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Mechanochemical Study of NaDNA And NaDNA-Netropsin Fibers in Ethanol-Water And Trifluoroethanol-Water Solutions

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ABSTRACT Highly oriented calf-thymus NaDNA fibers, prepared by a wet-spinning method, were complexed with netropsin in ethanol-water and trifluoroethanol (TFE)-water solutions. The relative fiber length, L/L_0, was measured at room temperature as a function of ethanol or TFE concentration to obtain information on the B-A conformational transition. The B-A transition point and transition cooperativity of the fibers were calculated. The binding of netropsin to NaDNA fibers was found to stabilize B form and to displace the B-A transition to higher ethanol concentration, as indicated by its elongational effect on the fiber bundles. An increased salt concentration was found to reduce netropsin binding. In netropsin-free ethanol solution, the dissociation of bound netropsin from the DNA fibers was observable. Pure B-NaDNA fibers were found to be more stable in TFE solution than in ethanol solution. This was interpreted as being due to a different steric factor and a larger polarity of TFE compared with ethanol, resulting in its smaller capacity to reduce the water activity and dielectric constant of the medium in the immediate vicinity of DNA fibers. Therefore, the effect of netropsin binding on the B-A transition of NaDNA fibers became less obvious in TFE solution. In another series of experiments, L/L_0 was measured as a function of temperature to obtain information on the helix-coil transition, or melting, as well as the B-A transition of NaDNA and NaDNA-netropsin fibers. The melting temperature and helix-coil transition width were calculated from the melting curves. A phenomenological approach was used to describe the melting behavior of the fibers in and around the B-A transition region. The effect of netropsin on the melting of DNA fibers was attributed mainly to the stabilization of B-DNA and to a higher melting cooperativity in the B-DNA region.

INTRODUCTION

DNA conformation can be affected by the water activity, the nature of the counterion, the salt content, the nucleotide sequence, and the binding of ligands. The B-A conformational transition in dissolved DNA is induced, for instance, by adding nonelectrolytes such as ethanol or trifluoroethanol (TFE) to the aqueous DNA solution (Ivanov et al., 1973, 1974; Malenkov et al., 1975). In solid DNA, the conformational transition may be induced by varying the relative humidity of the ambient atmosphere (Lindsay et al., 1988; Fuller et al., 1965; Premilat et al., 1990; Albiser et al., 1988; Arnott, 1970; Rupprecht and Forslind, 1970) or by immersing the DNA fibers in certain nonelectrolyte solutions, which decreases the water activity in the DNA (Wyckoff, 1955). Counterions, such as Na^+, K^+, Cs^+, Li^+, and Mg^{2+}, possess different capacities to stabilize B-DNA, with Na^+ being the least effective. The interaction between DNA and counterions can be understood with reference to the groove binding model of Ivanov (Ivanov et al., 1973) and Skuratovskii et al. (Skuratovskii and Bartenev, 1979; Bartenev et al., 1983), and to the interhelical interactions studied by Lindsay and coworkers (Lindsay et al., 1988). The conformational flexibility of DNA is more or less inhibited by intercalative or nonintercalative binding of small, biologically relevant ligands (Pohle and Fritzsche, 1984; Fritzsche et al., 1984a; Fritzsche, 1994).

The interaction of DNA with netropsin (Fig. 1) has stimulated a variety of studies. Although too toxic for clinical use, this antibiotic is one of the most potent binding agents of DNA. The binding is nonintercalative (Zimmer, 1975; Zimmer and Wähnert, 1986). Various techniques, including ultraviolet (Zimmer et al., 1971a; Wartell et al., 1974), circular dichroism (Zasedatelev et al., 1974; Luck et al., 1974), FTIR spectroscopy (Liquier et al., 1989; Adnet et al., 1992), viscosity measurement (Reinert, 1972), and foot-printing analysis (Ward et al., 1987), all revealed the high binding preference of netropsin for the A-T base pairs of DNA. The binding is thermodynamically enthalpy-driven with large negative ΔH value (Marky and Breslauer, 1987; Patel et al., 1992; Marky and Kupke, 1989; Rentzeperis et al., 1993a; Marky et al., 1983). X-ray crystallography studies (Kopka et al., 1985a–c; Coll et al., 1989; Larsen et al., 1991; (Tabernero et al., 1993) and NMR studies (Patel and Shapiro, 1985, 1986a, b; Fritzsche and Crothers, 1983; Pardi et al., 1983; Patel et al., 1982; Fagan and Wemmer, 1992) have provided detailed structural information on certain oligonucleotide-netropsin complexes. The results indicate that the netropsin molecule binds to the minor groove of B-DNA through the combination of three types of interaction: hydrogen bonding and Van der Waals contacts between the netropsin amide groups and A-T base pairs at the bottom of the groove; close Van der Waals contacts with the sugar-phosphate backbone at the side wall of the groove; and electrostatic attraction to base atoms and/or phosphodiester groups with its positive charged ends. From IR linear dichroism studies of oriented DNA samples (Pohle and

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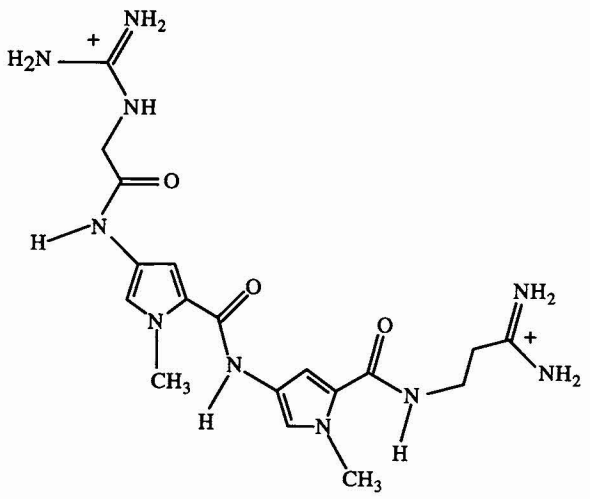

FIGURE 1 The netropsin molecule.

Fritzsche, 1984; Fritzsche et al., 1984a, b; Fritzsche, 1994; Pohle, 1990), it has been concluded that the binding of netropsin effectively suppresses the formation of A-DNA, even at rather low relative humidities. This conclusion was further confirmed by combining results from different methods (Fritzsche et al., 1992; Lee et al., 1993) and agrees with previous circular dichroism data (Ivanov et al., 1974). Recently, a viscometric investigation gave evidence that the interaction between DNA and netropsin would also influence the tertiary structure of DNA by the abolition of intrinsically bent DNA structure components in A-T clusters (Reinert, 1993a, b), in agreement with an earlier report (Wu and Crothers, 1984).

Mechanochemical study of oriented DNA fibers in ethanol-water solutions has been carried out in our laboratory. By analyzing the macroscopic change of fiber length in this unique system, we have obtained in great detail information on conformational transitions and the heat-induced helix-coil transition, or melting, of Li-, Na-, K-, Cs- and MgDNA fibers (Rupprecht et al., 1994; Schultz et al., 1994). We now have extended the mechanochemical study to the NaDNA-netropsin complex in various ethanol-water and TFE-water solutions, to elucidate the interaction between NaDNA fibers and netropsin and to obtain information on the conformational transition and melting properties of the complex.

MATERIALS AND METHODS

High molecular mass (1.17×10^7 Da) calf-thymus NaDNA (approximately 58 mol% A+T content) from Pharmacia Biotech Norden AB (Sollentuna, Sweden) was used without further purification. Research grade netropsin hydrochloride ($C_{18}H_{26}N_{10}O_3 \cdot 2HCl$, M_r = 503.4 Da, ϵ_{max} = 23,500 M^{-1} cm^{-1} at 296 nm) was purchased from Serva Feinbiochemica (Heidelberg, Germany). 99.5% (v/v) ethanol and 2,2,2-TFE were from Kemetyl AB (Stockholm, Sweden) and Sigma Chemical Co. (St. Louis, MO), respectively.

