A Mechanochemical Study of MgDNA Fibers in Ethanol-Water Solutions

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ABSTRACT Highly oriented calf-thymus MgDNA fibers, prepared by a wet spinning method, were studied with a simple mechanochemical set-up. The relative fiber length, L/L_0, was measured with the fibers submerged in ethanol-water solutions. In one type of experiment L/L_0 was measured as a function of ethanol concentration at room temperature. No substantial decrease in L/L_0 with increasing ethanol concentration was observed, indicating that MgDNA fibers stay in the B form even when the water activity is very low. For low ethanol concentrations the fiber structure is stable and does not dissolve even at very high water activities. In a second type of experiment, the heat-induced helix-coil transition was manifested by a marked contraction of the fibers. The transition temperature decreases linearly with increasing ethanol concentration between 52 and 68% ethanol. At higher ethanol concentrations the helix-coil transition temperature increases due to strong aggregation within the DNA fibers, and above 77% ethanol the fibers do not contract at all, not even at the upper temperature limit of the experiments, ~80°C. This behavior is discussed with reference to dried DNA and the P form of DNA. The helix-coil transition temperature of the MgDNA fibers in 70% ethanol does not show any dependence on the $MgCl_2$ concentration. It is shown that the Poisson-Boltzmann cylindrical cell model can account qualitatively for this lack of salt dependence.

INTRODUCTION

Numerous physicochemical studies of DNA have been performed in order to elucidate the influence of the water activity and the type of counterion on the structure of DNA. The water activity may be decreased in an aqueous DNA solution by the addition of nonelectrolytes such as ethanol. In solid DNA fibers it is possible to vary the water activity by changing the relative humidity of the ambient atmosphere. It is well known that at low water activities the structural form of the DNA depends on the identity of the counterions (Marvin et al., 1961; Ivanov et al., 1973, 1974, 1983; Malenkov et al., 1975; Zimmerman and Pheiffer, 1979; Skuratovskii and Bartenev, 1979). For example, Skuratovskii and Bartenev used solid phage T2 and calf-thymus Li- and MgDNA fibers for x-ray investigations over a broad range of relative humidities and found that Li(B)DNA transforms to C-DNA, whereas Mg(B)DNA stays in the B form at low water activity. This is in contrast to NaDNA, for example, which undergoes a B-A transition at low relative humidity.

The interaction between magnesium, an important physiological cofactor also taking part in the compactization of chromatin, and DNA has been studied mainly in aqueous DNA solutions. The Mg^{2+} ions have then been found to interact with DNA by a predominantly electrostatic mechanism (Record et al., 1981), and the fraction of bound Mg^{2+} ions has been found to be in accordance with the counterion condensation model (Manning, 1978) in solutions of low ionic strength (Record, 1975). All the Mg^{2+} ions are expected to be fully hydrated–not site-specifically bound, but rather territorially associated to DNA (condensed) (Manning, 1978). Possibly, a small fraction of Mg^{2+} ions are specifically coordinated to the bases (Murk Rose et al., 1980; Braunlin et al., 1991, 1992).

Based on x-ray work performed on DNA fibers, Skuratovskii and co-workers (Skuratovskii and Bartenev, 1979; Bartenev et al., 1983), inspired by the work of Ivanov et al. (1973), considered the binding of Mg^{2+} and alkali ions in the minor groove, where the spatial concentration of negatively charged phosphate groups is highest and where a direct coordination with the bases is possible. It was suggested that the smaller ions Mg^{2+} and Li^+ are incorporated in the minor groove of B-DNA in such a way that their interaction with the base atoms should proceed via water bridges (Bartenev et al., 1983). In contrast, Na^+, K^+, and Cs^+ would be able to coordinate directly with the bases. The absence of direct coordination between Mg^{2+} and base atoms has been demonstrated by a monocrystal x-ray diffraction study of a B-DNA dodecamer (Drew and Dickerson, 1981). Further, according to a recent molecular dynamics simulation (York et al., 1992), Mg^{2+} ions have a greater tendency to interact also with the phosphate anions as fully hydrated cations rather than by direct coordination.

The influence of the Mg^{2+} concentration on the stability of DNA has been subject to a number of studies. Increasing the Mg^{2+} concentration in a dilute aqueous MgDNA solution has been observed to stabilize the double helix and thus increase the helix-coil transition temperature, T_m (Eichhorn and Shin, 1968). In studies where higher DNA concentrations were used, T_m was observed to pass through a maximum as the Mg^{2+} concentration increased (Baba and Kagemoto, 1974; Ott et al., 1975; Vlasov et al., 1991).

Received for publication 1 March 1993 and in final form 20 December 1993.

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0006-3495/94/03/810/10 $2.00

If ethanol is added to an aqueous DNA solution, T_m decreases approximately linearly with increasing ethanol concentration with both Na^+ counterions (Srivastava et al., 1979) and Mg^{2+} counterions (Matsuoka et al., 1990). Eventually, at high enough ethanol concentration, the DNA precipitates.

Here we present an investigation of the influence of the water activity and the $MgCl_2$ concentration on the conformation and thermal stability of highly oriented calf-thymus MgDNA fibers submerged in ethanol-water solutions of various concentrations. The mechanical properties of the DNA fibers are used as a method to determine T_m and to detect conformational transitions (if any). The fibers were prepared by a wet spinning method (Rupprecht, 1966, 1970b) and, in contrast to dissolved DNA, can be studied in very high ethanol concentrations.

In an early mechanochemical study (Rupprecht, 1970a) it was observed that a force was built up temporarily in wet-spun LiDNA fibers submerged in ethanol-water solutions when the temperature was increased. This force was assumed to originate from the helix-coil transition of the DNA, thus reflecting the shorter average end-to-end distance in the coil state as compared to the double helical state. To enable measurements of the relative length of DNA fibers in ethanol-water solutions, a simple mechanochemical set-up was constructed (Rupprecht and Piskur, 1983). This set-up is used in the present investigation. The influence of different types of counterions can be deduced from a comparison of the results with an analogous mechanochemical study of Li-, Na-, K-, and CsDNA fibers published elsewhere (Rupprecht et al., 1994).

