Polymer Journal, Vol. 20, No. 9, pp 751—760 (1988)

# Preferential Hydration and B–A Transition of Deoxyribonucleic Acid in Ethanol–Water Mixtures

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(Received February 16, 1988)

ABSTRACT: The preferential solvent interaction of calf thymus deoxyribonucleic acid (DNA) in ethanol–water mixtures was investigated by a densimetric method at 25°C. For all solvent compositions up to 40% (w/v) ethanol, water molecule was preferentially bound to DNA, the extent of preferential hydration being maximum around 20% ethanol. The free energy of transfer of B-DNA from water to aqueous ethanol was calculated from the preferential hydration data, and found to be positive and to increase to 7.9 kcal mol$^{-1}$ of nucleotide with increasing ethanol content in the B–A transition region. The free energy of B–A transition induced by addition of ethanol was also estimated by circular dichroism analysis. On the basis of these results, the mechanism of stabilization of the B and A structures of DNA was discussed by means of the thermodynamics of multicomponent solutions (linked function analysis). The ethanol-induced B–A transition was successfully interpreted in terms of the different hydration levels of both structures as well as the changes in humidity.

KEY WORDS Preferential Hydration / B–A Transition / Ethanol Effect / Deoxyribonucleic Acid / Conformational Stability / Thermodynamic Analysis /

As revealed by X-ray diffraction studies, double-stranded DNA exists at least in two distinctly different forms, depending on the relative humidity: the high humidity B-DNA form (approx. 92% relative humidity) changes to the A-DNA form upon drying at about 75% relative humidity.[1–3] Similar conformational transition from B to A form can be also induced by addition of high concentration of ethanol, probably due to the reduced water activity in the mixed solvents.[4–6] These phenomena demonstrate that water plays an important role in stabilizing both conformations of DNA. In contrast to many detailed studies on DNA–water interaction,[7–10] however, the stabilization mechanism for the B and A conformations is as yet not fully understood from the thermodynamic standpoint (free energy level). From circular dichroism analysis, Ivanov *et al.*[11] proposed that the B-state is more stable in water by about 1 kcal mol$^{-1}$ of nucleotide, as compared to the A-state. Clearly, this difference in free energy decreases in the presence of ethanol, leading to the lower free energy level of the A-state relative to the B-state. According to the thermodynamic requirements, this process can be expressed by the following free energy diagram:

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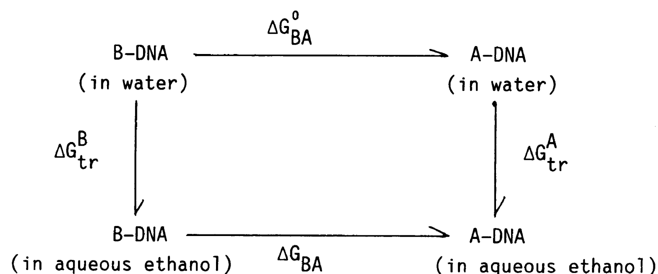


**Scheme 1.**

where $\Delta G^{0}_{BA}$ and $\Delta G_{BA}$ represent the standard free energies of B–A transition in water and aqueous ethanol, respectively; $\Delta G^{B}_{tr}$ and $\Delta G^{A}_{tr}$ are the standard free energies of transfer of B-DNA and A-DNA from water to aqueous ethanol, respectively. Therefore, further advanced understanding of the stabilization mechanism of both conformations should be reached by evàluating the free energy changes of each process in this diagram, especially the transfer process, since they are coupled with each other.

From this point of view, in this paper, we have measured the preferential interaction of solvent components with B-DNA in water–ethanol mixtures by a densimetric method. The free energy of B–A transition was also estimated from the circular dichroism analysis of DNA in these mixed solvents. On the basis of these results, the stabilization mechanism of both DNA structures shall be discussed in terms of the thermodynamics of multicomponent solutions (linked function analysis).[12-14]

## EXPERIMENTAL

### *Materials*

Calf thymus DNA was purchased from Worthington Biochemical Corp. (lot 33K820). This sample was sonicated in 0.2 M NaCl and freeze-dried according to the procedures previously reported.[15] Molecular weight of the sonicated DNA was estimated to be $2 \times 10^5$ from viscosity measurements ($[\eta] = 1.23\,\mathrm{dl\,g^{-1}}$ in 0.2 M NaCl at 25°C). Hyperchromicity of this sample upon heat denaturation was 35% at 260 nm, indicating the intactness of the native structure. Special reagent grade ethanol was purchased from Wako Pure Chemicals and purified by distillation before use.

