Unwinding of double-stranded DNA helix by dehydration
(DNA conformation/hydration/thermodynamics/gel electrophoresis)

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ABSTRACT

Conformational changes of the double-stranded DNA helix in response to dehydration were investigated by monitoring, by agarose gel electrophoresis, the linking number of covalently closed circular DNA generated by ligation of linear DNA in the presence of different organic solvents or at different temperatures. It was found that: (i) The DNA helix unwinds upon addition of certain organic solvents or elevation of temperature. (ii) The conformational change observed under the experimental conditions is a continuous process in response to the organic solvent concentration. (iii) The $\Delta H$ of unwinding one linking of the DNA helix is constant at approximately 12.2 $\pm$ 0.4 kcal/mol (1 kcal = 4.184 kJ); the corresponding $\Delta S$ and $d\Delta S/dn$ are 2$kR$ and 2$kR$, respectively. (iv) $d\Delta S/dn$, like $k$, is inversely proportional to the number of base pairs in DNA. (v) Double-stranded DNAs of different chain lengths have average $\Delta S = 33$ cal/mol-K for unwinding one linking under the experimental conditions; this corresponds to 127 $\pm$ 14 base pairs per "relative linking."

Hydration and dehydration of DNA has been studied for the past two decades (see refs. 1–3 for reviews). By using the analytical ultracentrifuge, DNA has been found to be dehydrated upon increase of temperature (4–6). It has been reported that DNA denaturation occurs in a number of organic solvents due to dehydration (7–11). Extensive studies of the effect of dehydration on DNA conformation have been carried out using circular dichroism (CD). The conformation of DNA in a thin film (12) or in aqueous organic solvent mixtures (13–18) has been correlated to the spectral change of CD. The decrease of the CD band at 270 nm in the presence of organic solvent has been attributed to the presence of C form DNA (C DNA) (12–18). On this basis, C DNA has been assumed to prevail at high salt concentrations (19–24) or under low temperature conditions (in the premelting region) (25, 26). However, the presence of C DNA is not supported by x-ray diffraction studies of the DNA in concentrated salt solutions (27) or in organic solvent/water mixtures (27, 28). DNA condensed from various ethanol/water solutions also failed to show the x-ray diffraction pattern of the C form (29).

Although a considerable amount of data has been accumulated, no conclusive results have been published to relate hydration of the DNA to its helical structure in solution. Depew and Wang (30) and Pulleyblank et al. (31) showed that the thermal effect on the DNA base-pair twisting angle could be accurately determined by agarose gel electrophoresis of covalently closed circular DNA generated at different temperatures. This type of system is useful for studying DNA structure in solution. For example, using a gel electrophoresis technique, Wang (32, 33) demonstrated that the DNA double helix has 10.4 $\pm$ 0.1 base pairs per helical turn in dilute solution. [It should be pointed out that Zimmerman and Pfeiffer (34) found 9.9 base pairs per turn for DNA in concentrated solution.] In the present studies, we have explored the effect of hydration on the helical structure of DNA in solution, using phage T4 DNA ligase to covalently close the linear plasmid DNA (pBR322 and pNT7) at various levels of dehydration by addition of methanol, ethanol, glycerol, ethylene glycol, dimethyl sulfoxide, and tetrahydrofuran or exposure to different temperatures. The thermodynamic properties of superhelical formation and the conformational changes of DNA resulting from the different states of dehydration are discussed. An argument is made for the interpretation of the CD spectra of DNA in organic solvent/water mixtures and those in the premelting temperature regions. The implications of DNA hydration and dehydration and their effects on DNA–protein and DNA–drug interactions are discussed.

