Thermochimica Acta, 163 (1990) 147-154
Elsevier Science Publishers B.V., Amsterdam - Printed in The Netherlands

THE CONFORMATIONAL CHANGE OF DNA IN AQUEOUS ALCOHOL SOLUTIONS CONTAINING METALLIC IONS DETERMINED BY USING DIFFERENTIAL SCANNING CALORIMETER

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SUMMARY

In order to obtain information about the conformational change of DNA by changing the environment surrounding DNA molecule, we measured the helix-coil transition of DNA solutions with various concentrations of ethanol at a given concentration of various kinds of metallic ion by means of an adiabatic differential scanning calorimeter. The observed heat of transition, ΔH increases, but the transition temperature, T_1 decreases with increasing the concentration of ethanol, demonstrating that the conformation of DNA may considerably change by environment surrounding DNA. In order to confirm the conformational change of DNA, we also measured the CD spectra at room temperature under the same experimental conditions as the DSC measurement. The concentration range of ethanol where molecular ellipticity ratio, $[\theta]_{max}/[\theta]_{min}$ of positive maximum, $[\theta]_{max}$ to negative minimum, $[\theta]_{min}$ changes is in good agreement with that obtained from DSC measurements.

We will discuss the conformational change of DNA in solutions with various proportions of ethanol and a definite concentration of metallic ion by combining the results obtained by the calorimetric and spectral measurements.

INTRODUCTION

The helix-coil transition of DNA takes place by changing pH, temperature and ionic strength of the solution and the thermal stability of DNA depends considerably on both the kinds and the

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0040-6031/90/$03.50

concentrations of metallic ions(refs. 1-3). Further, DNA molecule seems to exist in various conformations which are determined by some external conditions and are classified into the forms such as A-, B-, C-forms and so on. Ivanov et al.(ref. 4) observed that DNA transforms from B-form to A-one in the solution containing 70∿80% of ethanol and/or isopropanol, and also from B-form to C-one by changing the concentration of alkali metallic ions. And Usatyi et al.(ref. 5) reported the helix-coil transition of A- and B-forms in aqueous ethanol solution by means of CD and UV spectral methods.

In order to obtain further information about the helix-coil transition of these conformations of DNA molecule, we will report the heat of helix-coil transition of DNA in solutions containing various proportions of ethanol and a definite concentration of metallic ions by using the differential scanning calorimeter in this paper.

EXPERIMENTAL

Materials

DNA samples used in this study were calf thymus DNA (DNA(I), GC content 42%) and clostridium perfringens DNA (DNA(II), GC content 26.5%) which were purchased from Sigma and Miles Laboratories Inc., (USA). All other materials were commercial preparations of analytical reagent grade. Solvent used was 1×10^{-3} mol dm^{-3} aqueous $NaNO_3$ solution(pH 5.3) containing various concentrations of ethanol at a given concentration of 1×10^{-3} mol dm^{-3} $MgCl_2$, 1×10^{-5} mol dm^{-3} $Cu(NO_3)_2$, and 1×10^{-5} mol dm^{-3} $HgCl_2$, respectively.

In addition, pH of the solutions containing ethanol and metallic ion is a definite value of 5.3, which is nearly independent of the kinds of metallic ion and the concentration of ethanol.

Apparatus and Procedure

Differential scanning calorimeters(DSC-1, Model 8005, Rigaku Denki Co. Ltd., Japan and DSC-2, DASM-4, USSR) were used in this study. The DNA sample was dissolved in aqueous ethanol solution containing metallic ions and allowed to stand overnight at about 278 K to obtain the homogeneous solution. The concentrations of

DNA solution for the DSC measurement used in this study were 0.5 wt% for DSC-1 and 0.05 wt% for DSC-2. The heating rate of DSC is about 1 K/min. DNA concentration was determined by a phosphorus analytical method(ref. 6). CD spectra were taken with a spectropolarimetry (JASCO,J-20A). The DNA concentration used for the CD spectral measurement was about one-tenth that for DSC measurement (DSC-2).

RESULTS AND DISCUSSION

1.DSC measurements

The thermal behaviours of DNA(concentration: 0.5 and 0.05 wt%) in aqueous ethanol solutions containing metallic ions were studied by using an adiabatic differential scanning calorimeter (DSC-2).