### *Density Measurements*

The preferential solvent interaction with DNA in aqueous ethanol solutions was studied in 1 mM $NaH_2PO_4/Na_2HPO_4$ buffer (pH 7) at 25°C, using a precision density meter (Anton Paar DMA 02C). This experiment was carried out at ethanol concentrations below 40% (w/v) to avoid the precipitation of DNA, since the DNA concentration used was considerably high (0.1—0.5%). The partial specific volume of DNA, $\phi_2$, was determined from the density increment of the solution under two experimental conditions.

First, $\phi_2^0$ was determined as $\phi_2$ under the condition that the molal concentration, $m_3$, of diffusible component 3 (here, ethanol) remains to be the same in the solvent and the DNA solutions. A given amout of DNA sample was dissolved in a phosphate buffer adjusted to 1 mM in final alcohol solutions and exhaustively dialyzed against the same solvent at 4°C. The dialysate was exchanged once and dialysis was then allowed to proceed without interruption for two days. After equilibrium dialysis, the DNA concentration in the dialyzed solution was determined by absorption measurement: this allowed calculation of the weight of solvent component and of DNA in the buffer. In

the next step, a given amount of cold ethanol was carefully weighed into the DNA solution in tightly closing glass bottles on a Mettler balance sensitive to 0.01 mg. This solution was used as DNA stock solution. In a similar way, the dialysate was diluted with ethanol at an exactly identical weight ratio of ethanol to buffer component in DNA solution: molality of ethanol in the solvent thus prepared was then precisely identical with that in DNA stock solution. Then, the DNA stock solution was diluted with this solvent to prepare five sample solutions of different DNA concentrations (0.1—0.5%). The sample solutions were introduced into the density meter cell using a 1-ml syringe and after temperature equilibration the time lapse was determined.

Second, $\phi_2^{0'}$ was determined as $\phi_2$ under the condition that the chemical potential of the component 3, $\mu_3$, was kept identical both in the DNA solution and in the reference solvent. Following the procedures mentioned above, five sample solutions of different DNA concentrations (1.5 ml each) were freshly prepared by using aqueous ethanol solutions made up to 1 mM phosphate. Each of the solutions was placed in a cellulose bag (Union Carbide Corp.) and exhaustively dialysed against the same solvent for 2 days at 4°C. The dialyzing system was shifted to 25°C for 5 h prior to the density measurements. Just before the measurements, dialysis bags were taken out individually from the dialysis system with stainless-steel forceps and the DNA solution in the bag was removed with 1-ml syringe with needle for transfer into the density meter cell. Density of the reference solvent was first measured, then the samples, and finally the solvent again. In cases where a significant change of time lapse was observed for solvent before and after measurements of the samples, the data were discarded. In most cases, the partial specific volume was determined within an experimental error of $\pm 0.005\ \mathrm{ml\,g^{-1}}$. Detailed procedures for the densimetric measurements of preferential solvent interaction were described for protein systems in previous papers.[16,17]

*Determination of DNA Concentration*

The DNA concentration was determined from the absorption measurements of the solution with a JASCO 505 spectrophotometer after the density measurements. The molar extinction coefficient of DNA was assumed to be $6400\ \mathrm{M^{-1}\,cm^{-1}}$ at 260 nm in 1 mM phosphate buffer and the average molecular weight of a nucleotide was taken as 331 for the Na salt. This extinction coefficient was corrected for each mixed solvent according to the procedures described in previous paper[17]: it was 6592, 6784, 7040, and 6719 $\mathrm{M^{-1}\,cm^{-1}}$ for ethanol concentrations of 10, 20, 30, and 40% (w/v), respectively. At high DNA concentrations, the sample solutions were gravimetrically diluted with solvent on a Mettler balance before the absorption measurments. The gravimetrically obtained dilution factor was converted to a volumetric one from density data on the solvent and solutions, by assuming the additivity of their volumes. This factor was used for calculating the DNA concentration in the parent sample solutions.

*Circular Dichroism Measurements*

The B–A transition of DNA molecule in ethanol–water mixture was studied by circular dichroism (CD) measurements with a JASCO J-40A spectropolarimeter. DNA sample solutions were prepared as follows. First, the aqueous solution of DNA ($1\ \mathrm{mg\,ml^{-1}}$) was prepared by dissolving freeze-dried DNA sample in 1.5 mM sodium citrate containing 5 mM NaCl (pH 7.0) and exhaustively dialyzing against the same solvent. 0.2 ml of DNA solution thus obtained was initially intoduced into a 5 ml flask and diluted with a predetermined amount of water. Then, the required quantity of cold ethanol was added step by step, each time with careful stirring, and finally the flask was filled up with a very small amount of water. In this way, DNA was

dissolved in aqueous ethanol solutions with different ethanol content up to 69% (w/v) without precipitation. The DNA solution thus prepared was introduced into a 1 cm path-length cell and the CD spectra were measured as a function of ethanol content. The CD magnitude was expressed in terms of difference in the molar absorptivity, $\Delta\varepsilon$ ($M^{-1}\,cm^{-1}$).

