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SHORT COMMUNICATION

The Helix—Coil Transition of DNA by Mercuric Ion

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As reported in a previous paper,[1] we have measured the heat of the helix—coil transition of DNA solutions with different concentrations of Mg^{2+} ions by using a modified DSC, and found that the transition temperature of DNA depends on both the concentrations of Mg^{2+} ion and of DNA; the heat of the helix—coil transition of DNA is estimated to be about 40 kJ per base pair mole at a mole ratio of 1 for Mg^{2+} to DNA(P), corresponding to 1 mol of Mg^{2+} binding to 1 mol of phosphate of DNA.

In 1961, the influence of mercuric ion on DNA was studied by Yamane, *et al.*,[2] using a spectrophotometer; they reported that DNA in $HgCl_2$ solution may form a DNA—Hg^{2+} complex.

In this paper, in order to substantiate this possibility and to obtain more information about the behavior of DNA in the $HgCl_2$ solution, the influence of Hg^{2+} on the helix—coil transition of DNA has been studied by using the modified DSC and the microcalorimeter.

Calf thymus (GC content 43%) was purchased from Miles Co. Ltd. The buffer solution used in this study was 1 m*M* acetate solution (pH 5.72). The DNA sample was dissolved in the buffer solution with different $HgCl_2$ concentrations and allowed to stand overnight at 278K in order to obtain a homogeneous solution.

For the DSC measurement, the DNA concentration was about 0.2%; this was determined by measurement of the absorption spectrum at 260 nm taking $E(P)=6300$ at this wavelength.

For the calorimetric measurement, the DNA concentration was about one-tenth that for the DSC measurement.

The DSC (Model M8005, Rigaku Denki Co. Ltd.) used in this study was modified so as to be able to measure slight changes in the heat of a small quantity of a sample; the calorimeter used in the measurement of the heat of mixing of the DNA—$HgCl_2$ system was similar to the LKB batch micro calorimeter. The cell for mixing was made of glass instead of gold.

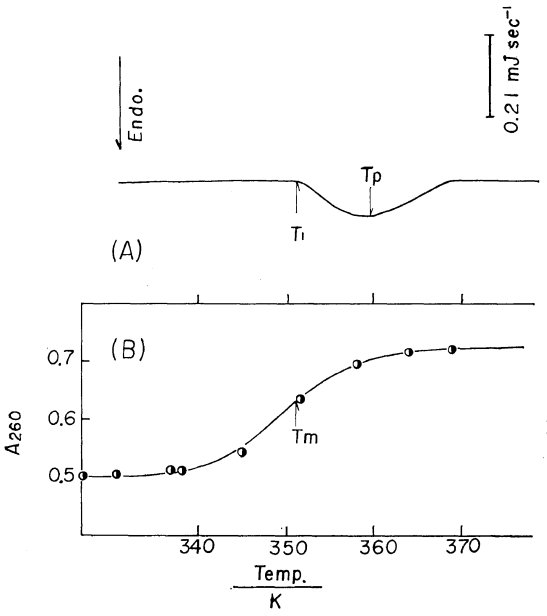

Figure 1. (A) A typical DSC curve of DNA solution: DNA, 0.24%; $HgCl_2$, 2×10^{-4} mol; buffer solution, 1 m*M* acetate (pH 5.72). T_i and T_p are the initial departure and peak temperatures, respectively. (B) The plot of absorbance at 260 nm against the temperature. T_m is the helix—coil transition temperature.

The thermal behavior for DNA dissolved in

1 mM acetate buffer solution with Hg^{2+} was studied by the modified DSC. The heating rate in this study was about 10K/min. The DSC curve obtained is shown in Figure 1A; it has an endothermic peak with a temperature width of 15 to 20K. To confirm the endothermic peak obtained from the DSC curve, the dependence of the absorbance on the temperature was studied by using the spectrophotometer.

The plot of the absorbance at a wavelength of 260 nm against the temperature is shown in Figure 1B, together with the DSC curve. As seen in Figure 1B, the helix—coil transition temperature (T_m) of DNA obtained is about 353K. Accordingly, T_m corresponds to the temperature (T_1) where the peak begins to appear rather than the peak temperature (T_p) on the DSC curve. Thus, an endothermic peak appearing on a DSC curve may be considered to correspond to the helix—coil transition of DNA.

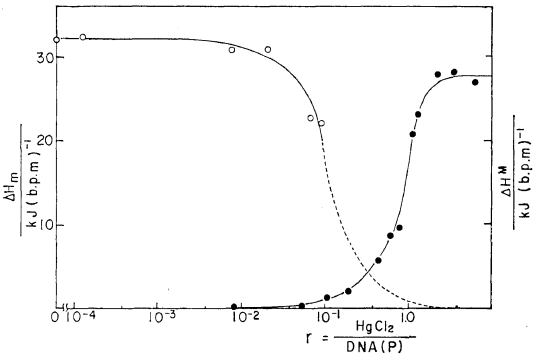

Figure 2. The plots of ΔH_m (○) and ΔH^M (●) of DNA—Hg^{2+} system against r (=mole added $HgCl_2$/mole phosphate of DNA). The dotted line of ΔH_m gives the expected phase diagram; it can not be measured because of the amalgam between the aluminum DSC cell and mercuric ion in excess $HgCl_2$.