MATERIALS AND METHODS

High molecular weight calf-thymus DNA (Pharmacia Biotech Norden AB, Sollentuna, Sweden) was used without further purification. 99.5% (v/v) ethanol was purchased from Kemetyl AB (Stockholm, Sweden). All ethanol concentrations quoted in this paper are given in percent by volume. Oriented fibers of NaDNA were prepared by a wet spinning method (Rupprecht, 1966, 1970b) that was slightly modified (Rupprecht, 1970a). The cylinder with the spun NaDNA was bathed for several days in two successive baths containing 75% ethanol and 1.0 M $MgCl_2$ in order to exchange Na^+ for Mg^{2+}. This exchange of univalent to divalent ions is expected to be very efficient. This procedure was necessary because direct spinning of MgDNA was not possible due to gelation (Rupprecht et al., 1991). About 70 equivalent fiber bundle samples, typically 12–15 cm, could be taken from the cylinder. Each fiber bundle consisted of about 5760 DNA fibers (the figure is based on the use of eight sweeps and a spinneret with 720 holes in the wet spinning process), and the cross-section of the fiber bundle contained about 3.3×10^8 DNA helices. The diameter of an individual fiber in the fiber bundle in 70% ethanol was approximately 0.7 μm.

Much thicker fiber bundles were spun for x-ray diffraction. After exchange to MgDNA as described above, the cylinder was stored in 75% ethanol with 0.15 M $MgCl_2$. A 2 cm long piece of the fiber bundle was cut off and pushed into a thin-walled capillary tube with an inner diameter of 1.5 mm. All of these operations were performed with the fiber bundle in 75% ethanol with 0.15 M $MgCl_2$. The x-ray diffraction pattern was somewhat diffuse, but the reflections showed the presence of B-DNA. In an earlier preparative paper (Rupprecht et al., 1991) a distinct x-ray diffraction pattern of wet-spun MgDNA fibers equilibrated in 75% RH and showing the B form can be found. Also, as was noted in the Introduction, Skuratovskii and Bartenev found that MgDNA in fibers is in the B form when equilibrated in relative humidities ranging from 65 to 95% (Skuratovskii and Bartenev, 1979). From an x-ray diffraction study on DNA fibers either equilibrated at a certain relative humidity or in an ethanol-water solution, Wyckoff (1955) found that an 85% ethanol solution is roughly equivalent to 75% relative humidity and that 70% ethanol is roughly equivalent to 92% relative humidity. Thus, all things considered, we feel confident that our MgDNA in 75% ethanol is in the B form.

The mechanochemical set-up is shown in Fig. 1. The DNA fiber bundle was mounted by winding its ends a few turns around the glass hook and the Pt-weight, respectively. The weight serves the dual purpose of keeping the fiber bundle straight and being a marker when determining its length. To keep the fiber bundle satisfactorily straight a 30-mg weight was needed, which corresponds to a force of about 9×10^{-13} N/helix. In water solution this force has been found to straighten a DNA helix to about 90% of its contour length (Smith et al., 1992). This force may be compared with the force needed to prevent a B-A transition in NaDNA fibers pulled from a gel, 7.5×10^{-11} N (Harmouchi et al., 1992), which is about 80 times larger. The reference length, L_0, was determined in 75% ethanol and 0.15 M $MgCl_2$ at room temperature after permitting the fiber bundle to relax for at least 24

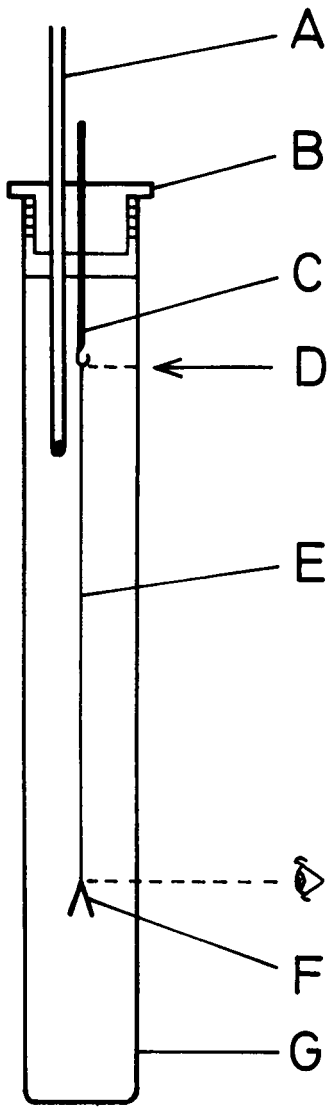

FIGURE 1 The mechanochemical set-up. (*A*) Thermometer. (*B*) Teflon-plug (cross-section). (*C*) Glass hook. (*D*) Upper index of the measuring cylinder scale. (*E*) DNA fiber bundle. (*F*) V-shaped Pt-weight. (*G*) 250-ml glass measuring cylinder with ethanol-water solution.

h in the bath. The use of a simple device with two parallel hairs reduced parallax errors. This set-up was used for two kinds of mechanochemical experiments.

Method 1. Observation of relative length, L/L_0, at room temperature as a function of ethanol concentration

After the determination of L_0 in the reference bath, the MgDNA fiber bundle was placed in a measuring cylinder containing a bath of 75% ethanol and 0.01 M $MgCl_2$. Some fiber bundles exhibited a small (up to 2%) contraction at this point. Thereafter, the Teflon-plug with glass hook and attached DNA fiber bundle was lifted and brought into another measuring cylinder with a different ethanol concentration but the same salt concentration (0.01 M). This salt concentration was chosen in order to use the same salt concentration as in the mechanochemical study of DNA fibers with various alkali counterions (univalent) (Rupprecht et al., 1994). The DNA fiber length, L, changed immediately and reached a constant value within a few hours. By performing several parallel experiments, L/L_0 could be determined as a function of ethanol concentration. Because the axial rise per base pair in B- and A-DNA is different, an ethanol-induced B-A transition should be noticeable if it occurs (Rupprecht and Piskur, 1983; Rupprecht et al., 1994).