Oriented NaDNA fibers were prepared by a wet-spinning method (Rupprecht, 1966, 1970a) that was specially modified (Rupprecht, 1970b) to provide fiber bundle samples. The spinning was achieved by using a spinning bath containing 75% ethanol, 0.15 M NaCl, a 720-hole spinneret (70-μm-hole diameter) and 8 sweeps. The oriented fiber bundle thus consisted of about 5760 thin, parallel DNA fibers, corresponding to about 3.3×10^8 DNA helices in its cross section.

The mechanochemical setup is shown in Fig. 2. The measuring cylinder was designed with 14-mm diameter, much smaller than that used earlier (Rupprecht et al., 1994; Schultz et al., 1994), so as to reduce its volume and, consequently, the required amount of chemicals, in particular netropsin. The results were found to be unaffected by this change. The way of mounting DNA fiber bundle and measuring equilibrated fiber length, L, has been described elsewhere (Rupprecht et al., 1994; Schultz et al., 1994). The complexation of the DNA fibers with netropsin was also achieved in the setup.

In the following text, ethanol and TFE are referred to collectively as alcohol. The measurements in alcohol-water solutions proceeded as follows. After equilibration in 75% ethanol (0.15 M NaCl), the fiber bundles were moved into other 75% ethanol or TFE solutions with desired NaCl concentrations. A slight change of fiber length (about 1%) occurred occasionally because of the effect of varied salt concentration and/or the transferring from ethanol to TFE. Thereafter, three types of experiment were performed.

Experiment (i)

The NaDNA fibers were placed in alcohol-water solutions with the alcohol concentration ranging from 60 to 95%. The solutions were either netropsin-free or they contained 0.8 mM netropsin. Fiber elongation or contraction was observed until an equilibrium reached within a few hours (without netropsin) or days (with netropsin).

Experiment (ii)

Some of the fiber bundles, handled in various alcohol solutions, were further moved into new solutions containing 0.8 mM netropsin but with the same alcohol and salt concentration. A net elongation due to netropsin binding occurred, depending on the alcohol and NaCl contents. In the case of ethanol, a few samples were later returned to their original netropsin-free solutions for observing any possible dissociation of the NaDNA-netropsin complex.

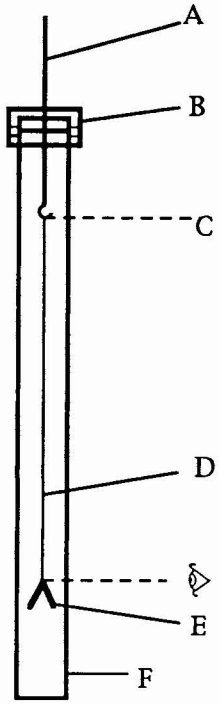

FIGURE 2 The mechanochemical set-up. (A) Glass hook; (B) cap with Teflon-liner; (C) upper index of the measuring cylinder scale; (D) DNA fiber bundle; (E) V-shaped Pt-weight; (F) measuring cylinder with alcohol solution.

Experiment (iii)

The mechanochemical setups were submerged into a thermostated waterbath and subjected to a linear heating rate of 0.1°C/min. A drastic fiber contraction was observed both for NaDNA and NaDNA-netropsin in the various alcohol solutions, corresponding to the helix-coil transition, or melting, of the DNA fibers.

The observation in each experiment above is specified by the relative fiber length, L/L_0, where L_0 and L are the fiber lengths before and after the experiment, respectively. The notation of L_0 here differs from that used in our previous paper (Rupprecht et al., 1994), which essentially was the initial fiber length in the spinning bath (75% ethanol, 0.15 M NaCl) and, consequently, a constant characterizing each particular fiber bundle sample. Both definitions of L/L_0 can be used for analyzing the results, but the present one emphasizes the change arising directly from the individual experiment. To obtain information on the B-A transition from experiments (i) and (ii), L/L_0 was plotted as a function of alcohol concentration. From experiment (iii), on the other hand, L/L_0 was plotted versus temperature to allow calculation of the melting temperature, T_m, and the melting cooperativity, ΔT, characterizing the helix-coil transition.

In experiments (i) and (ii), the Pt-weights used were either 30 mg (in ethanol-water) or 10 mg (in TFE-water). Comparing the present results of NaDNA fibers in ethanol-water solutions with the earlier ones, it was found that using 30-mg instead of 10-mg weights caused only slight shift of L/L_0 curve toward higher ethanol concentration, with the B-A transition point (Z_{BA} % alcohol, as defined later) 0.3% higher. In experiment (iii), the weights were all 10 mg. As addressed previously (Rupprecht et al., 1994), the melting temperature and melting cooperativity may also be influenced by the choice of weight, but the lighter weights were found necessary for experiment (iii) to avoid possible fiber elongation or breakage caused by using too heavy weights before the melting was completed.

RESULTS AND DISCUSSION

B-A transition of NaDNA and NaDNA-netropsin fibers in ethanol-water solutions observed by experiment (i)

The results of this experiment are displayed in Fig. 3, *a* and *b*. Fig. 3 *a* shows the variation of relative fiber length, L/L_0, with ethanol concentration for NaDNA and NaDNA-netropsin fibers (in the latter case, the bulk concentration of NaCl was 0.05 and 0.15 M, respectively). A locally weighted least-square error method was used to generate the solid lines from experimental data.

The NaDNA curve is analogous to that displayed in Fig. 3 in the previous paper (Rupprecht et al., 1994), although the two measurements were performed with different salt concentrations and Pt-weights. We attribute the change of fiber length in this experiment to an ethanol-induced B-A transition of DNA, caused by the decrease of the water activity. This conclusion is supported by various DNA investigations using x-ray diffraction (Lindsay et al., 1988; Premilat et al., 1990; Wyckoff, 1955; Zimmerman and Pheiffer, 1979, 1980, 1983) and other methods (Ivanov et al., 1973, 1974; Malenkov et al., 1975; Shlyakhtenko, 1984; Martin and Wartell, 1982). The fact that the B- and A-DNA helices have different translations per residue, about 0.34 and 0.26 nm, respectively, underlies the change of the end-to-end length when the B-A transition occurs. This is macroscopically manifested in our highly oriented DNA fiber bundles during the experiment.

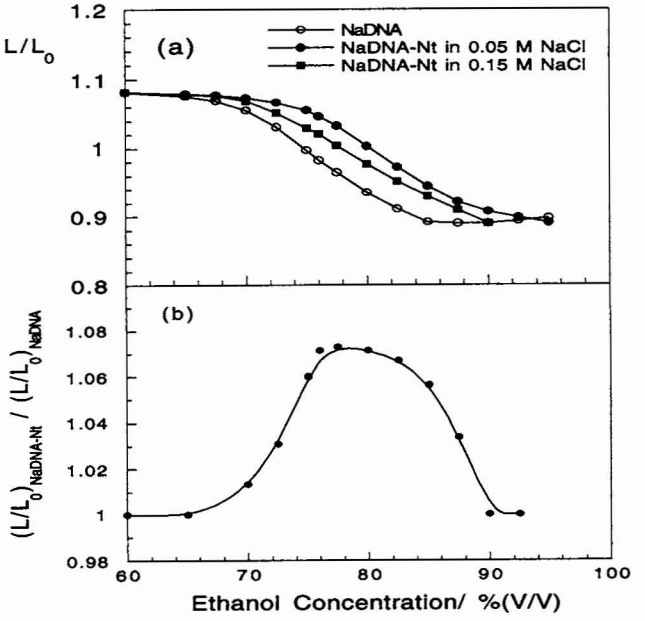

FIGURE 3 (*a*) Relative fiber length, L/L_0, of NaDNA and NaDNA-netropsin fibers in ethanol-water solutions with and without 0.8 mM netropsin, measured by experiment (i). (*b*) The ratio of L/L_0 for NaDNA-netropsin (0.05 M NaCl) and NaDNA fibers in ethanol-water solutions; the data are from Fig. 3 *a*.

