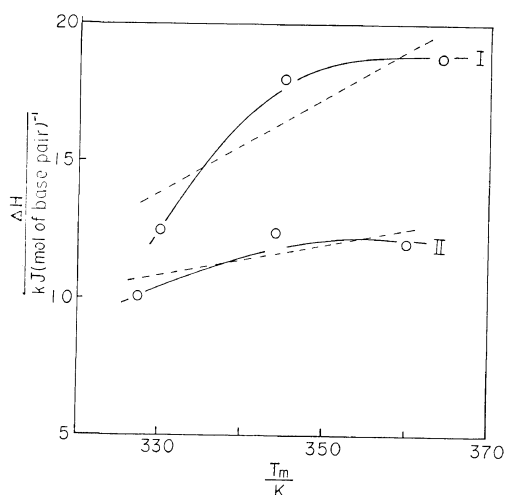


Figure 5. Dependence of the transition enthalpy on transition temperature for Poly A with a given NaCl concentration.

NaCl concn, I— 10^{-5} ; II— 10^{-2} mol/l.

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about 10^{-2} mol of salt is smaller than those in the lower concentration ranges of the salt. This difference may be due to the contribution from electrostatic interactions obtained above, and also the different conformations of Poly A between the lower and higher concentration regions of salt may be due to this same contribution.

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