Ultrasonic Scission of Deoxyribonucleic Acid in Aqueous Solution IV. Circular Dichroism of Sonicated and Unsonicated DNA in Water-Alcohol Mixed Solvents

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ABSTRACT: The circular dichroic (CD) spectra of calf thymus deoxyribonucleic acid (hDNA) and the sonicated fragment (sDNA) with weight-average molecular weights of 580×10^4 and 14.2×10^4 , respectively, were measured in the 220–320 nm wavelength region at 15°C in water-alcohol mixed solvents with an ionic strength of 0.4×10^{-3} . The effect of ionic valence on CD spectra was examined with sodium or magnesium ions as the counterion and $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ or $\text{Mg}(\text{CH}_3\text{COO})_2/\text{CH}_3\text{COOH}$ as the buffer reagent. The CD spectra of both hDNA and sDNA with the Na^+ counterion showed a B-form pattern in 0–70 vol% ethanol and transformed to an A-form profile in 72–90 vol%, the spectral change of hDNA being more cooperative than that of sDNA. Trifluoroethanol (TFE) induced changes in the CD spectra of sDNA as ethanol. In the case of the Mg^{2+} counterion, the CD spectra of h- and sDNA revealed the B-form pattern between 0 and 40% ethanol. A further addition of ethanol induced another spectral change, but fibrous precipitates appeared above 56% ethanol. From circular dichroic point of view, both hDNA and sDNA behave identically toward counterion, ethanol and TFE, their secondary structure probably remaining much the same.

KEY WORDS Deoxyribonucleic Acid / Ultrasonic Scission / Melting / Circular Dichroism / B–A Structural Transition / Ethanol / Trifluoroethanol /

While the secondary structure of crystalline DNA has been unraveled with developments in synthesis and crystallization of oligomeric DNA,^{1–3} the conformation of polymeric DNA in solution remains still unresolved. The circular dichroism (CD) technique has been used to study the thermal stability and the effects of counterion, ionic strength, organic solvent, etc., on the higher-order structure of nucleic acids.^{4–22} Ivanov *et al.*, for example, reported that the addition of excess salt or methanol induces a structural transition from B to C form but the addition of ethanol induces the B to A transition.⁴ This transition has also been studied by other techniques, *e.g.*, X-ray diffraction,^{23,24} Raman scattering,²⁵ nuclear

magnetic resonance,²⁶ and electric linear dichroism.^{27–29} Although the CD is a sensitive means that can detect many subtle changes, it is actually an empirical one to identify the secondary structure of DNA in solution.

The helical structure of DNA is defined by the axial translation per base pair, the tilt, roll and twist angles of the base pair plane, the pitch of helix, the sugar puckering, etc.³ If the absolute molecular weight and the chain length of unperturbed (completely straight) DNA helix are available, those structural parameters may be determined mostly by viscosity and light scattering or their combination with CD, or more preferably with the pulsed electrooptic techniques.^{27–29} The overall conformation of

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high-molecular-weight DNA (hDNA) is, however, complex because of chain flexibility, depending on the valence of counterion, salt concentration, and other solution properties. Hence, the DNA molecule should be in a rigid rodlike or ellipsoidal shape for a simpler interpretation of the hydrodynamic results.

We have established a method of preparing low-molecular-weight DNA samples with the narrow molecular weight distribution, in large quantities, by sonication.³⁰⁻³² In this work, we report the effect of the chain length of the DNA helix on the CD spectra which sensitively reflect environmental changes. We clarify the relationship between the CD spectra and the alcohol content for hDNA and its sonicated fragment (sDNA) with two objectives: (1) if the DNA length and counterion species affect the ethanol-induced transition and (2) whether trifluoroethanol, a far-UV transparent alcohol,³³ induces the CD spectral change as ethanol. In order to avoid the problem, which would arise from molecular flexibility even for short sDNA,³² we chose an extremely low ionic condition to *straighten out* the chain backbone by electrostatic repulsion.