## RESULTS AND DISCUSSION

*Preferential Solvent Interaction with B-DNA*

The partial specific volumes of DNA in ethanol–water mixtures, $\phi_2^0$ and $\phi_2^{0'}$, where the superscript 0 refers to the state of infinite dilution of DNA, were measured under the respective conditions of identical molality and identical chemical potential of ethanol between the solvent and the DNA solutions. The experimental results are shown in Table I and plotted against ethanol content in Figure 1. The $\phi_2^0$ and $\phi_2^{0'}$ were found to be 0.545 ml g$^{-1}$ in dilute buffer solution without ethanol, almost identical with literature values.[18] It is seen that $\phi_2^0$ increases with increasing ethanol content while it decreases slightly at low ethanol content. This indicates that the volume change in the transfer of DNA from water to aqueous ethanol solutions is essentially positive: the volume increase on transferring into 30% ethanol is estimated to be 12 ml mol$^{-1}$ of nucleotide. This volume change is evidently the net one due to the desorption of bound water and the adsorption of ethanol (the contribution of conformational change of DNA is not involved here, since the conformation is still in the B form within this low ethanol concentration region). Although it is impossible to estimate each contribution separately, we may ascribe a fair part of the volume increase to dehydration of the DNA molecule. In contrast with $\phi_2^0$, $\phi_2^{0'}$ shows a dramatic dependence upon ethanol concentration, suggesting a complicated mode of DNA-solvent interaction. These are the first experimental data for $\phi_2^{0'}$ of DNA in aqueous ethanol solutions, while some values have been assumed in ultracentifugal analysis.[19]

From a combination of $\phi_2^0$ and $\phi_2^{0'}$, the preferential interaction parameter of ethanol (component 3) with DNA (component 2), $(\partial g_3/\partial g_2)_{T,\mu_1,\mu_3}$, was calculated by use of the following equation[12]

$$(\partial g_3/\partial g_2)_{T,\mu_1,\mu_3} = \rho_0(\phi_2^0 - \phi_2^{0'})/(1-\rho_0 v_3) \quad (1)$$

where $g_i$ is the concentration of component $i$ in gram per gram of principal solvent, water (component 1); $\rho_0$ is the density of the mixed solvent; and $v_3$ is the partial specific volume of component 3. The $v_3$ values were determined from the density measurements to be 1.160, 1.159, 1.194, and 1.223 ml g$^{-1}$ at ethanol concentrations of 10, 20, 30, and 40% (w/v), respectively. The preferential interaction parameter on a molal basis, $(\partial m_3/\partial m_2)_{T,\mu_1,\mu_3}$, *i.e.*, the number of moles of component 3 bound to a mole of component 2, is calculated as follows by using the molecular weights of component $i$, $M_i$.

$$(\partial m_3/\partial m_2)_{T,\mu_1,\mu_3} = (M_2/M_3)(\partial g_3/\partial g_2)_{T,\mu_1,\mu_3} \quad (2)$$

As shown in Table I, the calculated values of $(\partial g_3/\partial g_2)_{T,\mu_1,\mu_3}$ or $(\partial m_3/\partial m_2)_{T,\mu_1,\mu_3}$ are negative, indicating a deficiency of ethanol molecules in the immediate domain of DNA, *i.e.*, preferential binding of water. The corresponding preferential hydration parameter, $(\partial g_1/\partial g_2)_{T,\mu_1,\mu_3}$, was calculated from

$$(\partial g_1/\partial g_2)_{T,\mu_1,\mu_3} = -(1/g_3)(\partial g_3/\partial g_2)_{T,\mu_1,\mu_3} \quad (3)$$

The $(\partial g_1/\partial g_2)_{T,\mu_1,\mu_3}$ values obtained are listed in Table I and plotted against ethanol concentration in Figure 1. Clearly, the degree of preferential hydration increases with increasing ethanol content, reaches a maximum value at around 20% ethanol, and then decreases. It is interesting to note that this maximum appears at around the ethanol concentration that ethanol shows the minimum partial molal volume and the maximum activity coefficient in water. This suggests that the DNA-solvent interaction in ethanol–water mixtures is di-