The typical DSC curves obtained for DNA(I) and/or DNA(II) in aqueous ethanol solutions containing Mg^{2+} ion show the endothermic peaks as shown in Fig. 1a and 1b, respectively. Those peaks may correspond to the helix-coil transition of DNA from the results obtained by UV spectral measurements. As seen in Fig. 1a, the DSC curves for DNA(I) show the characteristic thermograms having the double shoulders at high temperature and these shoulders of DSC curve at high temperature become the smaller with an increase of the

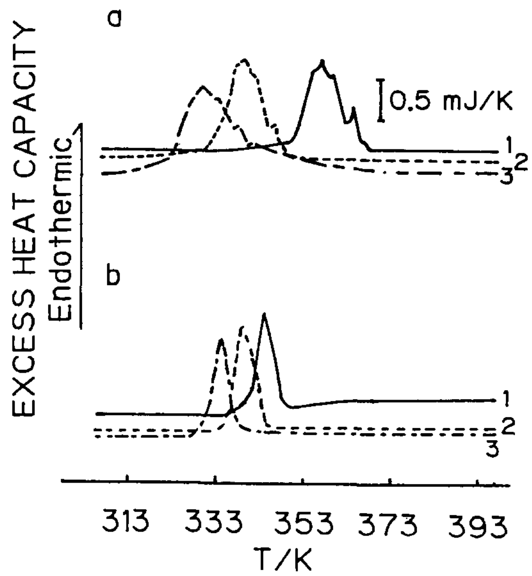

Fig. 1. The typical DSC curves of DNA in aqueous ethanol solutions containing Mg^{2+} ion:
a: DNA(I), b: DNA(II)
1, 2, and 3 in figure represent 0, 10, and 20 vol.% of ethanol, respectively.

volume percent (vol%) of ethanol. And the transition temperature estimated from the endothermic peaks on the DSC curve decreases with increasing the vol.% of ethanol, demonstrating that the conformational change of DNA brings about by the addition of ethanol. It may be suggested that ethanol molecule may play an important role for the conformational change of DNA. On the other hand, DSC curves for DNA(II) are similar to those of DNA(I). However, the double shoulder at high temperature as seen in DSC curve for DNA(I) does not appear for DNA(II), suggesting that the double shoulders of DSC curve at high temperature seem to depend on the GC content of DNA. On the other hand, the thermal behaviours of DNA(I) (concentration 0.5 wt%) solutions containing metallic ions such as Mg^{2+}, Na^{+}, Cu^{2+}, and Hg^{2+} were also measured by DSC-1. The peak temperature of DSC curves for Mg^{2+} ion mea-

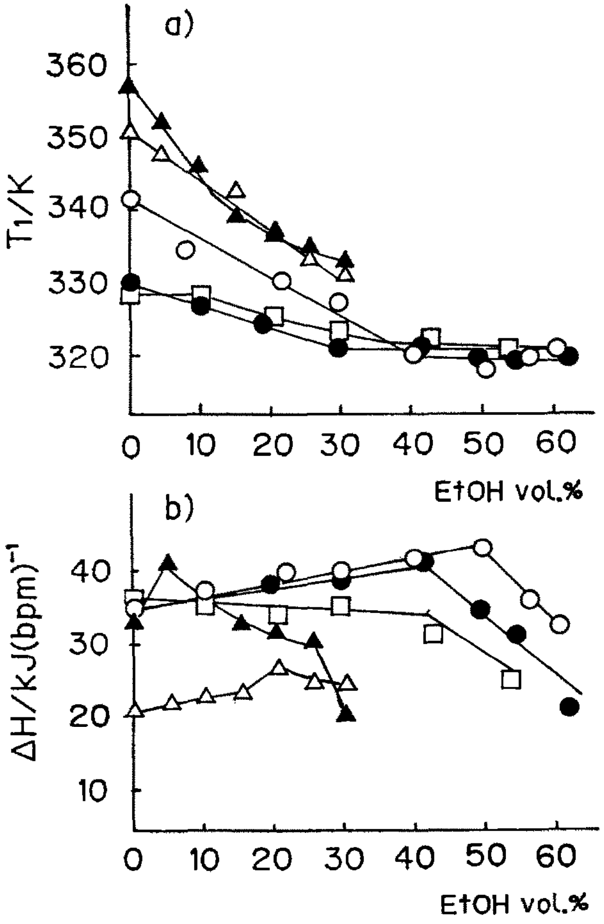

Fig. 2. The dependence of (a) the transition temperature, T_1, and (b) enthalpy change, ΔH, of DNA on concentration of ethanol:

●: Na^{+}, ▲: Mg^{2+} DNA(I), ○: Cu^{2+},
□: Hg^{2+} and △: Mg^{2+} DNA(II) systems

sured by DSC-1 is in good agreement with that determined from DSC-2 as shown in Fig. 1a. Then, DSC curves for Na^+, Cu^{2+}, and Hg^{2+} obtained from DSC-1 are analyzed. The transition temperature, T_1 determined from the endothermic peak is shown in Fig. 2a, where T_1 is plotted against the vol.% of ethanol. As seen in Fig. 2a, T_1 seems to depend considerably on the metallic ions and decreases as the concentration of ethanol increases.