Method 2. Observation of relative length, L/L_0, as a function of temperature to obtain information on the helix-coil transition

As before, the reference length, L_0, was determined in a bath containing 75% ethanol and 0.15 M $MgCl_2$. Thereafter, the fiber bundles were transferred to baths with various ethanol and $MgCl_2$ concentrations as described below. The measuring cylinder was immersed in a thermostated bath, the temperature of which was increased linearly with time (0.10°C/min). The heat-induced helix-coil transition of the DNA fibers was followed by measuring the fiber bundle length, L, as a function of temperature. Because the average end-to-end distance in the coil state is much shorter than in the helix state, the helix-coil transition is manifested by a marked contraction of the DNA fiber bundle. A representative example of such a L/L_0 versus temperature curve is presented in Fig. 2. Some premelting contraction is also seen. The measurements were speeded up by running up to 10 mechanochemical set-ups in parallel in the water bath.

Using method 2, the dependence of the DNA fiber helix-coil transition temperature, T_m, on the ethanol concentration was studied. The $MgCl_2$ concentration in the ethanol-water solutions was kept constant (0.01 or 0.4 M). The salt dependence of T_m was studied as well, using a constant ethanol concentration of 70%. The $MgCl_2$ concentration in the measuring cylinders then ranged from 0.00004 to 0.4 M. For the lower salt concentrations the bath was renewed 3 times before starting the heating to ensure that salt brought with the fiber from the previous bath did not increase the salt concentration. The helix-coil transition temperature, T_m, and the helix-coil transition width, ΔT, are defined as follows:

$$T_m = \int_{T_1}^{T_2} T\left(-\frac{d\theta}{dT}\right)dT \approx \sum_{k=k(T_1)}^{k(T_2)} \frac{t_k(R_{k+1} - R_k)}{R_{k(T_2)} - R_{k(T_1)}} \quad (1)$$

$$\Delta = \left[\int_{T_1}^{T_2} (T - T_m)^2\left(-\frac{d\theta}{dT}\right)dT\right]^{1/2} \approx \left[\sum_{k=k(T_1)}^{k(T_2)} \frac{(t_k - T_m)^2(R_{k+1} - R_k)}{R_{k(T_2)} - R_{k(T_1)}}\right]^{1/2} \quad (2)$$

where T_1 and T_2 are the temperatures between which the degree of helicity, θ, is assumed to change from 1 to 0. The integrals were evaluated as the respective sums, where $t_k = (T_k + T_{k+1})/2$, and R_k and T_k are the relative length, L/L_0, and the temperature, T, at the kth data point, respectively. The sums are normalized between T_1 and T_2, and it is assumed that $d\theta/dT = d(L/L_0)/dT$. The equations above are analogous to the expressions used in spectroscopic studies of melting of DNA in solution (Wada et al., 1980).

When calculating T_m and ΔT some iteration was required. An initial value of T_m was estimated by eye, and the T_2 value was set where the main melting had stopped (where L/L_0 changed with less than 0.01/°C). We kept this T_2 value constant during the iteration. The premelting contraction made the T_1 value rather approximate and was set, therefore, at a temperature displaced with an equal amount to the left (low temperature) of the initial T_m as T_2 is displaced to the right (high temperature). Inserting these numbers in Eq. 1 gave a second T_m value that was used to obtain an improved T_1 value, etc.

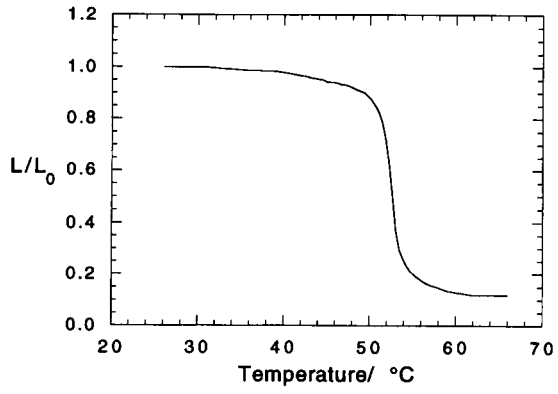

FIGURE 2 The relative length, L/L_0, of a MgDNA fiber bundle in 70% (v/v) ethanol and 0.01 M $MgCl_2$ as a function of temperature.

RESULTS AND DISCUSSION

Method 1

Fig. 3 *A* shows the variation of relative length, L/L_0, with ethanol concentration for MgDNA fiber bundles. The $MgCl_2$ concentration was 0.01 M. In an additional experiment, L/L_0 of the MgDNA fibers was seen to be independent of the $MgCl_2$ concentration in the bath (not shown).

In our mechanochemical work with alkali counterions (Rupprecht et al., 1994), a decrease in L/L_0 of the DNA fiber bundle was observed with increasing ethanol concentration. With the exception of LiDNA, the behavior was interpreted as a B-A transition of the DNA, reflecting the difference in axial rise per base pair (3.38 Å and 2.56 Å for B- and A-DNA, respectively). As can be seen in Fig. 3 *A*, L/L_0 of MgDNA fiber bundles is practically unaffected by a change to baths with ethanol concentrations between 60 and 95%. The absence of a change of L/L_0 indicates that no change of conformation occurs in this range of ethanol concentration. Skuratovskii and Bartenev (1979) explained the reluctance of MgDNA to change conformation by the strong binding of Mg^{2+} ions to the minor groove of B-DNA where the spatial concentration of phosphate groups is highest (Ivanov et al., 1973). The minor groove narrows with decreasing water activity so that one Mg^{2+} ion may neutralize both the phosphate groups situated at the "edges" of the minor groove and thus stabilize a twisted B form. The formation of this twisted B form could explain the small contraction observed at the highest ethanol concentrations.