**Table I.** Partial specific volumes and preferential interaction parameters of DNA in aqueous ethanol solutions at 25°C[a]

| Ethanol concn. | | $\phi_2^0$ | $\phi_2^{0\prime}$ | $(\partial g_3/\partial g_2)_{T,\mu_1,\mu_3}$ | $(\partial g_1/\partial g_2)_{T,\mu_1,\mu_3}$ | $(\partial m_3/\partial m_2)_{T,\mu_1,\mu_3}$ [b] | $(\partial \mu_2/\partial m_3)_{T,P,m_2}$ [c] |
|---|---|---|---|---|---|---|---|
| w/v% | $m_3$ | ml g$^{-1}$ | ml g$^{-1}$ | g g$^{-1}$ | g g$^{-1}$ | mol mol$^{-1}$ | |
| 0 | 0 | 0.545 | 0.545 | | | | |
| 10 | 2.47 | 0.535 | 0.510 | −0.179 | 1.58 | −1.29 | 390 |
| 20 | 5.68 | 0.559 | 0.305 | −2.07 | 7.91 | −14.9 | 980 |
| 30 | 10.1 | 0.580 | 0.321 | −1.87 | 4.03 | −13.4 | 340 |
| 40 | 16.6 | 0.655 | 0.420 | −1.66 | 2.18 | −12.0 | 110 |

[a] Solvent: 1 mM $NaH_2PO_4/Na_2HPO_4$ (pH 7).
[b] $M_2=331$.
[c] (cal/mol · nucleotide)/(mol · ethanol/1000 g $H_2O$).

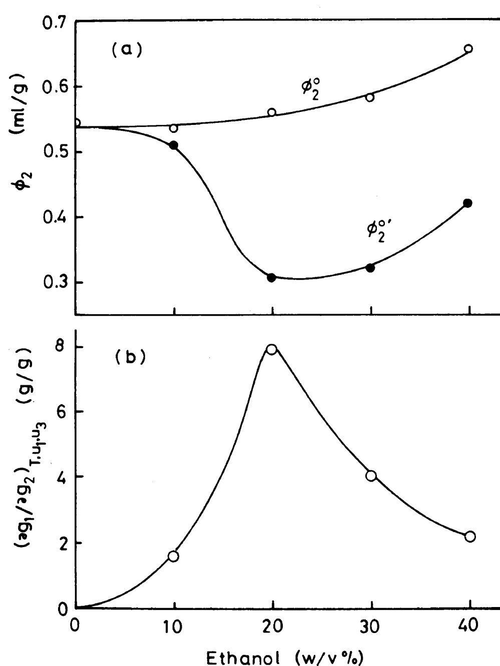


**Figure 1.** The partial specific volumes (a) and the preferential hydration parameters (b) of DNA in water-ethanol mixtures (1 mM phosphate buffer, pH 7) at 25°C. $\phi_2^0$ and $\phi_2^{0\prime}$ represent the partial specific volumes under the respective conditions of identical molality and identical chemical potential of ethanol between the solvent and DNA solutions.

rectly affected by the thermodynamic properties of these mixed solvents.

Preferential solvent interaction is strictly a thermodynamic effect as shown by the following relationships:

$$(\partial m_3/\partial m_2)_{T,P,\mu_3} = -(\partial \mu_3/\partial m_2)_{T,P,m_3}/(\partial \mu_3/\partial m_3)_{T,P,m_2} \qquad (4)$$

$$(\partial \mu_3/\partial m_2)_{T,P,m_3} = (\partial \mu_2/\partial m_3)_{T,P,m_2} = -RT(M_2/M_3)(1/m_3 + \partial \ln \gamma_3/\partial m_3)(\partial g_3/\partial g_2)_{T,\mu_1,\mu_3} \qquad (5)$$

where $\gamma_3$ is the activity coefficient of component 3 (ethanol). Therefore, the chemical potential change of DNA with ethanol, $(\partial \mu_2/\partial m_3)_{T,P,m_2}$, can be calculated by using the preferential interaction data and by estimating the term $(\partial \ln \gamma_3/\partial m_3)$ from the vapor pressure data of ethanol–water mixtures (International Critical Table, 1928). The results of such calculations are listed in the last column of Table I and plotted against molal concentration of ethanol in Figure 2. The positive values of $(\partial \mu_2/\partial m_3)_{T,P,m_2}$ indicate that an introduction of ethanol into the aqueous DNA solution enhances the chemical potential of DNA molecule and the system is thermodynamically destabilized. This is also expected from the fact that DNA precipitates at high concentrations of DNA and ethanol.