On the other hand, under the assumption that the area under the peak of the DSC curve corresponds to the transition enthalpy from the helical conformation to the coiled one of DNA, the transition enthalpy, ΔH determined from the area is plotted against the vol.% of ethanol as shown in Fig. 2b. It is seen from Fig.2 that ΔH for DNA(I) and/or DNA(II) in an aqueous ethanol solution containing Mg^{2+} increases at first and decreases, but T_1 decreases with an increase of vol.% of ethanol. However, the ethanol content where ΔH shows maximum is about 5 vol.% for DNA(I) and about 20 vol.% for DNA(II), demonstrating that ΔH at maximum may depend on the GC content of DNA and ethanol content, respectively. While, ΔH estimated from DSC-1 for DNA(I) in aqueous ethanol solutions with Na^+, Cu^{2+},and Hg^{2+} is also plotted against vol.% of ethanol as shown in Fig. 2b. As seen in Fig. 2b, ethanol dependence of ΔH for Na^+, and Cu^{2+} systems is similar to the tendency for Mg^{2+} system. But, the concentration range of ethanol which ΔH increases may differ by the kinds of metallic ion; that is $0\sim40$ vol.% for Na^+, $0\sim50$ vol.% for Cu^{2+}. But, for Hg^{2+} system, ΔH shows a definite value which is nearly independent of the vol.% of ethanol and then decreases with increasing vol.% of ethanol. The concentration range of ethanol showing a definite value of ΔH for Hg^{2+} system is the zero to about 40 vol.%. It is worth noting that all ΔH obtained for DNA(I) at zero ethanol concentration have the same values which is nearly independent of the various kinds of metallic ion, except for that of DNA (II).

2. CD spectra

In order to obtain further information about the dependence of ΔH on the concentration of ethanol, CD spectra of DNA solutions with various proportions of ethanol containing a definite concentration of metallic ions were measured at room temperature by using a spectropolarimeter. The results obtained are shown in Fig. 3, where molecular ellipticity ratio, $[\theta]_{max}/[\theta]_{min}$ of positive maximum, $[\theta]_{max}$ to negative minimum, $[\theta]_{min}$ is plotted against the vol.% of ethanol. As seen in Fig. 3, for Na^+, Cu^{2+}, and Hg^{2+} systems, $[\theta]_{max}/[\theta]_{min}$ decreases at first and reaches a definite value with an increase of the vol.% of ethanol. The concentration range of ethanol where $[\theta]_{max}/[\theta]_{min}$ decreases is $0\sim40$ vol.% for Na^+, $0\sim50$ vol.% for Cu^{2+} and $0\sim40$ vol.% for Hg^{2+}. On the other hand, $[\theta]_{max}/[\theta]_{min}$ for DNA(I) and/or DNA(II) with Mg^{2+} ion has a definite value at first and then decreases with increasing the vol.% of ethanol. The behaviours of $[\theta]_{max}/[\theta]_{min}$ of all metallic ions for DNA (I) and Mg^{2+} system for DNA (II) seem to correspond to those in the concentration range where ΔH increases. The decreasing tendency of $[\theta]_{max}/[\theta]_{min}$ for DNA(I) seems to be due to a conformational change from B-form to B'-one which is intermediate conformation between B-form and C-one for Mg^{2+}, from B'-form to C'-one as intermediate conformation between B'- and C-forms for Na^+ and Cu^{2+}, from B-form to C'-one for Hg^{2+} and from C'-form to C-one for Mg^{2+} of DNA(II), respectively. In these cases, B'- and C'-forms are considered to be closely allied to B- and C-forms, respectively. From the results of CD spectral measurements, in the concentration range of ethanol where ΔH increases, a complicated conformational change of DNA molecule seems to bring about, and ethanol molecules may be expected to play an important role for conformational change of DNA.