EXPERIMENTAL

Materials and Preparation of DNA Solution

The characterization of a high-molecular-weight calf thymus DNA (hDNA) sample (Worthington Biochemical Corp., U.S.A.) and a sonicated and fractionated DNA (sDNA) sample was reported in previous papers.³⁰⁻³² The weight-average molecular weights used in this work were 580×10^4 for hDNA and 14.2×10^4 for sDNA. Trifluoroethanol (Aldrich Chemical Co., U.S.A.) was used without further purification and all other chemicals were of reagent grade.

Stock hDNA and sDNA solutions (the concentration of DNA *ca.* 0.5 mg ml^{-1} in 0.2 M NaCl) were prepared as described previously.^{30,34} Each solution was dialyzed for 72 h at 4°C against a buffer solution (pH *ca.* 7), whose

composition was either $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ or $\text{Mg}(\text{CH}_3\text{COO})_2/\text{CH}_3\text{COOH}$ at an ionic strength of 4×10^{-3} ; totally 8 liters of the buffer solution were changed. To prepare a DNA solution for CD measurements, distilled water and then alcohol (ethanol or trifluoroethanol) were added dropwise to the dialyzed solution which was stirred gently at *ca.* 0°C (the content of alcohol was expressed in vol%). The final solution was stood at 4°C for 5–24 h prior to use for CD measurements. No DNA precipitate appeared in the solutions which contained less than 90% ethanol for NaDNA or less than 56% ethanol for MgDNA.

Methods and Measurements

Absorption Spectra and Melting Profile.

The absorption spectra of DNA in water-alcohol mixtures were measured in 1-cm long quartz cells on a Shimadzu UV-250 spectrophotometer. The temperature of a sample solution was varied from 10 to 80°C , by circulating temperature-regulated water. The following characteristic parameters were evaluated from the measured melting profile at 260 nm: the fractional absorbance change, *f*, defined as $(A - A_n)/(A_d - A_n)$, where A_n is the absorbance of a native DNA sample, A_d is the absorbance of heat-denatured DNA sample, and *A* is the absorbance at a given temperature; the hyperchromicity, *H*, defined as $(A_d - A_n)/A_n$; the melting temperature, T_m , defined as the temperature at *f* = 0.5; the breadth of melting transition, ΔT , defined as the difference in temperatures where *f* = 0.9 and 0.1.

Circular Dichroism. The ellipticity, ψ , of a DNA solution was measured at 15°C in a 1-cm long quartz cell on a DICHROGRAPH MARK III-J spectropolarimeter (Union Giken Co.) in the 220–320 nm region. The CD data were accumulated, averaged, and then recorded on a microcomputer. The molar dichroic absorption, $\Delta\epsilon$, in $\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1}$ is related to ψ as

$$\Delta\epsilon = \epsilon_L - \epsilon_R = (4\pi/180)(1/\ln 10)(\psi/cd) \quad (1)$$

where ϵ_L and ϵ_R are the molar absorption coefficients for the left and right circularly-polarized light beams, c is the molar concentration of DNA in mol dm^{-3} , expressed in terms of mononucleotide unit, and d is the optical pathlength in cm.

RESULTS AND DISCUSSION

Thermal Stability of Double-Stranded DNA

Effect of Counterion on Melting. Figure 1a shows the melting curves of sDNA and hDNA in aqueous solutions containing the counterion Na^+ or Mg^{2+} . The fractional absorbance change is plotted against temperature. The f -value cooperatively increases with an increase in temperature, indicating that DNA remains as the double-stranded helix at 15°C , where the CD spectra were measured. The melting temperature T_m is high at 83°C for Mg-hDNA but low at 41°C for Na-hDNA under the same ionic strength (0.4×10^{-3}), whereas the ΔT values are 10°C and 26°C ; thus, MgDNA is thermally denatured more cooperatively than NaDNA. Clearly Mg^{2+} ions stabilize the helical structure more effectively than Na^+ , probably because of the neutralization of negative charges on DNA, which results from the electrostatic attractive force by positively charged counterions.³⁵ It is surprising indeed that H -, T_m -, and ΔT -values were all affected only slightly by the difference in molecular weights (580×10^4 versus 14.2×10^4) of hDNA and sDNA irrespective of the Na^+ or Mg^{2+} counterion. These results indicate that the ultrasonic irradiation simply cuts the hDNA helix laterally to yield shorter fragments but does not denature them; sDNA is optically indistinguishable from hDNA.