When the MgDNA fiber bundles were submerged in baths containing less than 60% ethanol, a substantial contraction

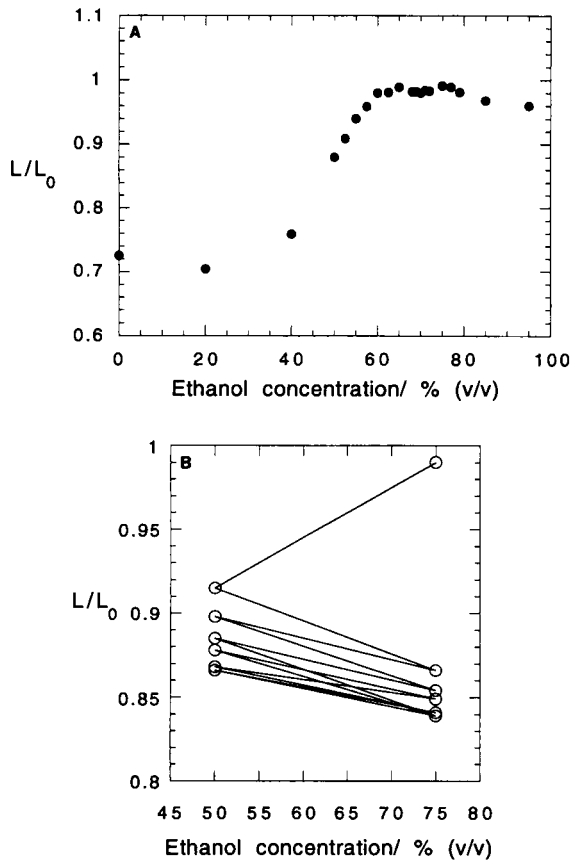

FIGURE 3 (*A*) The relative length, L/L_0, of different MgDNA fiber bundles transferred to different ethanol concentrations. [$MgCl_2$] = 0.01 M. (*B*) L/L_0 of one MgDNA fiber bundle transferred alternately every 24 h between baths containing 75% (v/v) and 50% (v/v) ethanol. [$MgCl_2$] = 0.01 M. The starting point of the experiment is at the top right.

occurred. As the measurements were repeated several times it was found that the L/L_0 values at low ethanol concentrations (<60%), as opposed to the L/L_0 values at higher ethanol concentrations, showed a considerable spread. The L/L_0 values at low ethanol concentrations seem to differ between MgDNA fibers that were taken from the same batch but prepared on different occasions. Thus, the L/L_0 values in Fig. 3 *A* come from measurements on MgDNA fiber bundles prepared on the same occasion.

To learn more about this contraction we measured the length of one fiber bundle sample, which was transferred every 24 h alternately between a 75% ethanol bath and a bath containing 50% ethanol. The result is presented in Fig. 3 *B*. As can be seen, the contraction is absolutely irreversible. This is in contrast to Na-, K-, Li-, and CsDNA, where a nearly full or at least partial reversibility was observed depending on counterion. Eventually, an "equilibrium length" was reached. The L/L_0 equilibrium value is slightly higher in 50% ethanol than in 75% ethanol. This behavior, together with the earlier mentioned uncertainty in the L/L_0 values at low ethanol concentrations, indicates that the contraction of the MgDNA fiber bundles at low ethanol concentrations is of macroscopic origin and, as such, not due to a DNA conformational change. At these relatively high water contents the strength of the aggregation between the DNA helices must have decreased considerably. The result of the cycling experiment presented in Fig. 3 *B* can be partly interpreted, then, in terms of the degree of order within the fibers. The DNA molecules may become more disordered when submerged in lower ethanol concentrations. When transferred back to the higher ethanol concentration, the stronger aggregation present could "freeze in" some of that disorder. After several cycles, a constant degree of disorder could be expected. Further, the difference in the "equilibrium length" can also be explained by the difference in the strength of the aggregation because the entire fiber structure will lose strength in lower ethanol concentrations. That loss of strength was also observed in the mechanochemical investigation with alkali counterions (Rupprecht et al., 1994).

The fiber bundle will break only after being submerged in pure water and 0.01 M $MgCl_2$ at room temperature for more than a month. This stability differs markedly from the case with alkali counterions where the DNA fiber bundles will break at room temperature when submerged in baths containing between 59 and 69% ethanol and 0.01 M salt, depending on counterion. The DNA is obviously much more strongly aggregated when Mg^{2+} ions are present than with any of the alkali counterions. The need of a higher weight to keep the fiber bundle straight reflects the higher degree of aggregation as well (30 vs. 10 mg for the alkali counterions).

This difference in the strength of the aggregation between DNA with univalent and divalent counterions indicates that the aggregation is predominantly an electrostatic effect. In the present dense system it is likely that Mg^{2+} ions are involved in intermolecular crosslinking (i.e., one Mg^{2+} ion linking two phosphate groups from two DNA molecules). Because the electrostatic interaction energy between charges increases as the dielectric constant of the medium decreases, the interhelical interactions mediated by the Mg^{2+} ions should contribute significantly to the aggregation. Also, the screening of the phosphate groups is more efficient with divalent ions present than with univalent ions (Trohalaki et al., 1991). Possibly, there is also an attractive contribution to the force between the adjacent DNA polyelectrolytes in the presence of divalent counterions (Guldbrand et al., 1986). Parsegian and coworkers, on the other hand, have shown that the interhelical distance in counterion-condensed DNA systems in methanol-water solutions is practically independent of the size of the multivalent counterion (Rau and Parsegian, 1992). This result implies that direct intermolecular crosslinking is not the attractive force keeping the DNA helices together in that system. The attractive force is proposed, instead, to originate from the organization of the water between the DNA helices, which may create attractive long-range "hydration forces." However, the high ethanol concentrations used in this work and the subsequent strong electrostatic interactions should tentatively render the contribution from electrostatic interactions to the aggregation more important than hydration forces.