MATERIALS AND METHODS

Closed-circular plasmid DNA (pBR322 or pNT7), purified from Escherichia coli host cells, was cut by endonuclease EcoRI (Bethesda Research Laboratory, Rockville, MD) to the linear form. Linear plasmid pBR322 DNA (0.2 $\mu$g) was then sealed at 15°C to form the covalently closed circular form, using T4 DNA ligase (2 units) (Bethesda Research Laboratory) in the presence of various concentrations of organic solvents—methanol, ethanol, glycerol, ethylene glycol, dimethyl sulfoxide, and tetrahydrofuran. The ligation mixture (40 $\mu$l) was in 66 mM Tris-HCl (pH 7.6)/6.6 mM MgCl2/1 mM KCl/1 mM dithiothreitol. Similar reactions were conducted at 0°C, 15°C, 30°C, and 38°C without addition of organic solvent for pBR322 and pNT7 DNA. The DNA ligation products, after ethanol precipitation, were dissolved in 10 $\mu$l of solution containing 10% (vol/vol) glycerol, 0.1% sodium dodecyl sulfate, and 0.01% bromophenol blue and loaded onto a 7% agarose gel (30 $\times$ 30 $\times$ 0.3 cm). After electrophoresis at about 2 V/cm in Peacock's buffer (90 mM Tris/90 mM boric acid/2.5 mM Na2EDTA, pH 7.8) for 20 hr, the gel was stained with ethidium bromide (0.5 $\mu$g/ml) in the dark for 1 hr. Photographs of the gel were taken with a Polaroid camera under UV illumination. The negative of the gel photograph was scanned by a Gilford spectrophotometer to determine the relative population of molecules of each closed circular DNA, and also to estimate the Gaussian center of the DNA distribution.

In order to resolve the closed circular DNA bands near the open circular DNA position, a second-dimension electrophoresis, 90° to the first dimension, was conducted in the same buffer, but containing 0.03 $\mu$M ethidium bromide, at 1.25 V/cm for 18 hr.

Theoretical and experimental research of the topology of circular DNA has been considerably developed recently (30–33, 38).
Consider two linear DNAs, a and b, that differ only in their average conformations in the ligation mixture. Their corresponding ligated closed-circular forms have $Lk_a$, $Tw_a$, and $Wr_a$, and $Lk_b$, $Tw_b$, and $Wr_b$ as the linking, twisting, and writhing numbers, respectively. The respective topological parameters are $Lk'_a$, $Tw'_a$, and $Wr'_a$, and $Lk'_b$, $Tw'_b$, and $Wr'_b$ when they are transferred into the electrophoresis system. From the relationship

$$Lk = Tw + Wr$$

(35-37) for closed circular DNA, the following equations are established:

$$Lk_a - Lk_b = (Tw_a - Tw_b) + (Wr_a - Wr_b)$$

$$Lk'_a - Lk'_b = (Tw'_a - Tw'_b) + (Wr'_a - Wr'_b).$$

Because the linking number of the closed-circular DNA is independent of the conditions, i.e., $Lk_a = Lk'_a$ and $Lk_b = Lk'_b$, from Eq. 2 and 3 we obtain

$$(Lk'_a - Lk'_b) = (Tw'_a - Tw'_b) + (Wr'_a - Wr'_b).$$

Thus, the difference between the conformations of two linear DNAs in the ligation mixtures $(Tw'_a - Tw'_b) + (Wr'_a - Wr'_b)$ can be determined by monitoring the difference between the linking numbers of their respective closed-circular DNA $(Lk'_a - Lk'_b)$ under the electrophoresis conditions (30, 38). Hereafter, the term "linking" or "equivalent linking number" used in the text refers to the conformation of the linear DNA, even though it is originally defined as a topological parameter for closed-circular DNA (35-37). Also, in this paper, "winding" or "unwinding" of a DNA duplex refers to changes of $(Tw + Wr)$, while "twisting" or "untwisting" refers to changes of $Tw$ only.

The equilibrium constant $K$ for unwinding one equivalent linking number or one turn of the DNA helix—i.e., $Lk_a \rightarrow Lk_{a-e}$—at a given temperature was determined by using the ratio of the population of the linear DNAs with one equivalent linking number difference $[(Lk_{a-e})/(Lk_{a-e})]$. They were observed as two neighboring closed-circular DNA bands in gel electrophoresis. From the slope and the intercept of the van't Hoff plot of $\ln K$ vs. $1/T$ ($T$ being absolute temperature), $\Delta H$ and $\Delta S$ were obtained, respectively. In a similar way, the thermodynamic parameters for unwinding turns of DNA helix—i.e., $Lk_a \rightarrow Lk_{a-e}$—were determined.

RESULTS

Unwinding of DNA Helix by Organic Solvents. As shown in Fig. 1, when the methanol concentration in the ligation mixture

![Fig. 1. Gel electrophoresis patterns of closed-circular pBR322 DNA samples generated at different methanol concentrations. The methanol concentrations are, in % (vol/vol), 0, 5, 10, 15, 20, 25, 30, 35, and 40 for samples in lanes A, B, C, D, E, F, G, H, and I, respectively. OC and L stand for open-circular and linear DNA, respectively.]

increases, the Gaussian center of the ligated discrete closed-circular DNA bands moves up to the open-circular DNA band position and then descends. Such a shift of the Gaussian center was determined by tracing the negative of the gel photograph (Fig. 2). In one-dimensional gel electrophoresis, the resolution

![Fig. 2. Tracings for the gel samples E and F in Fig. 1. OC and L denote the open-circular and the linear DNA peaks, respectively. The Gaussian center (shown by an arrow) shifts by -1.7 linkings from sample E to sample F.]