Definition of the B-A transition point and the cooperativity

Because the B-A transition is a cooperative process in general, we define the B-A transition point, Z_{BA} (% alcohol), as the mean concentration of ethanol (or TFE) at which the B-A transition takes place, and the transition cooperativity, ΔZ_{BA} (% alcohol), as the mean-square deviation from the mean concentration, analogous to Eqs. 2 and 3 presented previously (Rupprecht et al., 1994). That is,

$$Z_{BA}\,(\%\ \text{alcohol}) = \sum_{k(Z_1)}^{k(Z_2)} \frac{0.5\,(Z_k + Z_{k+1})\,[(L/L_0)_{k+1} - (L/L_0)_k]}{(L/L_0)_{k(Z_2)} - (L/L_0)_{k(Z_1)}} \tag{1}$$

$$\Delta Z_{BA}\,(\%\ \text{alcohol}) = \left\{ \sum_{k(Z_1)}^{k(Z_2)} \frac{[0.5(Z_k + Z_{k+1}) - Z_{BA}]^2\,[(L/L_0)_{k+1} - (L/L_0)_k]}{(L/L_0)_{k(Z_2)} - (L/L_0)_{k(Z_1)}} \right\}^{1/2}, \tag{2}$$

where k denotes the order-number of the measured data point ranging from $k(Z_1)$ to $k(Z_2)$. The calculation is achieved by iteration as follows. After choosing Z_2, which corresponds to a minimum L/L_0, and estimating an approximate Z_{BA} (% alcohol) from each solid line in Fig. 3 *a*, a preliminary Z_1 is obtained by $Z_2 - Z_{BA} = Z_{BA} - Z_1$. Applying the L/L_0 values corresponding to these Z_1 and Z_2 to Eq. 1 gives a more accurate Z_{BA} (% alcohol), which in turn is used to obtain an improved Z_1, etc. This method may have a limited accuracy but does give a useful comparison. The results of these calculations are listed in Table 1. Although the B-A transition points of NaDNA and NaDNA-netropsin fibers in ethanol-water solutions differ from each other, the

TABLE 1 Summary of certain experimental results from fibers of DNA and DNA-netropsin (Nt) in ethanol-water and TFE-water solutions containing 0.05 (with *) and 0.15 M NaCl

Experiment and parameter	In ethanol-water		In TFE-water	
	NaDNA	NaDNA-Nt	NaDNA	NaDNA-Nt
Experiment (i)				
B-A transition point, Z_{BA}/% alcohol	76.1	80 81.9*	85.3	85.6
B-A transition cooperativity, ΔZ_{BA}/% alcohol	4.7	5.4 5.7*	2.13	2.62
Experiment (ii)				
Maximum L/L_0 and corresponding % alcohol		1.05 at 75.5% 1.07 at 76.5%*		1.02 at 85%
Experiment (iii)				
K_B/(°C/% alcohol)	−0.72	−0.52	−0.83	−0.45
$T^0_{m,B}$/°C	115.5	105.5	135.8	113.3
K_A/(°C/% alcohol)	−2.10	−1.91	−4.15	−4.63
$T^0_{m,A}$/°C	231.9	213.7	421.3	464.7
Helix-coil transition width, ΔT/°C (mean value from Figs. 13 and 14):				
B-DNA	3.22	2.38	2.61	1.35
B-A transition region	1.6	1.54	1.52	1.96
A-DNA	2.21	1.73	4.2	4.7
B-A transition point $\frac{1}{2}(Z_A + Z_B)$/% alcohol	75	80	80.7	79
B-A transition width $(Z_A - Z_B)$/% alcohol	10	10	6.5	8
$\Delta Z^{(iii)}_{BA}$/% alcohol	2.9	2.9	1.9	2.3

Estimated accuracy of % alcohol given in the table = ±1%.

cooperativity data are similar and comparable with the transition interval (about 6% alcohol) defined by Ivanov et al. (Ivanov and Krylov, 1992).

The stabilization of B-NaDNA by netropsin binding

As we already know (Rupprecht et al., 1994), the B-A transition of NaDNA fibers occurs at the lowest ethanol concentration, i.e., NaDNA has the largest tendency to adopt A form, compared with K-, Cs-, Li-, and MgDNA (the latter two do not adopt A form at all). The complexation of netropsin to NaDNA fibers, therefore, are the most informative for our purpose to study the influence of netropsin on the stabilization of B-DNA. From Fig. 3 *a*, it is clearly seen that transferring the fibers from 75% ethanol to a defined netropsin-containing solution gives rise to a larger L/L_0, as compared with the result obtained in the same solution without netropsin. Or to achieve the same L/L_0 for NaDNA and NaDNA-netropsin fibers, the latter has to be in more concentrated ethanol solutions. Thus, the entire L/L_0 curve of NaDNA-netropsin is displaced somewhat toward higher ethanol concentration.

It is found here, and previously (Rupprecht et al., 1994), that the B-A transition point, Z_{BA} (% alcohol), of NaDNA fibers is at about 76% ethanol. Thus, in the initial 75% ethanol solution without netropsin, the fraction of A- and B-DNA is nearly the same. Microscopically, the elongation of DNA due to netropsin binding might be attributed to its effects on both DNA secondary and tertiary structure, i.e., 1) the favoring of B-DNA; 2) a partial abolition of DNA local bending (Reinert, 1993a, b). Because experiment (i) involves the variation of ethanol concentration, which means that the role of ethanol to induce the B-A transition exists concomitantly, the first effect mentioned above must be dominant. In other words, the observed macroscopic change of fiber length arises mainly from the effect of bound netropsin on the DNA secondary structure. As compared with NaDNA fibers, the smaller contraction of NaDNA-netropsin fibers with increased ethanol concentration (>75%) corresponds to a partial inhibition of the ethanol-induced B-A transition. The larger elongation of NaDNA-netropsin at lowered ethanol concentration (<75%), on the other hand, gives the evidence that the binding of netropsin may even convert A-DNA to B-DNA (Minchenkova and Zimmer, 1980), and thus contributes an additional fiber elongation. As an illustration for the case with 0.05 M NaCl (see Fig. 3 *a*), the NaDNA fiber bundle previously immersed in 75% ethanol solution will not contract at all even if one moves it into 80% ethanol with 0.8 mM netropsin. In other words, fiber elongation may still occur within this region, in contrast to netropsin-free NaDNA fibers, only because the solution then contains 0.8 mM netropsin as well. The capacity of ethanol to induce the B-A transition seems to have been eliminated completely in this case by the formation of NaDNA-netropsin complex. Outside the concentration range of 65–90% ethanol, NaDNA and NaDNA-netropsin fibers come equally to their maximum and minimum L/L_0 values. The absence of visible binding effect on fiber length implies that the role of ethanol-water to keep the DNA fibers in pure B or A form prevails in such low and high ethanol concentration. The argument also agrees with the fact that the binding of netropsin to A-DNA is rather

weak. Otherwise, the elongation due to netropsin binding would be observable even at high ethanol concentrations.

As an alternative way to describe the relative effect of netropsin binding in various ethanol solutions, the ratio of two sets of L/L_0 data, those from NaDNA-netropsin (with 0.05 M NaCl) and NaDNA fibers in Fig. 3 *a*, is plotted versus ethanol concentration (see Fig. 3 *b*). It is apparent that the relative effect on fiber length, due to netropsin binding, shows its maximum at 75–80% ethanol, the main B-A transition region of NaDNA.

The salt effect on netropsin binding

Fig. 3 *a* also gives the insight into the effect of bulk salt concentration on netropsin binding to NaDNA fibers. Under the constant netropsin concentration (0.8 mM), the L/L_0 curve of NaDNA-netropsin fibers with 0.05 M NaCl (the lower content) shows a larger displacement toward higher ethanol concentration, with respect to NaDNA, whereas the curve representing NaDNA-netropsin with 0.15 M NaCl (the higher content) lies in between. The calculated B-A transition points become 81.9 and 80% ethanol, respectively (see Table 1). The salt dependence of netropsin binding was verified by experiment (ii) as well. It will be discussed further later.