Since $(\partial \mu_2/\partial m_3)_{T,P,m_2}$ is a function of ethanol concentration ($m_3$), integration of this parameter with respect to $m_3$ should yield the total free energy change of DNA when transferring it from water to aqueous ethanol, $\Delta G_{tr}^{B}$,

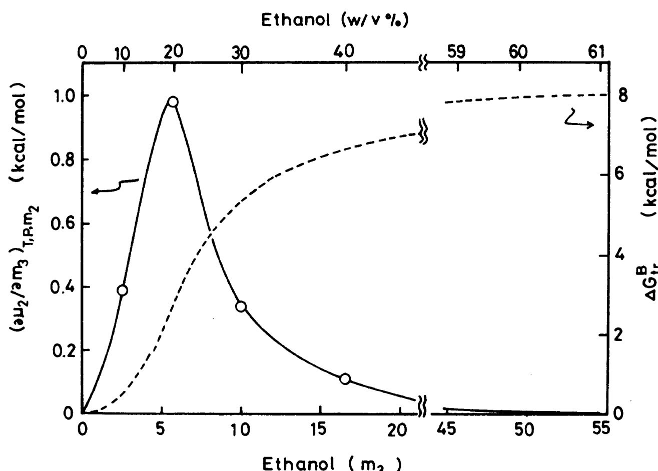


**Figure 2.** The chemical potential change of DNA by addition of ethanol at 25°C. $\Delta G_{tr}^{B}$ was obtained by integrating graphically the $(\partial\mu_2/\partial m_3)_{T,P,m_2}$–$m_3$ plots with respect to $m_3$. Both vertical coordinates are expressed in unit, kcal mol$^{-1}$ of nucleotide.

$$\Delta G_{tr}^{B}=\mu_2(m_3)-\mu_2(0)=\int_0^{m_3}(\partial\mu_2/\partial m_3)_{T,P,m_2}\mathrm{d}m_3 \qquad (6)$$

where $\mu_2(m_3)$ and $\mu_2(0)$ refer to the chemical potential of the DNA in aqueous ethanol and in water, respectively. As shown in Figure 2, the relationship between $(\partial\mu_2/\partial m_3)_{T,P,m_2}$ and $m_3$ cannot be expressed by a simple equation, then the integration was done graphically by estimating the area under the curve. It is seen from Figure 2, that $\Delta G_{tr}^{B}$ increases with increasing ethanol concentration in a sigmoidal pattern. By assuming simple extrapolation to higher ehtanol concentrations, the chemical potential of B-DNA can be expected to increase by about 7.9 kcal mol$^{-1}$ of nucleotide in the ethanol concentration range of B–A transition for the DNA molecule.

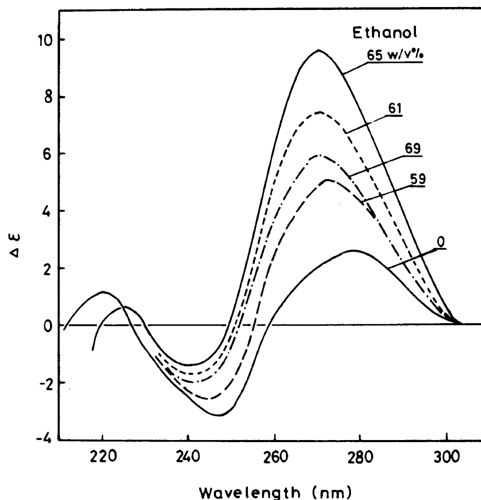


**Figure 3.** The circular dichroism spectra of DNA in water–ethanol mixtures at 25°C. The solvents contain 0.06 mM sodium citrate (pH 7) and 0.2 mM NaCl. The ethanol concentration for each curve is presented in the figure.

*B–A Transition*

Figure 3 shows the CD spectra of DNA in water–ethanol mixtures. The peak at around 270 nm shifts to shorter wavelength, accompanying an increase in intensity by addition of ethanol. This change in CD spectra is consistent with earlier observations[4,6,11] and thus demonstrates the conformational change of the DNA molecule from B form into A form. Through addition of a large amount of ethanol, however, the peak intensity oppositely decreased without affecting the peak wavelength, proportionally to the ethanol con-

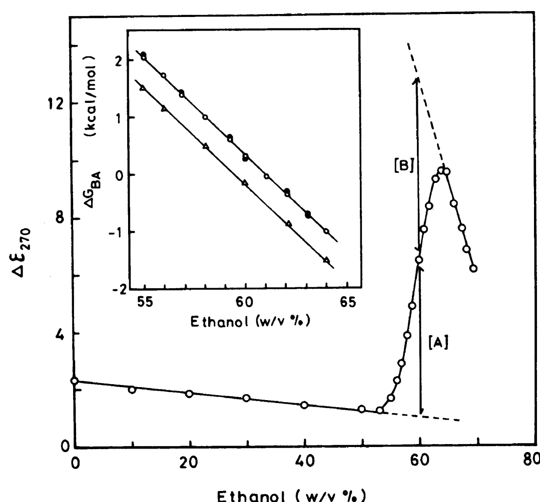


**Figure 4.** The CD magnitude of DNA at 270 nm as a function of ethanol concentration at 25°C. The solvents are same as those in Figure 3. [DNA] = 0.04 mg ml$^{-1}$. The fractions of B and A forms were calculated as indicated in the figure by assuming the same linear dependences of CD magnitude on ethanol concentration in the transition region as in the pure B and A states. Inset: the free energy change of B–A transition as a function of ethanol concentration. The $\Delta G_{BA}$ is expressed as the value per mole of a cooperative fragment. The symbols ○ and ● refer to the data at 270 and 280 nm respectively in 0.06 mM citrate (pH 7) containing 0.2 mM NaCl. The symbol △ refers to the data at 270 nm in 0.5 mM NaCl.