![Fig. 3. Dependence of the relative equivalent linking number $\Delta Lk$ of DNA on the organic solvent concentration. $\Delta Lk = 0$ is set for the standard condition: 0.2 M NaCl, 37°C (refs. 39 and 40), with an adjustment of +2.7 linkings for the presence of 6.6 mM Mg$^2+$ (unpublished data). The vertical axis on the right is expressed in terms of the base pair twisting angle (assuming no change for the writhe of DNA; i.e., $\Delta Wr = 0$) with 34.6° corresponding to 10.4 base pairs per helical turn (refs. 32 and 33).]

of the bands near the open-circular DNA position is poor and the assignment of positive or negative supercoiling of the DNA is unclear. This problem is solved by using two-dimensional gel electrophoresis (discussed in the next section). Thus the Gaussian center of each DNA sample can be determined within ±0.2 turns. Ethanol, glycerol, ethylene glycol, and dimethyl sulfoxide all show a similar unwinding of the DNA helix, although to different degrees. Fig. 3 shows the dose response of the Gaussian center shift of linear DNA for these organic solvents. The shifts of the Gaussian center reveal that the conformational change of DNA is continuous in response to the concentrations of the organic solvents. It shows a clear tendency for the DNA helix to unwind by as much as 9–15.5 equivalent linking numbers. Assuming no bending of DNA occurs—i.e., ΔWr = 0—this unwinding corresponds to base pair untwisting from 34.86° to 33.58°, on the basis of Wang's value of 10.4 base pairs per helical turn (32, 33). It is conceivable that the DNA helix would unwind further at higher organic solvent concentrations, but these are not accessible in the present system because the ligase loses its activity. More hydrophobic organic solvents such as tetrahydrofuran (data not shown because of difficulty of controlling the concentration in the experiment due to its volatility) exhibit a similar effect. Thus, such unwinding of the DNA duplex caused by dehydration is likely to be a common phenomenon in organic solvents (10).

Two-Dimensional Gel Electrophoresis. Fig. 4 shows the DNA band pattern of a two-dimensional gel electrophoresis of samples containing positive and negative supercoiled (relative to the open-circular DNA) DNAs. The two-dimensional gel pattern of several individual samples is shown in Fig. 5. In the first-dimension electrophoresis, most of the positive and the negative superhelical DNAs migrate faster than the open-circular DNA. During the second-dimension electrophoresis, the originally positive superhelical DNA in the first dimension binds with the ethidium bromide added in the buffer and gains positive superhelicity so that it migrates faster, while the originally negative superhelical DNA loses negative superhelicity and migrates slower (38). Because of such differentiation of the mobility of DNA:ethidium bromide complexes, the originally positive and the negative supercoiled DNAs are then separated on both sides of the arch formation and are clearly distinguishable in the second dimension. The positive superhelical DNA bands are on the right-hand side of the arch, while the negative ones are on the left-hand side. The top band in the first-dimensional gel electrophoresis contains open-circular DNA and relaxed closed-circular DNAs. They are resolved in the second-dimension gel electrophoresis. It is clear that the open-circular DNA in the first dimension migrates faster than the most relaxed closed-circular DNA bands (Figs. 4 and 5). This may be due to the fact that open-circular DNA has higher molecular flexibility so that it passes through the pores of the gel easier. The relative mobility of the positive and the negative superhelical DNAs is affected by the gel temperature (38), the ionic strength in the gel (which forms a gradient from cathode to anode), and the molecular dimensions (determined by the linking and the writting numbers). With two-dimensional gel electrophoresis and one-dimensional electrophoresis at different temperatures or in the presence of different concentrations of ethidium bromide (38), the measurement of the Gaussian center of each DNA sample was accurate within ±0.2 turn.