B-A transition of NaDNA and NaDNA-netropsin fibers in TFE-water solutions observed by experiment (i)

The TFE-induced B-A transition of dissolved DNA was studied early (Ivanov and Krylov, 1992; Ivanov et al., 1983, 1985; Charney and Chen, 1987; Minchenkova et al., 1986). For native DNA, the transition was found to occur in the region 65–70% TFE. According to Ivanov et al., the water activity data of TFE-water and ethanol-water solutions may not differ very much. Nevertheless, TFE is more polar than ethanol (Mikhailenko and Shlyakhtenko, 1984) with reported dielectric constant, ϵ, at 20°C equal to 27.68 (Llinas and Klein, 1975) and 25 (Åkerlöf, 1932), respectively.

The measurements on wet-spun oriented DNA fibers in TFE-water solutions were performed by us for the first time. The result was somewhat unexpected. As can be seen from Fig. 4, in 60–75% TFE solutions, the relative length, L/L_0, of NaDNA and NaDNA-netropsin fibers undergoes in fact only a small change. Larger contraction occurs when TFE concentration is raised to more than 78%. The calculated B-A transition point, Z_{BA} (% alcohol), is at about 85.3 and 85.6% TFE for NaDNA and NaDNA-netropsin fibers, respectively (see Table 1), which is higher than that of NaDNA-netropsin fibers in ethanol solution. It is noticed that the L/L_0 curve of NaDNA fibers is already displaced so much toward higher TFE concentration, no remarkable elongation was observed by the binding of netropsin, so that the Z_{BA} (% alcohol) values of both fibers are nearly the same. Comparing with NaDNA fibers in ethanol solution, performing the measurements under the same conditions of sample lot, salt content, and Pt-

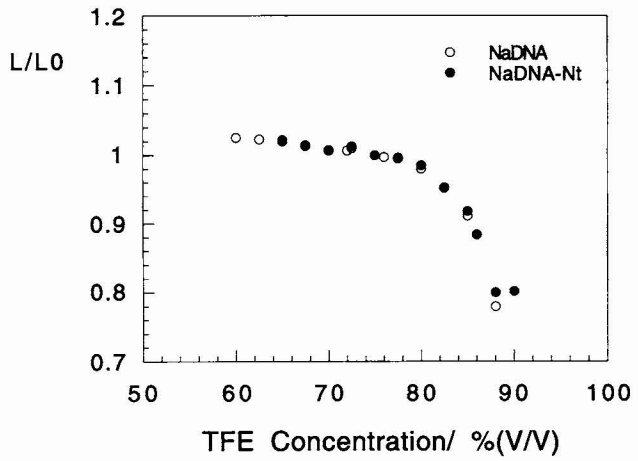

FIGURE 4 Relative fiber length, L/L_0, of NaDNA and NaDNA-netropsin fibers in TFE-water solutions with and without 0.8 mM netropsin, measured by experiment (i).

weights, it becomes certain that the displacement of the L/L_0 curve really is due to the use of TFE. Thus, it is concluded that the addition of TFE to water solution is not as efficient as ethanol to induce a B-A transition of NaDNA fibers, i.e., B-NaDNA fibers are more stable in TFE-water than in ethanol-water solutions. This result is in sharp contrast to what was observed in dissolved DNA (Ivanov and Krylov, 1992; Ivanov et al., 1985), where the B-A transition of native DNA occurs at a lower TFE concentration, as compared with ethanol. Referring to the analysis by Gekko et al. (1988), the preferential interaction between solvent and DNA depends in general on two factors: 1) the steric exclusion principle; and 2) the affinity between solvent components and binding sites on DNA. Because TFE molecules possess a larger molal volume than ethanol, they should be less accommodated statistically in the immediate vicinity of DNA helices as compared with ethanol, assuming the same alcohol-water concentration (v/v). As to the affinity, on the other hand, there are three types of binding sites on DNA molecules, i.e., ionic, polar, and nonpolar groups. Although the former two would favor the binding of a more polar solvent (TFE), the nonpolar surface might interact more strongly with ethanol. Thus, the accessibility of TFE or ethanol will influence directly the water activity around DNA. Because the water activity, in particular the hydration at the base sites of the DNA helix, is known to play an important role in the B-A transition of DNA, the difference caused by using TFE and ethanol can be understood. The water activities may not differ very much in equally concentrated TFE- and ethanol-water bulk solutions, so that the difference of using TFE and ethanol might be trivial for investigating the B-A transition of dissolved DNA. But the difference may become significant in aggregated DNA fibers, considering the effective water activity in the immediate vicinity of DNA molecules. This effect will also be discussed below.

Further evidence of the effect of netropsin binding on NaDNA fiber length. Influence of alcohol and salt concentrations: experiment (ii)

The effect of bound netropsin on the DNA conformation was further manifested by experiment (ii). Figs. 5 and 6 show the results, measured in various ethanol or TFE solutions containing 0.8 mM netropsin.

Netropsin-induced A-B transition

With the ethanol concentration remaining invariant, a net elongation caused by netropsin binding was observed in 67.5–87.5% ethanol (Fig. 5). This is in accordance with the results from experiment (i), but even more convincingly indicates the conversion of A- to B-DNA due to the effect of netropsin. As reported previously (Wartell et al., 1974; Zasedatelev et al., 1974; Luck et al., 1974; Liquier et al., 1989; Fritzsche, 1994; Fritzsche et al., 1984b), each bound netropsin molecule covers about 4–5 A-T bp. However, its influence, or quantitatively the "freezing" index according to Fritzsche (1994), may extend to about 24 or more bp along the double helix, which is longer than the cooperative length (about 16 bp) of the B-A transition of calf-thymus DNA (Ivanov and Krylov, 1992). We believe that the A-B transition caused by netropsin binding and represented macroscopically by the fiber elongation, probably takes place within the extended region in such a way that netropsin binds directly to A-T base pairs of B-DNA and thus forces the adjacent regions to adopt B form as well. In Fig. 5, each L/L_0 curve has its maximum near the B-A transition point, Z_{BA} (% alcohol), of NaDNA fibers. This is conceivable because the net elongation depends actually on the combined effect of netropsin, alcohol, and the relative amount of A and B form that existed originally. At high ethanol concentrations, for instance, the effect of ethanol to favor A-DNA is so strong that the B-DNA-dominated regions of the DNA helices are few and short. As a result, netropsin binding becomes weaker and the fiber elongation is reduced. The location of the maximum elongation in this plot may provide a complement in estimating the B-A transition point of netropsin-free NaDNA fibers.

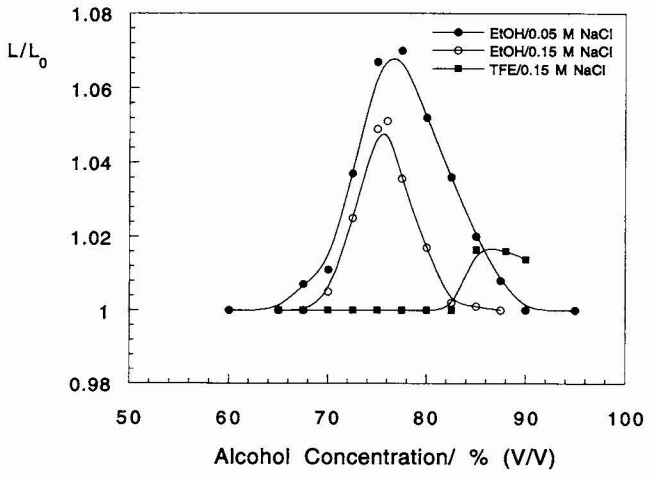

FIGURE 5 Fiber elongation due to netropsin binding to NaDNA fibers, measured by experiment (ii), in ethanol-water or TFE-water solutions containing 0.8 mM netropsin and 0.05 M or 0.15 M NaCl.