tent after going through a maximum at about 64% ethanol. This feature can be seen more clearly in Figure 4 where the CD magnitude at 270 nm was plotted against ethanol concentration. There could be two possible origins for such depressed spectra at high ethanol concentrations: one is solvent perturbation of CD spectra of A form and the other is precipitation of DNA molecules. However, no confirmative evidence could be found for the latter possibility: the absorbance of DNA solution is comparable at low and high ethanol concentrations and dilution of high ethanol-content solution gives CD spectra identical to those at corresponding low ethanol concentrations. In the present study, therefore, it was assumed that the depressed CD magnitude at high ethanol concentrations is due to the solvent effect on the CD spectra of A form, while DNA molecules may partially exist in aggregated form. Such depression of the magnitude by ethanol was also observed for the B form spectra in dilute ethanol solutions but with much less effect compared with the A form spectra. By assuming that the CD magnitudes of B and A forms in the transition region, $\Delta\varepsilon_{270}^{B}$ and $\Delta\varepsilon_{270}^{A}$, have the same linear dependences on ethanol concentration as in the pure B and A states (see Figure 4), the equilibrium constant of B–A transition, $K$, was calculated from the equation

$$K = [A]/[B] = (\Delta\varepsilon_{270} - \Delta\varepsilon_{270}^{B})/(\Delta\varepsilon_{270}^{A} - \Delta\varepsilon_{270}) \quad (7)$$

The free energy of B–A transition, $\Delta G_{BA}$ ($= -RT\ln K$), are plotted as a function of ethanol concentration in inset of Figure 4, in which the results obtained by monitoring the CD magnitude at 280 nm are also included. Evidently, $\Delta G_{BA}$ is a linearly decreasing function of ethanol concentration, allowing an evaluation of the free energy of B–A transition in water without ethanol, $\Delta G_{BA}^{0}$, by linear extraporation. The $\Delta G_{BA}^{0}$ value thus obtained was $21.2 \pm 0.8$ kcal mol$^{-1}$ in 0.06 mM sodium citrate (pH 7) containing 0.2 mM NaCl, and 19.8 kcal mol$^{-1}$ in 0.5 mM NaCl without buffer. Here, it should be noted that these values refer to the free energy per mole of a fragment cooperatively participating in the transition. Although no definite value is yet known for the number of base pairs involved in such a cooperative fragment, the order of ten base pairs has been proposed.[11,20,21] Assuming that this fragment consists of 20 base pairs as estimated by Ivanov *et al.*,[11] the $\Delta G_{BA}^{0}$ per nucleotide could be estimated to be about 1 kcal mol$^{-1}$ of nucleotide. This value is completely in agreement with the result obtained by Ivanov *et al.*[11] from the linear extrapolation of $\Delta G_{BA}$-water activity plots.

Since $\Delta G_{BA}$ and $\Delta G_{BA}^{0}$ have been evaluated above, we now could complete the free energy

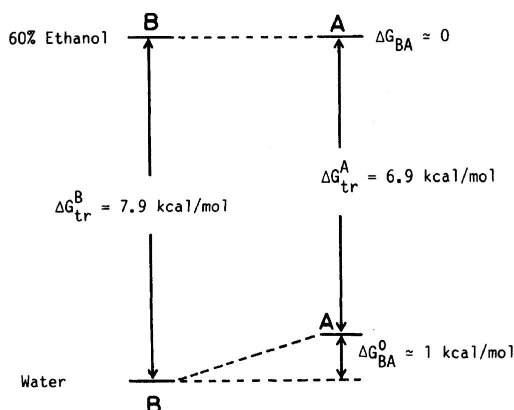


**Figure 5.** The free energy levels of B and A forms of DNA in water and 60% (w/v) ethanol at 25°C. Each free energy value is expressed in kcal mol$^{-1}$ of nucleotide.