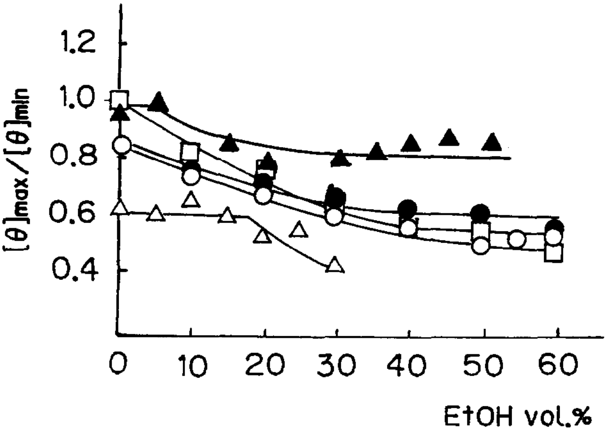

Fig. 3. The dependence of ratio, $[\theta]_{max}/[\theta]_{min}$, of molecular ellipticity of positive maximum, $[\theta]_{max}$, to that of negative maximum, $[\theta]_{min}$, on the concentration of ethanol:
●: Na^+, ▲: Mg^{2+} DNA(I), ○: Cu^{2+},
□: Hg^{2+} and △: Mg^{2+} DNA(II) systems

Table I The influence of ethanol concentration on the heat of helix-coil transition, ΔH_t, and heat of conformational change, ΔH_c of DNA in various metallic ion solutions

Ion	DNA GC cont. (%)	ΔH_t (kJ/bpm)1)	ΔH2) (kJ/bpm)1)	ΔH_c (kJ/bpm)1)	conformational change EtOH vol.% 0,5,10, 20, 30, 40, 50
Mg^{2+}	26.5	20.8	26.4	-5.6	$C' \longrightarrow C$
	42.0	32.7	41.0	-8.3	$B \rightarrow B'$
Na^+	42.0	34.8	40.8	-6.0	$B' \longrightarrow C'$
Cu^{2+}	42.0	34.4	42.4	-8.0	$B' \longrightarrow C'$
Hg^{2+}	42.0	36.0	36.0	0	$B \longrightarrow C'$

1) bpm here means mole of base pair of DNA.

2) ΔH here means enthalpy change at maximum for each metallic ion system as shown in Fig. 2b.

3. Enthalpy change for conformation of DNA

The transition enthalpy, ΔH estimated from DSC for DNA in aqueous solution with metallic ion is the difference between the heat of helix-coil transition of DNA, ΔH_t and the heat of transformation accompanying the conformational change of DNA, ΔH_c; that is, $\Delta H = \Delta H_t - \Delta H_c$. Where ΔH_t value is given as that of ΔH obtained when the concentration of ethanol is zero and the value of ΔH is determined as that at the discontinuous point of maximum for each system as seen in Fig. 2b. The values of ΔH_t, ΔH and ΔH_c obtained for each system are summarized in Table I. As seen in Table I, ΔH_c depends on the various kinds of metallic ions. In particular, it is worth noting that the value of ΔH_c for Hg^{2+} is zero although the conformation of DNA transforms from B-form to C'-one by the addition of ethanol. Thus, the B-form of DNA in aqueous solution with Hg^{2+} ion seems to be more stable than B'-one, although, in aqueous solutions containing Na^+ and/or Cu^{2+}, B'-form is more stable. However, the conformation of DNA seems to change to the same C'-form for all ions, except for Mg^{2+} systems.

On the other hand, in aqueous solution with Mg^{2+} ion, the conformation of DNA(I) transforms from B-form to B'-one with increasing the concentration of ethanol, although the thermal stability is high in comparison with the other ions used in this study. However, the conformation of DNA(II) changes from C'-form to C-one by addition of ethanol. To make clear this problem, further study will be needed for the GC content dependence of DNA on the metallic ions.

In the concentration region where the concentration of ethanol is less than characteristic value of the metallic ion in a solution, the reason why ΔH increases with decreasing T_1 as shown in Fig. 2a and 2b is very difficult to make clear. The possible explanation, however, may be due to the dissociation of the water hydrated around the bases of DNA and the breaking of the three dimensional structure of water surrounding DNA by the addition of ethanol. Then, the increase of ΔH as shown in Fig. 2b seems to be considered as sum of the heat of conformational change and the heat of dehydration around the DNA. And T_1 decreases based on lability of DNA by the dehydration and the breaking of the three dimensional structure of water surrounding DNA with increasing the concentration of ethanol, respectively.

On the other hand, when the concentration of ethanol is more than the characteristic value, the ΔH for all metallic ion systems indicates a drastic decrease with an increase of vol.% of ethanol, suggesting that ethanol may play an important role for the conformational change of DNA. However, to interpret the drastic decrease of ΔH at the concentrated ethanol solution may be very difficult to make clear from our present work only. Further study will be needed.

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