Method 2

The helix-coil transition temperature, T_m, and the helix-coil transition width, ΔT, of MgDNA fibers in solutions of varying ethanol concentrations and 0.01 M $MgCl_2$ are shown in Fig. 4. A comparison with reported T_m values for calf-thymus MgDNA dissolved in ethanol-water solutions (Matsuoka et al., 1990) (~84°C in pure water decreasing to ~60°C in a 30% ethanol solution) shows, if extrapolated to the present ethanol concentrations, that the fiber structure stabilizes the DNA double helix. As may be seen in Fig. 4, T_m decreases approximately linearly as the ethanol concentration increases up to about 68% ethanol. The same behavior is seen when the $MgCl_2$ concentration is 0.4 M (not shown). The slope (~−0.73°C/% ethanol) is similar to the slopes found for B-DNA with alkali counterions (Rupprecht et al., 1994). In that work it was also found that the ethanol concentration inducing the conformational change is independent of temperature and that B- and A-DNA have different slopes in the T_m versus ethanol concentration curve. Because there is no change in the slope between 52 and 68% ethanol for MgDNA, we conclude that no B-A transition occurs in this concentration range. The conclusion is in accordance with earlier work (Skuratovskii and Bartenev, 1979) and with the results of Method 1 reported above. In the same concentration range ΔT is observed to decrease with increasing ethanol concentration. This is most probably a consequence of the stronger interhelical interactions due to aggregation present at higher ethanol concentrations, which should favor the cooperativity of the helix-coil transition.

The stronger aggregation experienced by MgDNA fibers compared to fibers with alkali counterions discussed above is also manifested by the much lower ethanol concentration for which T_m starts to increase due to strong aggregation (~68% compared to 84–90% for alkali counterions) and the ethanol concentration above which the DNA does not contract at all at 80°C (77% compared to 88–95% for alkali counterions). At ethanol concentrations below 50% the fiber bundle breaks when heated (64–70% for alkali counterions).

The DNA polyelectrolyte exerts a considerable influence on the surrounding solvent. In aqueous solution, the water near the polar DNA surface is subject to ordering (Eagland, 1975). When a nonelectrolyte such as ethanol is added, the water structure is disrupted. This influences the stability of the DNA double helix because the solvent composition and structure should have an effect on noncovalent bonds such as hydrogen bonds, charge-charge interactions, and hydrophobic interactions. Thus, the decrease of T_m between 52 and 68% ethanol could be explained by a continuing destruction of the water structure with increasing ethanol concentration together with the changes of the electrostatic interactions accompanying the decrease of the dielectric constant of the solvent.

The T_m versus ethanol concentration curve for MgDNA is similar to the one for LiDNA (Rupprecht et al., 1994) except that the former is displaced to lower ethanol concentrations by nearly 20% ethanol (see Fig. 5). That Mg^{2+} and Li^+ behave similarly is in agreement with the model proposed by Skuratovskii and coworkers (Skuratovskii and Bartenev, 1979; Bartenev et al., 1983), in which Mg^{2+} and Li^+, in contrast to Na^+, K^+, and Cs^+, interact with the bases as fully hydrated ions via water bridges.

Provided that Mg^{2+} and Li^+ interact with DNA in a similar way, there are basically two possible explanations for the mutual displacement by nearly 20% ethanol of the curves: a) A difference in the water content and structure near the DNA surface mediated by the different hydration properties of the two counterions; and b) the greater ability of Mg^{2+} to form interhelical bridges in this dense system.

A model discussed by Eagland (Eagland, 1975), which we call the counterion-water-affinity model, would fit into the first category. Eagland argues that the greater the degree of hydration of the counterion, the more effective it becomes in

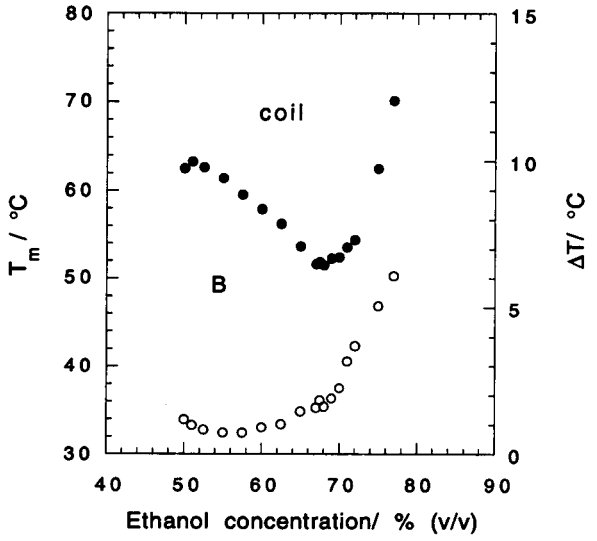

FIGURE 4 The helix-coil transition temperature, T_m (*filled circles*), and the helix-coil transition width, ΔT (*empty circles*), of MgDNA fiber bundles as a function of ethanol concentration. [$MgCl_2$] = 0.01 M. Coil and B-DNA have been indicated as in a quasi-phase diagram.

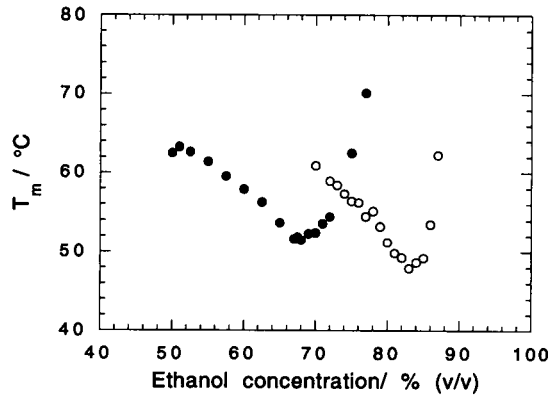

FIGURE 5 The helix-coil transition temperature, T_m, of Mg- (*filled circles*) and LiDNA (*empty circles*) fiber bundles as a function of ethanol concentration. [$MgCl_2$] = [LiCl] = 0.01 M. The LiDNA curve is taken from the analogous mechanochemical study of DNA fibers with alkali counterions (Rupprecht et al., 1994).

stabilizing the DNA double helix against heat-induced helix-coil transition by partial dehydration of the macromolecule. The model is discussed in connection with aqueous DNA solutions in which T_m increases as the salt concentration increases. At T_m, $\Delta G = 0$ and $\Delta H = T_m \Delta S$ where ΔS is the transition entropy and ΔH is the transition enthalpy. For this equality to hold with increasing salt concentration, ΔS must increase at a relatively smaller rate than ΔH, implying that the total increase in disorder created during the helix-coil transition tends to become smaller with increasing salt concentration. Because there is a loss of hydration during the helix-coil transition, it appears that an increased salt concentration produces a smaller loss of hydration during the transition. That is explicable if the salt has a tendency to dehydrate the DNA molecule, leaving less water to be lost during the helix-coil transition. If this is correct, then Li^+, the most strongly hydrated ion in the alkali metal series (Robinson and Stokes, 1965), should dehydrate the DNA molecule most effectively and hence give the highest T_m. That is observed, as well as that Mg^{2+}, which has an even higher affinity for water, gives an even higher T_m than Li^+ (Dix and Straus, 1972).