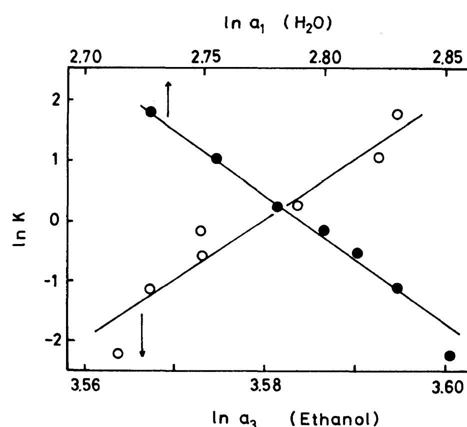


**Figure 6.** The equilibrium constant of B–A transition of DNA as a function of activity of solvent components in water–ethanol mixtures at 25°C (Wyman plots). The solvents contain 0.06 mM sodium citrate (pH 7) and 0.2 mM NaCl.

diagram as shown in Scheme 1 by coupling these values with the free energy of transfer, $\Delta G_{\rm tr}^{\rm B}$, found by measurement of preferential solvent interactions. As example, in Figure 5 are represented the free energy levels of B and A forms at the midpoint of equilibrium (about 60% ethanol). For the large $\Delta G_{\rm BA}^{0}$ relative to $\Delta G_{\rm BA}$, the free energy of transfer of A-DNA from water to aqueous ethanols, $\Delta G_{\rm tr}^{\rm A}$, should be smaller than that of B-DNA, $\Delta G_{\rm tr}^{\rm B}$, since $\Delta G_{\rm tr}^{\rm B}+\Delta G_{\rm BA}=\Delta G_{\rm BA}^{0}+\Delta G_{\rm tr}^{\rm A}$. Thus, the conformational stability of DNA in ethanol–water mixtures can be interpreted in terms of solvent interaction with its two conformational states. A more detailed discussion will be given below based on linked function analysis.

*Linked Function Analysis*

For the present three-component systems, the dependence of the equilibrium constant of B–A transition upon the solvent variables, $\mathrm{d}\ln K/\mathrm{d}\ln a_i$, is ascribed to the difference in the preferential binding of solvent components, $\Delta v_3$ and $\Delta v_1$, between the B and A states[13,14]

$$(\mathrm{d}\ln K/\mathrm{d}\ln a_3)=\Delta v_3 = (\partial m_3/\partial m_2)^{\rm A}_{T,\mu_1,\mu_3}-(\partial m_3/\partial m_2)^{\rm B}_{T,\mu_1,\mu_3} \quad (8)$$

$$(\mathrm{d}\ln K/\mathrm{d}\ln a_1)=\Delta v_1 = (\partial m_1/\partial m_2)^{\rm A}_{T,\mu_1,\mu_3}-(\partial m_1/\partial m_2)^{\rm B}_{T,\mu_1,\mu_3} \quad (9)$$

where $a_i$, $m_i$, and $\mu_i$ are respectively the activity, molal concentration and chemical potential of the component indicated by the subscript 1 (water), 2 (DNA), and 3 (ethanol); and the superscripts B and A refer to the two final states. As shown in Figure 6, plots of $\ln K$ *vs.* $\ln a_1$, or $\ln a_3$, give a linear relationship in the B–A transition region, where the activity of ethanol and water in their mixtures was calculated from the vapor pressure data. From the slopes of the lines, $\Delta v_1$ and $\Delta v_3$ were estimated to be $-27$ and 100 mol mol$^{-1}$ of a cooperative fragment, which correspond to $-1.4$ and 5 mol mol$^{-1}$ of nucleotide, respectively, by assuming 20 base pairs for the fragment.[11] On the other hand, coupling eq 5 to eq 8 yields

$$(\partial \mu_2/\partial m_3)^{\rm A}_{T,P,m_2}-(\partial \mu_2/\partial m_3)^{\rm B}_{T,P,m_2} = -RT\Delta v_3(1/m_3+\partial \ln \gamma_3/\partial m_3) \quad (10)$$

Since the values in parentheses on the right-hand side of this equation are always positive within the ethanol concentration region used, the positive $\Delta v_3$ leads to $(\partial m_3/\partial m_2)^{\rm A}_{T,\mu_1,\mu_3}>$

$(\partial m_3/\partial m_2)^{B}_{T,\mu_1,\mu_3}$ and $(\partial \mu_2/\partial m_3)^{A}_{T,P,m_2} < (\partial \mu_2/\partial m_3)^{B}_{T,P,m_2}$. This indicates that the ethanol molecule is preferentially excluded from the surface of B-DNA more strongly than from that of A-DNA and the resulting thermodynamic instability of the B form should be reduced by a displacement of the equilibrium towards the A form.