Further evidence of the effect of salt and TFE on netropsin-induced fiber elongation

A salt effect on the binding constant of netropsin has been reported by other authors for DNA dissolved in water solution (Rentzeperis et al., 1993a, b). The influence of salt concentration on the elongation of NaDNA-netropsin fibers is also manifested in Figs. 5 and 6. In two L/L_0 curves corresponding to NaDNA-netropsin fibers in ethanol solutions with 0.8 mM netropsin but different NaCl contents (0.05 and 015 M; see Fig. 5), the one with 0.15 M NaCl shows apparently a smaller fiber elongation, in accordance with what observed in experiment (i) discussed above. A few measurements of L/L_0 were also performed at constant 75% ethanol, 0.8 mM netropsin, but different NaCl concentrations (0.01, 0.05, 0.15, and 0.4 M, respectively). As may be seen from Fig. 6, there is clear salt effect. Because the NaCl concentration alone has a much smaller influence on the B-A transition of NaDNA fibers (Rupprecht et al., 1994), the salt effect on netropsin binding and, consequently, on the conformational transition of the NaDNA-netropsin complex must arise from a sort of competition between netropsin and salt in their interaction with DNA. In this context, we have to distinguish between the stronger binding sites of DNA consisting of 4–5 consecutive A-T base pairs, and weaker binding sites where the A-T base pairs are interrupted by one or more G-C base pairs. The weaker binding sites are be-

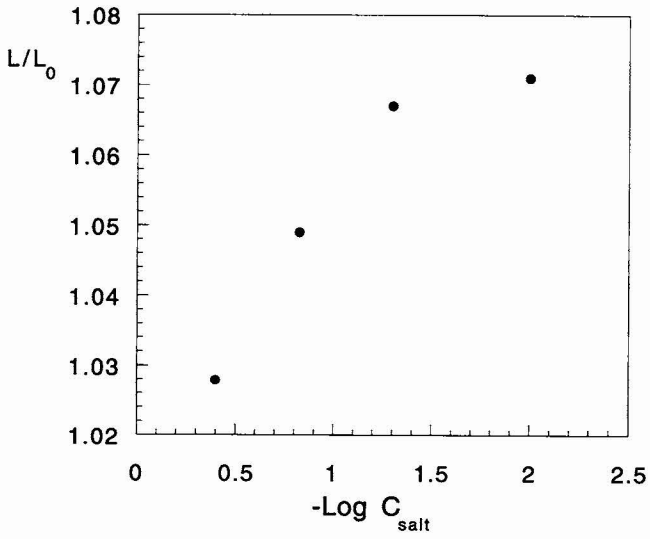

FIGURE 6 Salt effect on netropsin binding, as revealed by varied relative fiber lengths, L/L_0, of NaDNA-netropsin fibers, in 75% ethanol solutions with 0.8 mM netropsin and different NaCl concentrations. The measurements were performed according to experiment (ii).

lieved to be more sensitive to the increase of the ionic strength than the stronger ones.

In TFE solutions, the effect of netropsin on fiber elongation is relatively weak (see Fig. 5). It becomes visible when the TFE concentration is higher than 82.5% and reaches a maximum at about 85% TFE. This agrees qualitatively with the results from experiment (i), which shows that in netropsin-free TFE the B-A transition of NaDNA fibers is displaced to higher TFE concentration, as compared with ethanol, with the transition point of NaDNA and NaDNA-netropsin fibers at about 85.3 and 85.6% TFE, respectively.

The reversibility of netropsin binding in ethanol solution

A rough investigation was performed to estimate the time required for achieving binding equilibrium of NaDNA-netropsin fibers. Contrary to the ethanol-induced B-A transition, which is quickly achieved in general (within a few minutes), the process of netropsin binding is relatively slow. Thus, the elongation becomes gradually visible after a few hours, but the complexation needs 1–2 days to reach equilibrium. At room temperature and the constant netropsin concentration used (0.8 mM), the time needed for the complexation depended on the relative extent of elongation as well as on the ethanol concentration. For instance, when moving the fibers from 75% ethanol to 76–80% ethanol solutions containing netropsin, a quick fiber contraction was followed by a slow elongation. Fig. 7 shows some typical curves of L/L_0 versus time, illustrating the fiber elongation due to netropsin binding at certain ethanol concentrations.

To investigate the reversibility of netropsin binding, i.e., the possible release of bound netropsin from the fibers, a few measurements were performed by bringing some NaDNA-netropsin fiber bundles back to their original 65–87.5% ethanol solutions without netropsin. All of these fibers were slowly shortened, as typically illustrated in Fig. 8, indicating a dissociation of the NaDNA-netropsin complex in the ethanol solutions. The recovery of fiber length was almost complete, whereas the time needed for this experiment depended on the relative length, L/L_0, to be recovered and the ethanol concentration. Apparently, the bound netropsin is not firmly "fixed" on the DNA fibers. As the bulk concentration of netropsin falls down, which is achieved in our case by returning the NaDNA-netropsin fibers to netropsin-free solutions, the bound netropsin is released, depending on its binding site (weak or strong), and diffuses out from the fiber bundle, as revealed by the fiber shortening. Particularly at higher ethanol concentrations, where A-DNA is favored, the dissociation of the NaDNA-netropsin complex was faster. According to previous findings (Zimmer and Luck, 1972), some organic solvents (e.g., glycol), urea, and high salt concentration may serve as dissociative agents for NaDNA-netropsin complex dissolved in water solution. From our result, ethanol can be added to that category, at least for NaDNA-netropsin fibers in ethanol-water solutions within the concentration range studied here.

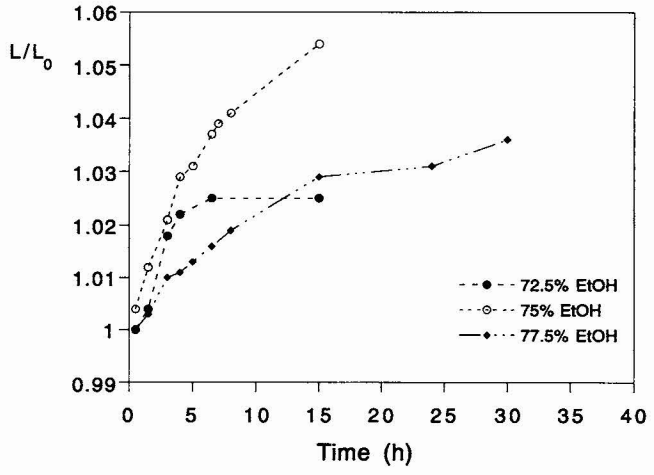

FIGURE 7 Complexation of NaDNA-netropsin in different ethanol-water solutions containing 0.8 mM netropsin, as revealed by the increase of the relative fiber length, L/L_0, with time (h) at room temperature.

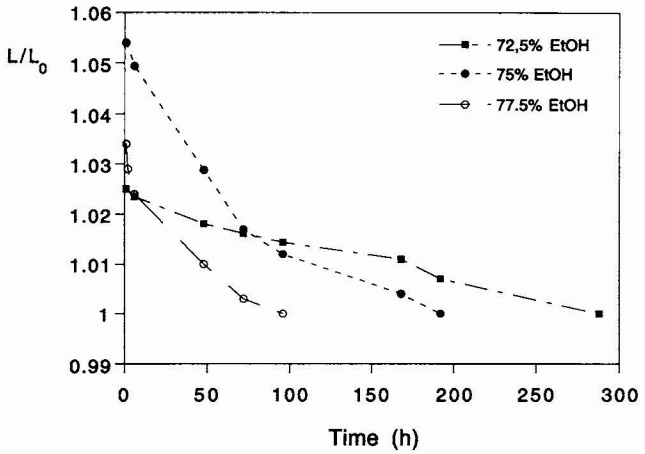

FIGURE 8 Dissociation of the NaDNA-netropsin complex in ethanol-water solutions without netropsin, as revealed by gradual fiber shortening and almost complete recovery to the original relative fiber length, L/L_0, of the NaDNA fibers.