In ethanol-water solutions there should be, at least when the ethanol concentration is high, a competition between the DNA molecules and the counterions for the available water. Thus, if the water affinity of the counterions is high, the DNA molecules will experience a lower water activity and, consequently, react as if the ethanol concentration was higher. The counterion-water-affinity model was able to account for most differences between the T_m versus ethanol concentration curves for the alkali counterions (Rupprecht et al., 1994). Also, the strength of the interhelical bonds studied in solid DNA with alkali counterions (Lindsay et al., 1988; Weidlich et al., 1988; DeMarco et al., 1985; Lavalle et al., 1991) follows the trend predicted by the counterion-water-affinity model.

The Mg^{2+} ions should, as a consequence of their higher charge, coordinate the water molecules more strongly than the Li^+ ions. Furthermore, the Mg^{2+} ions each coordinate six water molecules in aqueous solution as compared to four for Li^+ ions (Ohtaki and Radnai, 1993). Thus, it seems that the model is able to explain, at least partly, the mutual displacement of the T_m versus ethanol concentration curves for MgDNA and LiDNA. Furthermore, the water content of oriented Li- and NaDNA fibers prepared at this laboratory and equilibrated at different relative humidities has been investigated (Brandes et al., 1986; Lindsay et al., 1988). These investigations showed that at 92% relative humidity (which is roughly equivalent to 70% ethanol (Wyckoff, 1955)) there are 20–23 water molecules/nucleotide, and at 84% relative humidity (which is roughly equivalent to 77% ethanol if the results of Wyckoff are interpolated) there are 10–12 water molecules/nucleotide. Because DNA is fully hydrated with about 20 water molecules/nucleotide (Jeffrey and Saenger, 1991), the competition at 70% ethanol between the DNA molecules and the counterions for the available water cannot be so fierce. At 77% ethanol, however, where the MgDNA fiber bundles do not contract at 80°C, this competition may start to become relevant. It should be noted, however, that it is doubtful that the counterion-water-affinity model has physical reality. Several gravimetric studies performed on solid DNA with alkali counterions equilibrated at various relative humidities (Lindsay et al., 1988; Lavalle et al., 1990; Falk et al., 1962) have shown that the degree of hydration of the samples is independent of the identity of the counterion. If so, then part of the effect might be due to hydration forces influenced by the water affinity of the counterion.

The fact that Mg^{2+} ions form interhelical bridges more readily than Li^+ ions provides a more direct mechanism. As was mentioned above, this mechanism should prove more important as the ethanol concentration increases because the electrostatic interaction energy between charges increases (both attractive and repulsive) as the dielectric constant of the medium decreases. Provided that the attractive interhelical forces (like interhelical Mg^{2+} bridges) dominate, the onset of strong aggregation should occur at a lower ethanol concentration compared to DNA with alkali ions. This explanation of the mutual displacement of the Mg^{2+} and the Li^+ T_m curves is the simplest and could very well be the dominant mechanism.

ΔT is smaller for MgDNA than for LiDNA (Rupprecht et al., 1994). This is most probably due to the stronger interhelical links in MgDNA, which should increase the cooperativity.

At ethanol concentrations below ~77% the aggregation is not strong enough to prevent the DNA strands from slipping past each other during a heating experiment, and this leads to the macroscopic contraction of the fibers reflecting the shorter end-to-end distance of the coil. The aggregation will increase gradually with increasing ethanol concentrations, and above ~68% ethanol T_m and ΔT start to increase with increasing ethanol concentration. ΔT then turns into a measure of the aggregation (which slows down the contraction) rather than a measure of the cooperativity of the helix-coil transition.

Above ~77% ethanol the DNA strands are unable to slip past each other even when the temperature is as high as 80°C. This "thermostable" DNA should closely resemble solid DNA equilibrated at low relative humidities. Linear infrared and ultraviolet dichroism studies have indicated that the systematic base stacking is lost in "dry" DNA, but also that the structure is reformed on rehydration (Falk et al., 1963a; Falk et al., 1963b). A similar behavior has been observed for DNA dissolved in concentrated methanol-ethanol solutions (Nordén et al., 1978; Zehfus and Johnson, 1984). According to Zehfus and Johnson, the secondary structure of this so called P-DNA is denatured (i.e., no or little hydrogen bonding prevails, and the base stacking is absent), whereas the tertiary structure is condensed. Thus, it is possible for the complementary strands to renature on addition of water. The DNA helices in the dried DNA films are not condensed, however, but aggregated (Bloomfield, 1991).

To see if the base stacking disappears in our aggregated MgDNA fibers in high ethanol concentrations, we performed an x-ray experiment on a thick fiber bundle in 95% ethanol

and 0.15 M $MgCl_2$. When comparing the x-ray diffraction pattern with one where the ethanol concentration was 75%, it was seen that the reflections stemming from the base stacking still are present, albeit of considerably weaker intensity. Thus, it seems that the secondary structure in the MgDNA fibers is partially denatured in high ethanol concentrations. In an identical experiment (Rupprecht et al., 1994) it was found that the reflections stemming from the base stacking disappeared for LiDNA in 95% ethanol. This suggests that Mg^{2+} ions have a stabilizing effect on the base stacking compared to Li^+ ions.