Why then is B-DNA more preferentially hydrated than A-DNA in ethanol–water mixtures? It may be pertinent to discuss this reason at the molecular level, since such solvent interaction of DNA is directly related to the stabilization mechanisms of both conformations. Preferential interaction is related to the total binding number of solvent components with DNA molecule, $\nu$ (mol mol$^{-1}$ of nucleotide), by the following equation[22-24]

$$(\partial m_3/\partial m_2)_{T,\mu_1,\mu_3} = \nu_3 - (m_3/m_1)\nu_1 \quad (11)$$

By combining this equation with eq 8, $\Delta\nu_3$ can be expressed as

$$\begin{aligned}\Delta\nu_3 &= (\partial m_3/\partial m_2)^{A}_{T,\mu_1,\mu_3} - (\partial m_3/\partial m_2)^{B}_{T,\mu_1,\mu_3}\\ &= (\nu_3^{A} - \nu_3^{B}) - (m_3/m_1)(\nu_1^{A} - \nu_1^{B}) \quad (12)\end{aligned}$$

Thus, the difference in preferential solvent interaction between A and B states is decided by the balance of total interactions of water and ethanol with the respective states. Although $\nu_1$ and $\nu_3$ cannot be determined from preferential interaction data only, it would be possible to evaluate qualitatively the difference in the respective values for the two conformational states by taking into consideration the nature of the molecular surface of DNA accessible to solvents.

In general, preferential solvent interaction is determined by the two factors: (1) the steric exclusion principle[25] and (2) the affinity between solvent components and the binding sites on the polymer. Since the molal volume of ethanol is about three times larger than that of water, water molecules should be more dominantly accomodated statistically in the immediate domain of DNA molecules. According to Alden and Kim,[26] the solvent-accessible surface area of B-DNA is considerably larger than that of A-DNA. Therefore, B-DNA could bind a larger amount of water and ethanol molecules compared to A-DNA ($\nu_1{}^{B} > \nu_1{}^{A}$ and $\nu_3{}^{B} > \nu_3{}^{A}$). It is probable that since $\nu_1 > \nu_3$, the difference in $\nu_1$ between both conformations is larger than that in $\nu_3$, leading to $\Delta\nu_3 > 0$ in the transition region ($m_3/m_1 = 0.7-1.2$), that is, the enhanced preferential hydration of B-DNA comparing with A-DNA (see eq 12).

The second factor is the affinity of solvent components for the polymer. There are three types of water binding sites on the surface of DNA molecules, ionic groups, polar groups and nonpolar groups. Clearly, the ionic and polar groups are antagonistic to ethanol as expected from the precipitation of salts and sugars in aqueous ethanol solutions. On the other hand, the nonpolar surface is preferable for binding ethanol molecules, as judged from the negative transfer free energy of nonpolar groups from water to aqueous ethanol.[27] Therefore, the ionic and polar groups would contribute to preferentially exclude ethanol molecules from the surface of DNA and oppositely nonpolar groups would contribute to preferentially bind them. Thus, the greater exposure of the ionic and polar groups relative to nonpolar carbon exposure, as revealed by calculations of surface accessibility,[25] should be a dominant factor in the preferential hydration of DNA in ethanol–water mixtures. The increased preferential hydration of B-DNA could be reasonably explained by the fact that B-DNA has a greater exposure of ionic or polar groups and a lesser exposure of nonpolar surfaces compared to A-DNA.[25]

It is well known that the B conformation is stable at high humidity (water activity), changing to A conformation with decreasing humidity. This conformational change has been ascribed to the degree of hydration which is required to maintain the respective conformations: A form binds about 9 to 10 water

molecules per nucleotide but extra water molecules are required in B form, a total of about 18 per nucleotide.[7] The similar results were derived from the surface accessibility calculations.[25] If these amounts of hydration are assumed unchanged in ethanol–water mixtures, eq 12 predicts that $v_3^B$ is larger than $v_3^A$ by 0.6—6.7 molecules per nucleotide, depending on the ethanol content in the transition region. These values may be overestimated since the actual amount of hydration of DNA, especially in B form, would decrease by addition of ethanol. However, such calculation demonstrates that ethanol molecules bind more extensively to B-DNA than A-DNA in the thermodynamic sense, as predicted above. In conclusion, the enhanced preferential hydration of B-DNA compared to A-DNA can be ascribed to the relatively larger amount of hydration overcoming the binding of ethanol. In this sense, the B–A transition induced by addition of ethanol could be, in principle, interpreted in terms of the different hydration levels of both states as well as the changes in humidity.

*Acknowledgement.* The authors wish to thank Professor Kiwamu Yamaoka of Hiroshima University for DNA sonication and helpful discussion. This work was supported by a Grant-in-Aid for Scientific Research (No. 59470134) from the Ministry of Education, Science, and Culture of Japan.

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