Effect of Added Ethanol on Melting. Figure 1b shows the melting profile of NaDNA in water-ethanol mixtures. (The melting curves of MgDNA in water-ethanol mixtures could not be measured at elevated temperatures because of the scattered light by DNA precipitates.) The sDNA helix in NaCl solution melts

Figure 1. (a) Melting profiles of hDNA and sDNA in aqueous salt solution and (b) those of sDNA in the water-ethanol mixed solvent system. The fraction of absorbance change at 260 nm, f , was plotted against temperature, T . The ionic strength was kept at 0.4×10^{-3} with $\text{Mg}(\text{CH}_3\text{COO})_2/\text{CH}_3\text{COOH}$ ($\square$, $\blacksquare$) or $\text{NaH}_2\text{PO}_4/\text{Na}_2\text{HPO}_4$ ($\circ$, $\bullet$). In (a) hDNA sample: $\bullet$, ($H=41\%$, $T_m=41^\circ\text{C}$, $\Delta T=26^\circ\text{C}$); $\blacksquare$, ($H=41\%$, $T_m=83^\circ\text{C}$, $\Delta T=10^\circ\text{C}$). In (a) sDNA sample: $\circ$, ($H=36\%$, $T_m=38^\circ\text{C}$, $\Delta T=27^\circ\text{C}$); $\square$, ($H=41\%$, $T_m=82^\circ\text{C}$, $\Delta T=13^\circ\text{C}$). In (b) hDNA sample: $\blacktriangle$, ($H=47\%$, $T_m=46^\circ\text{C}$, $\Delta T=13^\circ\text{C}$). In (b) sDNA sample: $\circ$, ($H=36\%$, $T_m=38^\circ\text{C}$, $\Delta T=27^\circ\text{C}$); $\triangle$, ($H=44\%$, $T_m=46^\circ\text{C}$, $\Delta T=22^\circ\text{C}$); $\blacksquare$, ($H=21\%$, $T_m=43^\circ\text{C}$, $\Delta T=24^\circ\text{C}$); ∇ , ($H=32\%$, $T_m=38^\circ\text{C}$, $\Delta T=14^\circ\text{C}$). Numerals in (b) indicate the ethanol content in vol%.

cooperatively in the 0–90% ethanol range with a narrower ΔT value at higher alcohol content, but a double-helical structure is obviously maintained at 15°C . In the 0–75% ethanol range, T_m increases with increasing alcohol content. Values of T_m reach a maximum (46°C) at 75% ethanol but decrease gradually thereafter. The factors involved in stabilizing the DNA structure are the hydrogen

bond between bases, the shielding of electrostatic repulsion between charged phosphate groups by counterions, the hydrophobic bonding (base stacking), and the hydrated sheath by water molecules. The shielding may be increased with a decrease in the dielectric constant of the solvent, which may promote the charge neutralization by counterions, the helical structure being stabilized up to 75% ethanol, whereas the decrease in T_m above 75% may be caused by the collapse of the sheath, *i.e.*, the release of hydrated water from the DNA grooves. The fact that value of ΔT decreases, *i.e.*, the melting profile sharpens, with the increase in the alcohol content suggests the increase in the transitional cooperativity.

CD of NaDNA in Water-Alcohol Mixed Solvents

CD Spectra of hDNA and sDNA. Figures

Figure 2. CD spectra of (a) hDNA and (b) sDNA in water-ethanol mixed solvents containing Na^+ as the counterion at 15°C . The molar dichroic absorption: $\Delta\epsilon$ ($=\epsilon_L - \epsilon_R$). The numerals stand for the ethanol content in vol%. The ionic strength: 0.4×10^{-3} .