Further, to check whether the partially denatured MgDNA is renatured on addition of water, we performed a melting experiment on three fiber bundle samples that were pretreated in different ways (see Fig. 6). First, all three MgDNA fiber bundles were equilibrated in 75% ethanol and 0.15 M $MgCl_2$ to determine L_0. The subsequent treatment was as follows with all ethanol baths containing 0.01 M $MgCl_2$: One MgDNA sample, the reference, was equilibrated in 70% ethanol while the other two were equilibrated in 95% ethanol. Of these two latter samples, one was then equilibrated in 70% ethanol. The other one was slowly heated to ~80°C in the 95% ethanol solution and slowly cooled before being equilibrated in 70% ethanol. Finally, the three MgDNA fibers were slowly heated (Method 2) in 70% ethanol. As can be seen in Fig. 6, the resulting relative length versus temperature curves are very similar. The difference between the reference curve and the curve for the sample that was not preheated is within experimental errors. This result clearly indicates that the partial denaturation is reversible for these MgDNA fibers. The small difference between the reference curve and the preheated fiber, however, could be due to an incomplete reformation of inter-base hydrogen bonds upon cooling.

Calculations within the Poisson-Boltzmann Cylindrical Cell Model

Fig. 7 shows T_m and ΔT of MgDNA fibers in 70% ethanol as a function of the $MgCl_2$ concentration of the baths. Neither

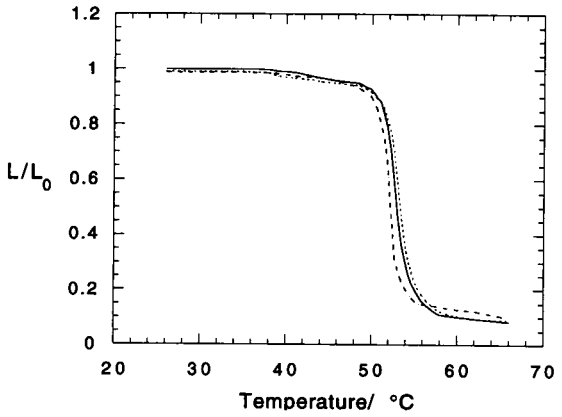

FIGURE 6 The relative length, L/L_0, of MgDNA fiber bundles in 70% (v/v) ethanol as a function of temperature. (·····) The reference fiber bundle, (——) the fiber bundle pre-equilibrated in 95% (v/v) ethanol, (- - - -) the fiber bundle pre-equilibrated and heated to 80°C in 95% (v/v) ethanol. [$MgCl_2$] = 0.01 M. See text.

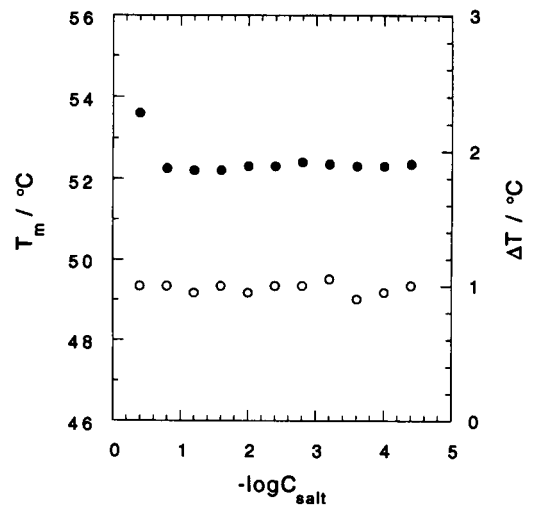

FIGURE 7 The helix-coil transition temperature, T_m (*filled circles*), and the helix-coil transition width, ΔT (*empty circles*), of MgDNA fiber bundles as a function of $MgCl_2$ concentration. The ethanol concentration was 70% (v/v).

T_m nor ΔT shows any salt dependence. To illustrate this lack of salt dependence, we have calculated ΔG_{el}, i.e., the difference in the electrostatic contribution to the free energy between a DNA molecule in a single strand (coil) and one in a fully double helical conformation, as a function of salt concentration at 25°C. The salt consists of divalent counterions and univalent coions (a 2:1 salt). This was done by a numerical solution of the full nonlinear Poisson-Boltzmann (PB) equation within the cylindrical cell model (Nilsson et al., 1985), which gave the electrostatic potential outside the charged DNA polyelectrolyte for the given experimental conditions. The result allowed us to calculate ΔG_{el} using the relation $G_{el} = E_{el} - TS_{el}$ and the equations of Marcus for E_{el} and S_{el} (the electrostatic part of the energy and the entropy, respectively) (Marcus, 1955). This kind of calculation has also been performed for a 1:1 salt (Rupprecht et al., 1994). A detailed account of the numerical procedure that we used to solve the PB equation is found in the work by Nilsson et al. (1985) of this laboratory.

For comparison, we first calculated ΔG_{el} for the helix-coil transition as a function of a 2:1 salt concentration for B-DNA in an aqueous semidilute solution (see Fig. 8). A phosphate concentration of 6 mM was assumed. For double helical B-DNA the following parameters were used: the relative permitivity $\epsilon_r = 78.3$, the DNA-radius $b = 1$ nm, the surface charge density $\sigma = -0.1509$ C/m^2, and the cylindrical cell radius $a = 22.830$ nm. For single strand coil DNA (Record et al., 1976), $b = 0.5$ nm, $\sigma = -0.1275$ C/m^2, and $a = 14.839$ nm (the phosphate concentration in the cell is kept constant). As was the case with a 1:1 salt, the calculations showed a strong salt dependence of ΔG_{el} so that a higher salt concentration stabilizes the double helical conformation (higher T_m). This behavior has been experimentally observed in dilute MgDNA solutions (Eichhorn and Shin, 1968). Consequently, the PB equation accurately describes this experimentally observed salt effect as being of an electrostatic origin.