Thermal melting of NaDNA and NaDNA-netropsin fibers in various alcohol solutions: experiment (iii)

The results of experiment (iii) are shown in Figs. 9–11. The heat-induced helix-coil transition was realized early by the observation of a contractile force that appeared temporarily in DNA fibers as the temperature was raised (Rupprecht, 1970b). Here, the transition is manifested macroscopically by a marked contraction of the DNA fibers because the average end-to-end distance in the coil state is much shorter than in the helix state. Fig. 9 shows representative melting curves for fiber bundles of NaDNA and NaDNA-netropsin in 70% ethanol and TFE solutions with and without netropsin.

The melting temperature, T_m, and the helix-coil transition width, ΔT, for fibers of NaDNA and NaDNA-netropsin in

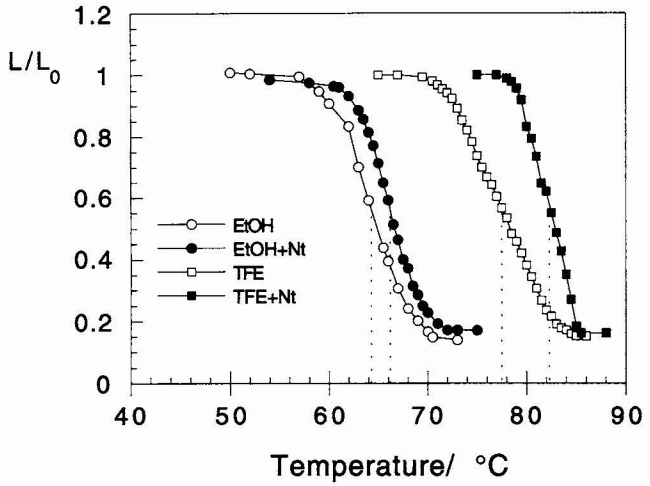

FIGURE 9 The melting curves of NaDNA and NaDNA-netropsin fibers in 70% ethanol or TFE solutions with and without 0.8 mM netropsin. The binding effect of netropsin on the melting temperature of B-DNA fibers is clearly seen. The vertical dashed lines indicate the respective T_m calculated with Eq. 1 from (Rupprecht et al., 1994).

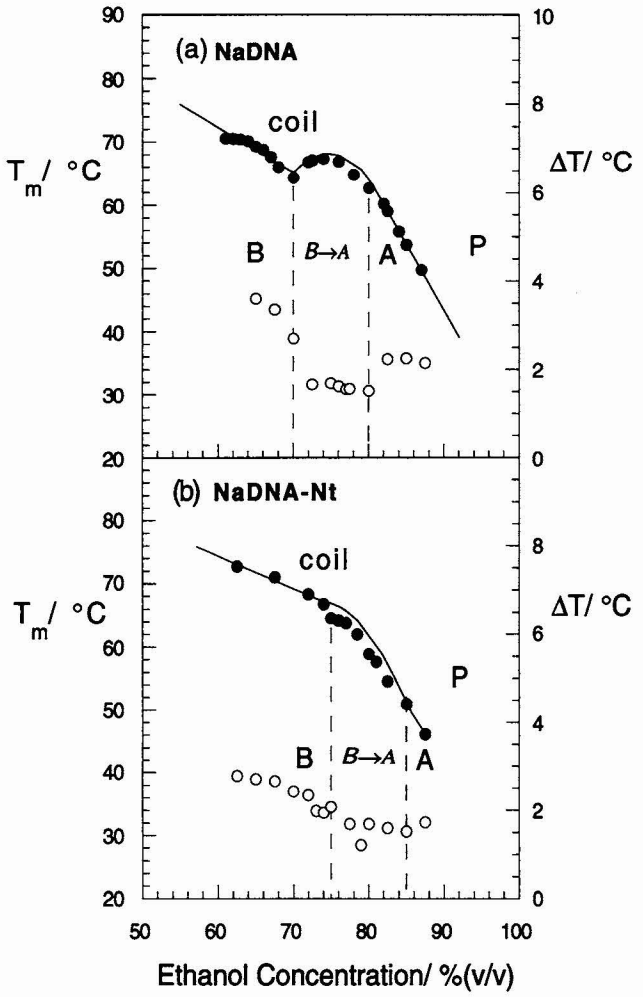

FIGURE 10 Melting temperature, T_m (●), and helix-coil transition width, ΔT (○), for fiber bundles of NaDNA (*a*) and NaDNA-netropsin (*b*) in ethanol-water solutions.

various ethanol and TFE solutions are calculated according to Eqs. 1 and 2 in (Rupprecht et al., 1994) (see Figs. 10 and 11 and Table 1). The melting behavior of NaDNA fibers in ethanol solution (Fig. 10 *a*) is in accordance with our previous result (Rupprecht et al., 1994).

The macroscopic melting properties of DNA fibers are affected by the stability of the DNA helix itself and by the aggregation, i.e., the interhelical interactions mediated by counterions, solvent molecules, etc. The aggregation effect is represented, for instance, by marked increase of T_m when DNA is precipitated in ethanol-water solution and, even more pronounced, by the formation of strongly aggregated P form of DNA (Nordén et al., 1978; Zehfus and Johnson, 1984) at very high ethanol concentrations (above 92% ethanol for NaDNA); see Fig. 12 presented earlier (Rupprecht et al., 1994). The strands of P-DNA are prevented from slipping past each other so that the fibers become thermostable (Rupprecht et al., 1994). In the B- and A-DNA regions, which lie on the left and right side of the B-A transition region, respectively, T_m decreases almost linearly with increasing alcohol concentration. This can be interpreted as being due mainly to the change of the electrostatic interaction, in particular the increased electrostatic repulsion among the phosphate groups of the DNA helix due to the lowering of the dielectric constant, and to the hydrophobic effect and the decreased water activity (change in the water structure).

A phenomenological approach for describing the melting temperature

The theory of Chogovadze and Frank-Kamenetskii on DNA melting in the B-A transition region (Chogovadze and Frank-Kamenetskii, 1983) was reported to agree experimentally with the melting of DNA dissolved in TFE-water solutions (Ivanov et al., 1983, 1985). However, as analyzed earlier (Rupprecht et al., 1994), this theory is not applicable for describing the melting properties of Li-, Na-, K-, and CsDNA fibers in ethanol solutions. Referring to the argument by Rupprecht et al. (1994), we found that this theory is not applicable to NaDNA fibers in TFE, or NaDNA-netropsin fibers in both ethanol- and TFE-water solutions either. Applying instead the phenomenological approach established earlier (Rupprecht et al., 1994), the melting temperatures of A- and B-DNA fibers can be described by linear equations

$$T_{m,J} = K_J Z + T^0_{m,J} \qquad (J = A \text{ or } B). \tag{3}$$

In the B-A transition region, the observed melting temperature is described as a weighted mean value

$$T_m = X_A T_{m,A} + (1 - X_A) T_{m,B}, \tag{4}$$

where Z is % alcohol; K_J is the slope, and $T^0_{m,J}$ is the intercept of the straight lines in Eq. 3; X_A, the fraction of A-DNA at a given Z value, is assumed to vary linearly