Table I. Transition temperature (T_1) and heat of transition (ΔH_m) estimated from DSC method (the second column), wavelength of maximum absorption (λ_{max}) and transition temperature (T_m) from spectrophotometer (the third column), and heat of mixing (ΔH^M) from micro calorimeter (the last column) for DNA—Hg^{2+} ion system, respectively

r=Hg^{2+}/DNA(P)	DSC		Spectra		Heat of mixing
	T_1, K	ΔH_m, kJ·(bpm)$^{-1}$	T_m, K	λ_{max}, nm	ΔH^M, kJ·(bpm)$^{-1}$
0	353.0	31.1	353	258	
1.20×10^{-4}	352.3	32.7	—	—	
7.80×10^{-3}	353.7	31.4	—	—	0
2.56×10^{-2}	351.6	32.3	350	259	—
6.86×10^{-2}	352.6	22.6	—	—	0.10
9.12×10^{-2}	354.1	22.5	—	—	—
0.11			351	260	—
0.15			350	262	1.07
0.18			350	263	1.75
0.45			350	265	5.57
0.60			—	—	8.53
0.71			350	268	9.54
1.05			not present	271	21.04
1.09					23.94
1.65			〃	275	—
1.92			〃	273	28.74
4.08					28.38
5.65					26.68

Assuming that an endothermic peak in the DSC curve is due to the helix—coil transition of DNA, the heat of the helix—coil transition of DNA can be estimated from the peak area of DSC curve. The results obtained are listed in the second column in Table I. Using Table I, the plot of the heat of helix—coil transition per base pair mole (ΔH_m) against r (=mole of $HgCl_2$/mole of DNA(P)) is shown in Figure 2. When r is more than 0.1, a DSC measurement is impossible because an amalgam is formed between the aluminum cell of DSC and the mercury. Then, the dotted line of ΔH_m is drawn from the results of the spectrophotometric measurement.

Where r is less than 10^{-2}, ΔH_m has a definite value of about 31 kJ which is nearly independent of the concentration of Hg^{2+}. When r is more than 10^{-2}, a drastic decrease of ΔH_m occurs and the wavelength of maximum absorption (λ_{max}) increases, as shown in Table I. In order to explain these facts, we may assume that the following equilibrium exists among DNA(helix)—Hg^{2+} complex, DNA(coil)—Hg^{2+} complex, and Hg^{2+}:

$$\text{DNA(helix)—Hg}^{2+}+\text{Hg}^{2+} \rightleftharpoons \text{DNA(coil)—Hg}^{2+}$$

When the concentration of Hg^{2+} is so low that r is less than 10^{-2}, the mainly existing molecules

may be DNA(helix)—Hg^{2+} and the ΔH_m accompanying the helix—coil change will be large. On the other hand, when the concentration of Hg^{2+} becomes so large that r is more than 10^{-2}, the equilibrium moves to the right and the number of DNA(helix)—Hg^{2+} becomes smaller as r increases, suggesting that ΔH_m decreases as r increases.

In order to obtain further information, the heats of mixing of DNA and $HgCl_2$ solutions have been measured by using the microcalorimeter at 298K. The results obtained are listed in the last column in Table I. Using Table I, the plot of the heat of mixing per base pair mole (ΔH^M) against r is shown in Figure 2, together with ΔH_m. This plot may be interpreted as follows. The heat of mixing of the DNA—$HgCl_2$ system may be assumed to consist of the following two steps:

DNA(helix)+a small amount of

$$Hg^{2+} \overset{\Delta H_1}{\rightleftharpoons} \text{DNA(helix)—}Hg^{2+}$$

DNA(helix)—Hg^{2+}+a large amount of

$$Hg^{2+} \overset{\Delta H_2}{\rightleftharpoons} \text{DNA(coil)—}Hg^{2+}$$

Then the heat of mixing ΔH^M as $r\to\infty$ will be equal to the sum of ΔH_1 and ΔH_2 of the above two steps, and ΔH_2 may be expected to increase with the concentration of Hg^{2+}, that is, with r, and to attain its maximum at a larger value of r, in close agreement with the curve in Figure 2. On the other hand, ΔH_m, when r is very small, corresponds to the process, DNA(helix)—$Hg^{2+}\to$DNA(coil)—Hg^{2+}, which is equal to the second step of the mixing. Therefore, ΔH_m as $r\to 0$ may be considered to be equal to the maximum value of ΔH_2. Using the relation $\Delta H_2=\Delta H^M-\Delta H_1$, the following value will be obtained:

$$\Delta H_m(r\to 0)=\Delta H^M(r\to\infty)-\Delta H_1$$

As $\Delta H_m(r\to 0)=31$ kJ and $\Delta H^M=28$ kJ, then ΔH_1 will be estimated to be -3 kJ.

REFERENCES

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2. T. Yamane and N. Davidson, *J. Amer. Chem. Soc.*, **83**, 2599 (1961).