2a and 2b show examples of the CD spectra of hDNA and sDNA in water-ethanol mixed solvents containing Na^+ as the counterion. In the absence of ethanol, the spectrum of hDNA, which has been assigned to a typical B-form conformation,^{4,16} exhibits a positive band ($\Delta\epsilon \approx 3$) at about 280 nm and a negative band ($\Delta\epsilon \approx -3$) at about 245 nm. As reported by Ivanov *et al.*,⁴ the spectral profile remains unaltered up to 75% ethanol, but then the positive band increases between 75 and 88%. The CD spectra transform from a B-form pattern to another rather nonsymmetric profile, which has been associated with an A-form structure of DNA.^{1,3} A further addition of ethanol to hDNA solution induces a new feature, *i.e.*, the decrease in the intensity of the positive band.²²

The molecular weight of sDNA is only one-fortieth that of hDNA, or 73 vs. 3000 nm in terms of the contour length; yet, the CD spectra of sDNA and the change with increasing alcohol contents are essentially identical with those of hDNA. This is an important finding, *i.e.*, the CD pattern is quite insensitive to the backbone flexibility and the continuous winding of the helical DNA chain in the entire alcohol content. The CD spectrum of sDNA also exhibits an A-form pattern with the positive band at 273 nm in 84% ethanol solution.

Dependence of CD Bands on Ethanol Content. The CD spectra of hDNA and sDNA exhibit a maximum at 270–280 nm and a minimum at 240–250 nm. Figure 3a shows the plots of the molar dichroic absorptions at 273 nm and 242 nm vs. the ethanol content. Both $\Delta\epsilon_{273}$ and $\Delta\epsilon_{242}$ values remain almost unchanged with ethanol up to 70%, but thereafter $\Delta\epsilon_{273}$ values increase cooperatively with the increase in the ethanol content, reaching the highest value ($\Delta\epsilon \approx 16$) at about 84% ethanol. This cooperative increase of $\Delta\epsilon_{273}$ in a narrow range of ethanol content (70–80%) may be interpreted as due either to the relative arrangement between DNA

Figure 3. Dependence of molar dichroic absorption, $\Delta\epsilon$, of (a) NaDNA and (b) MgDNA on ethanol content. Values of $\Delta\epsilon$ at 273 nm ($\circ$, $\bullet$) and at 242 nm ($\triangle$, $\blacktriangle$) for NaDNA and at 277 nm ($\circ$, $\bullet$) for MgDNA were evaluated from the spectra in Figures 2 and 6. Open and closed symbols denote hDNA and sDNA, respectively.

base-pairs^{6,7} or to the solvation on the bases. Although CD results are only circumstantial, such a rearrangement or a destruction of the hydrated sheath can possibly induce a structural transition of the DNA helix backbone, *e.g.*, the transition from B- to A-forms.²² With a further addition of ethanol (84–90%), $\Delta\epsilon_{273}$ decreases, while $\Delta\epsilon_{242}$ remains constant. As often reported by previous workers,^{10,22} the appearance of a maximum and a subsequent diminution in $\Delta\epsilon_{273}$ values is genuine, being difficult to be attributed to precipitation. No precipitates or fibrils were observed for both long (hDNA) and short (sDNA) samples during CD measurements.

Effect of DNA Chain Length on Band Intensities. If the B-form DNA is assumed, the contour lengths of sDNA and hDNA would be 73 and 3000 nm, respectively. The intrinsic viscosities of sDNA and hDNA are 0.87 and 46 dl g^{-1} in 0.2 M NaCl at 25°C, respectively, and the present sDNA molecule is regarded

Figure 4. CD spectra of sDNA in water-TFE mixed solvents containing Na^+ as the counterion at 15°C. The numerals denote the TFE content in vol%. The buffer reagent and ionic strength are the same as in Figure 3.

as the rigid rod.^{30,32} If hDNA was also rigid rodlike, the intrinsic viscosity, calculated from Simha's equation, should be about 10 times larger than the experimental value; thus the hDNA molecule is a flexible coil.³⁶ In spite of the large difference in the shape and size between sDNA and hDNA, the dependence of CD bands of sDNA on alcohol contents is very close to that of hDNA; thus the CD spectra are indifferent to the overall conformation. This fact leads to the conclusion that the helical structure remains much the same for sDNA and hDNA and is affected by alcohol in a parallel manner.

Effect of TFE on CD of DNA. Trifluoroethanol, an excellent solvent transparent in the far-UV region,³³ was examined if it also induces the same or a like spectral change which was observed for ethanol. Figure 4 shows some CD spectra of sDNA in water-TFE mixtures containing Na^+ as the counterion. In the 0–70% TFE solutions, the CD spectra of Na-sDNA remain hardly influenced and a further addition of TFE induces the spectral change in a similar manner, as was observed for ethanol; the spectral profile is again assignable to a B-form conformation (*cf.* Figure 2b).