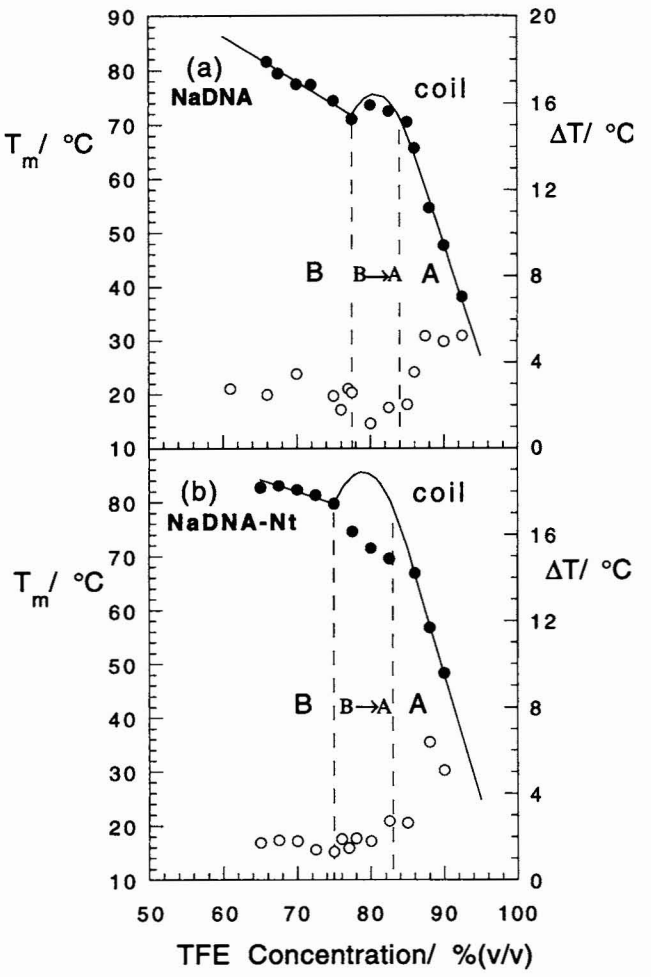

FIGURE 11 Melting temperature, T_m (●), and helix-coil transition width, ΔT (○), for fiber bundles of NaDNA (*a*) and NaDNA-netropsin (*b*) in TFE-water solutions.

between the beginning (Z_B) and end (Z_A) of the B-A transition region

$$X_A = \frac{Z - Z_B}{Z_A - Z_B}. \quad (5)$$

The values of $T^0_{m,J}$ and K_J defined in Eq. 3 are generated from experimental T_m data and are listed in Table 1. $T^0_{m,J}$ denotes the melting temperature of DNA fibers at a hypothetical state of 0% alcohol, and K_J characterizes the destabilization rate of the DNA helix with increasing alcohol concentration. Both $T^0_{m,J}$ and K_J depend on the types of counterions, as demonstrated for Na-, K-, or CsDNA fibers (Rupprecht et al., 1994), the DNA conformation, the complexation of DNA with netropsin, and the nonelectrolyte solution used. For each type of DNA fiber in defined alcohol solutions, $T^0_{m,A} > T^0_{m,B}$ and $|K_a| > |K_b|$ in general.

The solid lines in Figs. 10 and 11 are generated by using Eqs. 3–5. They fit the experimental data reasonably well except for the B-A transition region of NaDNA-netropsin fibers in TFE solutions (Fig. 11 *b*).

The influence of netropsin binding on T_m of NaDNA fibers in ethanol-water solutions

Comparing the T_m curves of NaDNA and NaDNA-netropsin in ethanol solution, it is found that the binding of netropsin raises the melting temperature of B-DNA fibers despite the fact that the calculated $T^0_{m,B}$ of NaDNA-netropsin is slightly lower than that of NaDNA. The reason is that the NaDNA-netropsin complex is characterized by a smaller $|K_B|$, which makes its T_m decrease less (with increasing ethanol concentration) than in the case of pure NaDNA. The smaller $|K_B|$ is another evidence of the stabilization of B-DNA by netropsin binding. However, the increase of T_m due to the formation of NaDNA-netropsin complex dissolved in aqueous solution is much more pronounced (Subra et al., 1991; Zimmer et al., 1971b, 1972). This difference might arise mainly from the different physical properties of precipitated and dissolved DNA. Because the aggregation of DNA in ethanol solution has already increased the melting temperature markedly, it is reasonable that a further increase of T_m due to netropsin binding should no longer be so pronounced. Besides, as the temperature increases, netropsin binding becomes less favored because of its large negative binding enthalpy as mentioned in the Introduction, in particular at the strong binding sites. Near the melting temperature, the input of netropsin in B-DNA fibers would be reduced as compared with the input at room temperature. This may also contribute to the smaller difference in T_m between B-NaDNA and its netropsin complex. Considering that the bulk concentration of netropsin is only 0.8 mM, the increase of T_m, nevertheless, is not trivial. In contrast, the salt concentration has no effect on T_m of pure DNA fibers. This was found experimentally and was justified by using the Poisson-Boltzmann cylindrical cell model, based on a pure electrostatic interaction between DNA and salt (Rupprecht et al., 1994; Schultz et al., 1994; Nilsson et al., 1985). Here the observed effect of netropsin on T_m of DNA fibers gives an additional indication that the interaction between DNA and netropsin is not purely electrostatic, but involves the combination of hydrogen bonding, Van der Waals contacts, and electrostatic attraction, as mentioned in the Introduction.

The appearance of a local maximum of experimental T_m in the B-A transition region of NaDNA fibers (Fig. 10 *a*) is well described by the phenomenological approach of Eqs. 3–5 (*solid line* in Fig. 10 *a*). For NaDNA-netropsin fibers in ethanol-water solutions (Fig. 10 *b*), this approach also gives a relatively good description of the principal behavior of their T_m, which lacks a local maximum in the B-A transition region. Referring to the analysis underlying Fig. 8 in the paper by Rupprecht et al. (1994), the lack of the local maximum is phenomenologically because of the fact that the two straight lines representing $T_{m,A}$ and $T_{m,B}$ cross near the left border of the B-A transition region (at 77.5% ethanol or 2.5% ethanol to the left of the B-A transition point). The reason is in part that the B-A transition region of the NaDNA-netropsin fibers is displaced to higher ethanol concentration, and in part that the crossing point of the straight lines is displaced to lower ethanol concentration (smaller $|K_B|$ and

$|K_A|$; see Table 1) compared with netropsin-free NaDNA fibers. It is clear, however, that the experimental T_m values lie somewhat below the solid line in the B-A transition region of Fig. 10 *b*. One possible explanation might be that within the B-A transition region, some netropsin molecules, in addition to groove binding to B-DNA, bind loosely to the outside of the DNA helix in fibers, thus reducing the stabilizing interhelical interaction and the melting temperature, T_m, of the DNA fibers as well.

In the A-DNA region, T_m decreases further as the ethanol concentration increases. The linear destabilizing rate is even higher than in the B-DNA region (see the larger $|K_A|$ value in Table 1). However, no significant difference in K_A is found between NaDNA and NaDNA-netropsin fibers. This is not surprising because the complex itself does not exist in the A-DNA region. According to Lindsay et al. (1988), the attractive interhelical interaction is stronger in A-DNA than in B-DNA. Nevertheless, the presence of netropsin slightly lowers $T_{m,A}$ of the fibers. This might be due to the reduction in the attractive interhelical interaction of DNA fibers caused by outside binding of netropsin, as was suggested above. But for both NaDNA and NaDNA-netropsin fibers in the A-DNA region, the lowering of the water activity and the dielectric constant lead to an increasingly stronger repulsive interaction between the negatively charged phosphate groups of DNA, which is now dominant and more directly responsible for the decrease of T_m.

The influence of TFE on T_m of NaDNA and NaDNA-netropsin fibers

Using TFE instead of ethanol gives two conspicuous effects. The first is that the phenomenological approach of describing T_m in the B-A transition region of NaDNA-netropsin fibers is no longer valid. A marked maximum in the phenomenological T_m in the B-A transition region has no counterpart in the experimental T_m (Fig. 11 *b*). Referring to a similar behavior of T_m of CsDNA fibers in ethanol solutions (Rupprecht et al., 1994), this indicates a decreased interhelical interaction, possibly due to the occurrence of some loosely bound netropsin molecules on the outside of the DNA helices (considering that the netropsin/DNA ratio might be low in our case). Comparing with the T_m curve of NaDNA-netropsin in ethanol solutions (Fig. 10 *b*), it can be realized that phenomenologically the difference is related to the larger negative value of K_A in the A-DNA region of Fig. 11 *b* and to the fact that the crossing point of the straight $T_{m,A}$ and $T_{m,B}$ lines lies to the right of the B-A transition region. The second effect of using TFE instead of ethanol is that the NaDNA and NaDNA-netropsin fibers do not become thermostable at very high TFE concentrations. Also, it is noticed that the difference in melting properties between NaDNA and NaDNA-netropsin fibers is in accordance qualitatively with the difference observed in ethanol solutions. Therefore, the relevant analysis above in principle can be extended to TFE solutions as well. Although in TFE solution the effect of netropsin binding on fiber elongation is less observable from experiments (i) and (ii) at room temperature, the binding effect of netropsin on fiber T_m is quite obvious in experiment (iii). This means that the complexation does occur in the B-DNA region and in the B-A transition region of TFE solution as well.