Thermal Effect on the Unwinding of DNA Helix. Depew and Wang (30) and Pulleyblank et al. (31) reported that the unwinding of the base pair of DNA is about −0.012° per base pair.
The decrease or of the relative for each mol-K for the studied conditions. We have found this is not the case. Increasing the salt concentrations (39) or lowering the temperature (30, 31) of the sample does cause unwinding of DNA. However, on the contrary, we observe that the DNA helix is unwound by organic solvents despite the fact that this unwound DNA shows CD spectra similar to those observed in high salt concentrations or at low temperature (13-26). The previous interpretation of CD spectra for C DNA in the 270-nm region is thus questionable. We believe that multiple factors such as DNA conformation (Tw + Wr), ionic environment, and the state of solvation, as well as the specific forms from the B to the C form upon introduction of organic solvents (13-18) or high salt concentration (19-24) into the system or upon lowering of the temperature (25, 26) of the system.

The physical representation of a DNA duplex at the state of zero entropy for unwinding (i.e., \( \Delta S = 0 \)) is of interest. In the previous paragraph, we designated this state as the reference point for counting the relative linking number of a DNA. Under the experimental conditions, we obtained \( \Delta S = 35 \pm 5 \text{ cal/mol-K} \) for unwinding one linking, and the corresponding \( n \) values for each DNA studied are listed in Table 1. For instance, the relative linking number of pBR322 DNA is 33. Our calculations also show that there are about 127 \pm 14 base pairs per relative linking in each DNA studied (Table 1). Other implications of the relative equivalent linking of a DNA duplex (linear or nicked circular) for its structures and conformations are still unclear.

**Form C DNA in Solution?** It has been suggested from the decrease of the CD band in the 270-nm region that DNA transduces from the B to the C form upon introduction of organic solvents (13-18) or high salt concentration (19-24) into the system or upon lowering of the temperature (25, 26) of the system. We have found this is not the case. Increasing the salt concentrations (39) or lowering the temperature (30, 31) of the sample does cause unwinding of DNA. However, on the contrary, we observe that the DNA helix is unwound by organic solvents despite the fact that this unwound DNA shows CD spectra similar to those observed in high salt concentrations or at low temperature (13-26). The previous interpretation of CD spectra for C DNA in the 270-nm region is thus questionable. We believe that multiple factors such as DNA conformation (\( \Delta U + \Delta W \)), ionic environment, and the state of solvation, as well as the specific forms from the B to the C form upon introduction of organic solvents (13-18) or high salt concentration (19-24) into the system or upon lowering of the temperature (25, 26) of the system.
nal properties (e.g., the $n \to \pi^*$ transition), etc., may be responsible for the CD change in this region. X-ray diffraction studies (27–29) have also concluded that there is no transition to the C form DNA.

**DISCUSSION**

Unwinding of the DNA helix has been believed to be a characteristic behavior of DNA-intercalating drugs since it was reported by Lerman (43) and Keller (38). However, winding or unwinding of DNA by nonintercalating agents such as metallic ions (39), DNA gyrase (44–46), DNA polymerase (47), and histones (48–50) has also been observed. Our finding of DNA unwinding by organic solvents (nonintercalating agents) indicates that dehydration leads to a structural perturbation (i.e., decrease of the linking number) similar to that caused by the intercalating agents. However, there is evidence that organic solvents (7–10) and elevation of temperature, as opposite to the intercalating agents, tend to destabilize or denature (in the severe cases) the DNA double helix in solution. This suggests that the water activity in the microenvironment of DNA is related to the stability of the DNA duplex and is expressed in terms of DNA conformation ($Tw + Wr$, or supercoiling). The degree of DNA supercoiling, which is affected by dehydration as well as protein binding, is probably an important factor in the biological mechanisms involving DNA such as replication, transcription, and the packing of chromosome or phage head particles, etc. For example, Wang observed that the binding of enzymes (DNA and RNA polymerases, DNase, etc.) to DNA is stronger when DNA is more supercoiled (51).

Another important aspect of DNA hydration is the interactions between DNA and drugs, including carcinogens, mutagens, and chemotherapeutic agents. Perturbation of the water activity around DNA and the consequent structural transformation may result in a change in binding efficiency as well as binding specificity of the chemical agents. In turn, the structure and the stability of the DNA-drug complexes are influenced by the hydration conditions of the system. We have found that the recognition of antibodies against the DNA-benz[a]pyrene metabolite complex increases drastically when an organic solvent, used in the present study, is added to unwind the DNA (unpublished data). This phenomenon is not observed for the control experiment using the heat-denatured DNA-benz[a]-pyrene metabolite complex. We speculate that the actions of DNA repair enzymes may also be related to changes in the conformation at the target sites due to changes in hydration state induced by an abnormal structure (52).

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