Figure 5 shows the dependence of the

Figure 5. Dependence of molar dichroic absorption, $\Delta\epsilon$, on alcohol content. Values of $\Delta\epsilon$ at 273 nm ($\circ$, $\bullet$) and at 242 nm ($\triangle$, $\blacktriangle$) were evaluated from the spectra in Figure 4. Alcohols: TFE (closed symbols); ethanol (open symbols).

positive and negative CD bands of sDNA on the alcohol content on a comparative basis; $\Delta\epsilon_{273}$ values in TFE or ethanol remain constant ($\Delta\epsilon \approx 3$) in the 0–70% alcohol range, cooperatively increasing with a further addition of alcohol. The maximum $\Delta\epsilon (\approx 18)$ is reached at about 82% TFE and 84% ethanol, the CD band decreasing again thereafter. The ability and behavior of TFE to induce the change in CD spectra are identical with those of ethanol. This conclusion suggests that the possible structural transition induced to DNA in the presence of 70–90% alcohol would also be identical. Hence, the use of TFE as an ultraviolet solvent is recommended for the spectrophotometric and circular dichroic studies of the conformational changes of polynucleotides and nucleic acids.

CD of MgDNA in Water–Ethanol Mixed Solvents

The CD Spectra of hDNA and sDNA. Figure 6 shows some CD spectra of hDNA and sDNA in water-ethanol mixtures containing Mg^{2+} as the counterion. In the 0–40% ethanol range, the CD spectra of hDNA and sDNA are essentially the same as those assigned to the B-form conformation, closely resembling the spectra of NaDNA (*cf.* Figure 2). A further addition of ethanol results in an

Figure 6. CD spectra of (a) hDNA and (b) sDNA in water-ethanol mixed solvents containing Mg^{2+} as the counterion at 15°C. The ionic strength: 0.4×10^{-3} with $\text{Mg}(\text{CH}_3\text{COO})_2/\text{CH}_3\text{COOH}$. The numerals denote the ethanol content in vol%. Note that slight precipitates appeared in the solutions of hDNA and sDNA in 70% ethanol after standing overnight.

increase in the positive band intensity, indicating that some structural changes are induced to hDNA and sDNA. The CD spectral profiles of Mg-hDNA and Mg-sDNA in 50–70% ethanol are similar to those observed for NaDNA at a higher alcohol content of about 82% (*cf.* Figure 2). With respect to the ethanol-induced spectral change, Mg-sDNA behaves identically with Mg-hDNA; again, the molecular weights of sonicated and unsonicated MgDNA samples have no influence on the CD spectra.

The effect of ethanol on the magnitudes of the positive band at 277 nm and the negative band at 246 nm are shown in Figure 3b. A comparison of plots shown in Figures 3a and 3b indicates that the lesser amount of ethanol is capable of inducing the CD spectral change for MgDNA.

CONCLUDING REMARKS

The condition for CD spectral change of hDNA and sDNA induced by alcohol is determined. The most important finding is that the induced CD change is independent of the molecular weight or chain length, flexibility or rigidity (*cf.* Figure 2). Hence, the secondary helical structure of sDNA probably remains the same as that of hDNA, however unknown the structure may be. A UV-transparent TFE also induces the CD spectral change in the same manner as ethanol. Hence, TFE will serve to study an alcohol-induced B-A transition spectrophotometrically in the far-UV region. Contrary to a wide belief, it is actually difficult to identify whichever the secondary structure of DNA is present in aqueous solution, on the basis of the CD spectrum alone, since the CD is a short-range optical phenomenon and insensitive to the chain length or the chain flexibility. Thus, it is necessary to clarify simultaneously the overall double-helical chain conformation, the disposition of the base pairs with respect to the helical axis, and the chain length of nonflexible DNA fragments. The transient pulsed electric birefringence^{37,38} and dichroism³⁷ techniques may be utilized fully for this purpose. By so doing, therefore, the relationship between the CD spectra and the electrooptical properties can be established to elucidate the precise secondary structure of DNA in solution.

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