For both NaDNA and NaDNA-netropsin fibers, T_m in TFE solution is higher than in ethanol solution, characterized also by their higher $T^0_{m,A}$ and $T^0_{m,B}$ (Table 1). The interpretation used in experiment (i) above also seems applicable here. That is, in TFE solution the water activity and the dielectric constant of the medium in the immediate vicinity of the DNA helices are not reduced as efficiently as in ethanol solution. Thus, the double helix is more stable than in ethanol solution of the same concentration. In other words, the DNA fibers in TFE solution are somewhat stabilized against both the conformational B-A transition and the helix-coil transition. In the A-DNA region, $T_{m,A}$ decreases more rapidly with increasing TFE concentration, expressed by a larger $|K_A|$ than in ethanol solution. Therefore, the difference between $T_{m,A}$ in TFE and ethanol solutions is reduced as the alcohol concentration increases. Concerning the interaction of DNA fibers with TFE or ethanol, however, there is one big difference when the water content is decreased further. Contrary to the formation of P-DNA, resulting in thermostable NaDNA fibers at high ethanol concentrations (Rupprecht et al., 1994), T_m becomes very low (below 40°C) when the TFE concentration is increased up to 95%. That is, no sign of the formation of P-DNA is found at this high TFE concentration. This reflects the more polar nature of TFE with respect to its effect on DNA fibers.

The helix-coil transition width

Concerning the helix-coil transition width of the fibers, ΔT, it is found that ΔT of B-DNA is lowered as the ethanol concentration increases. In the B-A transition region, ΔT reaches a minimum and in the A-DNA region, it goes up again; see Table 1 for the mean ΔT values in each region. The melting cooperativity of the fiber bundle is partly affected by the interhelical interactions or aggregation in the fibers. The low ΔT, or high cooperativity, in the B-A transition region can be correlated to the fact that only a common T_m, instead of separate $T_{m,A}$ and $T_{m,B}$, is measured during the melting process. As we interpreted recently (Rupprecht et al., 1994), the contractile force exerted by the B-DNA regions of the fiber (with lower T_m) would create a potential to drag the A-DNA regions (with higher T_m) into melting, whereas the A-DNA, stabilized by interhelical interactions, would have a tendency to delay the melting of B-DNA. As a result, a joint melting occurs and a common T_m is observed macroscopically. This mutual influence between A- and B-DNA leads to a higher melting cooperativity in the B-A transition region. For NaDNA-netropsin, the variation of ΔT with alcohol concentration has the same tendency as observed for NaDNA fibers in the corresponding netropsin-free solutions. As a main effect on melting cooperativity, the binding of netropsin gives lower ΔT in the B-DNA region. This is true for NaDNA-netropsin fibers in both ethanol and TFE solutions. Besides,

for either NaDNA or NaDNA-netropsin fibers in TFE solution, the ΔT value in the B-DNA region is less affected by TFE concentration. This indicates again the smaller sensitivity of DNA to TFE than to ethanol. At high TFE concentrations, the larger ΔT of both NaDNA and NaDNA-netropsin fibers is very likely an effect of increasing aggregation. However, as pointed out above, this aggregation is not strong enough in TFE to yield P-DNA and thermostable fibers, as was observed for all DNA fibers with alkali counterions in concentrated ethanol solutions (Rupprecht et al., 1994).

Information on the B-A transition from melting experiments

In addition to obtaining information on the helix-coil transition, experiment (iii) also provides us with a way to evaluate the B-A transition of DNA fibers at higher temperature. That is, the boundary concentrations of the B-A transition region, Z_B (% alcohol) and Z_A (% alcohol), can be analyzed from the solid lines in Figs. 10 and 11 and used to specify the B-A transition width of the DNA fibers near the melting temperature. The corresponding B-A transition point is approximated to $(Z_A + Z_B)/2$, and the values are listed in Table 1. Compared with the results from experiment (i) at room temperature, calculated by Eq. 1, the two approaches agree with each other in particular for NaDNA and NaDNA-netropsin fibers in ethanol-water solution, implying that the B-A transition of DNA is temperature-independent (Ivanov et al., 1973, 1974; Rupprecht et al., 1994). The fact that in TFE solutions the binding of netropsin actually has no effect on the B-A transition of NaDNA fibers can also be verified from the melting curves. But there are some discrepencies between these two approaches. In TFE solution, the B-A transition point of NaDNA and NaDNA-netropsin fibers estimated from melting temperature is somewhat lower than that calculated at room temperature. This result is somewhat uncertain because of experimental uncertainty. However, a partial abolition of the inhibition of the humidity-induced B-A transition at elevated temperature (40–45°C) has been observed in DNA films by other authors (Pohle et al., 1984; Kursar and Holzwarth, 1976). The transition width $Z_A - Z_B$ (% alcohol) evaluated from Fig. 10 is higher than ΔZ_{BA} (% alcohol) defined by Eq. 2. The deviation should mainly be due to their different definitions. A more direct comparison can be made by applying the assumed linear behavior of X_B $(=1 - X_A)$ of Eq. 5 in Eq. 2, which gives $\Delta Z_{BA}^{(iii)}$ (% alcohol) $\approx 0.29\,(Z_A - Z_B)$. As may be seen from Table 1, the resulting $\Delta Z_{BA}^{(iii)}$ (% alcohol) agrees with the ΔZ_{BA} (% alcohol) values in TFE solutions, but it is smaller than the corresponding ΔZ_{BA} (% alcohol) in ethanol solutions.

CONCLUSIONS

This comparative mechanochemical study of oriented NaDNA and NaDNA-netropsin fibers has given information on the B-A conformational transition and the helix-coil transition of the NaDNA-netropsin complex in fibers. The results support previous findings. In ethanol solutions, the binding of netropsin to NaDNA fibers will stabilize B-DNA and even convert part of A-DNA into B-DNA, as may be concluded from the fiber elongation and a displacement of the B-A transition region to higher ethanol concentrations. A maximum relative fiber elongation, due to the formation of NaDNA-netropsin complex, occurs around the B-A transition point of NaDNA fibers. The elongation demonstrates the combined effects of netropsin and ethanol, which in turn influence the fraction of B- and A-DNA. An increase of bulk NaCl concentration reduces the binding of netropsin, as revealed by a smaller fiber elongation and smaller displacement of the B-A transition region, compared with NaDNA-netropsin fibers at low salt concentration. The salt effect is interpreted as being due to competitive binding of counterions and netropsin to DNA. The complexation of DNA fibers with netropsin is nearly reversible. The bound netropsin can be released almost completely in netropsin-free ethanol-water solutions. TFE-water solution stabilizes B-DNA fibers, which makes the binding effect of netropsin on the conformational transition of NaDNA fibers less observable. From the melting experiments performed in ethanol- and TFE-water solutions, it is found that netropsin binding gives a higher fiber T_m in the B-DNA region and a lower T_m in the B-A transition region, indicating a stabilization of B-DNA. The higher B-A transition point, Z_{BA} (% alcohol), and higher T_m of NaDNA fibers in TFE-water solution are both interpreted with reference to the smaller capacity of TFE, as compared with ethanol, to decrease the water activity and the dielectric constant of the medium. The strongly aggregated P-DNA is not formed in TFE-water solution, not even in 95% TFE. Binding of netropsin in the B-DNA region increases the cooperativity of the helix-coil transition. The evaluation of the B-A conformational transition of NaDNA and NaDNA-netropsin fibers made from melting experiments gives results comparable with those obtained at room temperature.

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