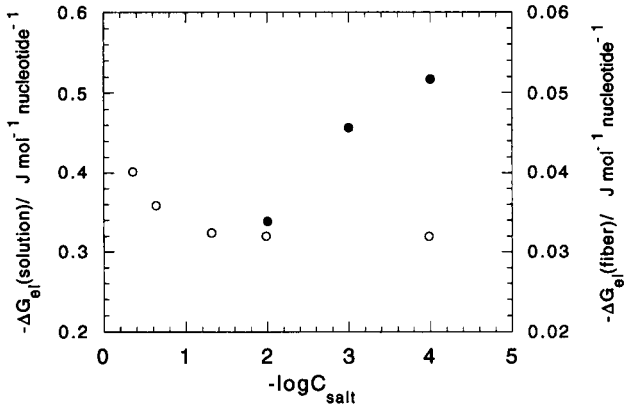

FIGURE 8 The negative of the electrostatic free energy difference between coil and helix, $-\Delta G_{el}$, calculated from the Poisson-Boltzmann cylindrical cell model for an aqueous solution of B-DNA at 6 mM phosphate concentration (*filled circles*), and for oriented B-DNA fibers submerged in a 70% (v/v) ethanol bath (*empty circles*), as a function of divalent (2:1) salt concentration in the respective solution.

When performing the calculations for a B-DNA molecule in a fiber, we considered the system as a cylindrical cell in equilibrium with an outside solution of a given salt concentration (Guldbrand et al., 1986). To allow a direct comparison, b and σ were given the same values as used above for a semidilute aqueous solution, although some discrepancy in these values between the two states cannot be excluded. A different choice of values for these parameters will not change, however, the conclusion as regards the difference in the salt dependence of ΔG_{el} in solution compared to the fiber (see below). ϵ_r is set to 46.8, which corresponds to a 70% ethanol-water solution (Åkerlöf, 1932) and is presumed to be uniform in the system. From x-ray measurements on fibrous phage T2 DNA drawn from a moistened gel, the interhelical distance in Mg(B)DNA fibers at 92% RH (which roughly corresponds to 70% ethanol (Wyckoff, 1955)) was found to be 25.5 Å (Skuratovskii and Bartenev, 1979). Using this value gives a cylindrical cell radius of 12.75 Å, to which we have added 1 Å to account for the extra space in the grooves, i.e., a = 13.75 Å for double helical DNA. To keep the phosphate concentration constant in the cell, a = 8.9375 Å for single-strand coil DNA.

The calculated ΔG_{el} values for the fiber in equilibrium with different salt solutions is shown in Fig. 8 as well. It can be seen that the calculated ΔG_{el} does not show any dependence on the salt concentration of the bathing solution, except for concentrations above 0.1 M, where the double helical conformation is slightly destabilized. The thermodynamic origin of this difference between semi-dilute DNA and ordered, highly concentrated DNA is that the favorable contribution to the entropy of mixing, caused by release of bound counterions during the helix-coil transition, will be insensitive to the added salt because the total concentration of neutralizing counterions is extremely high (~0.7 M) in this dense system. It should also be noted that the G_{el} values for both the helix and the coil state for DNA fibers in 70% ethanol are considerably smaller than the corresponding G_{el} values calculated for a DNA molecule in an aqueous semidilute solution. Thus, a calculated salt dependence of ΔG_{el} would have a negligible or very small effect on T_m in any case. The origin of the small absolute values of G_{el} is a thermodynamic consequence of the highly concentrated system. At this high salt concentration the average molecular contribution to E_{el} and S_{el} as obtained from the mean field PB equation is small compared to a dilute system. In the PB model this can be seen as an effect of the improved screening of the DNA charge distribution caused by the high ion concentration, and the concomitant loss of counterion ordering around the DNA cylinder. An additional possible origin of the lack of salt dependence, not inherent in the above calculations, is the formation of ion pairs Mg^{2+}-phosphate, which is favored by the presence of ethanol (Beronius et al., 1970). Consequently, a much lower amount of counterions is needed to "neutralize" the phosphate groups in high ethanol concentrations compared to aqueous solutions where the counterions screen the phosphate groups. The possibility that the lack of salt dependence is due to an entrapment of the counterions inside the DNA fibers can be ruled out because the conformation of wet-spun LiDNA in an ethanol-water solution has been shown to depend on the amount of LiCl present in the bathing solution (Rupprecht and Forslind, 1970; Schultz et al., 1992).

Finally, it should be pointed out that applying the PB equation on this dense system with divalent ions must be regarded as highly approximative. The PB equation is known to be less accurate when applied to systems containing divalent counterions. Also, the presumption of a uniform ϵ_r in the present system is certainly not valid. Lerner et al. (1984) found that no alcohol molecules are present in the vicinity of phosphate groups, so ϵ_r should be higher in the fiber than in the bathing solution. However, additional calculations showed that a higher ϵ_r will not affect the magnitudes of the G_{el} values to a great extent. Despite these uncertainties regarding the applicability of the model to the present system, we believe that the qualitative difference between the results of the calculations in solution as compared to the fiber, has correctly captured the origin of the different experimental salt dependencies.

CONCLUSIONS

The mechanochemical method is able to give information on structural changes and the helix-coil transition in oriented MgDNA fibers. We conclude that no B-A transition of the DNA takes place in the fibers when submerged in ethanol-water baths ranging from 52 to 95% ethanol. This conclusion is based on the observations that the relative fiber length does not decrease substantially when the fiber bundle is submerged in baths with an ethanol concentration ranging from 60 to 95% and that the helix-coil transition temperature of the MgDNA fibers is linearly dependent on the ethanol concentration of the baths between 52 and 68%. Above 77% ethanol the MgDNA fibers do not contract at all–not even at the highest temperature of the experiments, ~80°C–due to the strong aggregation present. The strong aggregation is suggested to be primarily due to electrostatic interactions. Nei-

ther the helix-coil transition temperature nor the helix-coil transition width of MgDNA fibers exhibit a dependence on the $MgCl_2$ concentration in the bath. Calculations within the Poisson-Boltzmann cylindrical cell model indicate that the lack of a salt dependence is a thermodynamic consequence of the high salt concentration present in this very dense system.

The work has been supported by the Swedish Medical Research Council and the Swedish Natural Science Research Council.

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