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The formation of A-DNA in NaDNA films is suppressed by netropsin

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ABSTRACT

Oriented films of NaDNA complexed with netropsin were studied with deuterium nuclear magnetic resonance (^{2}H NMR), X-ray diffraction and ultraviolet (UV) linear dichroism to obtain information about the influence of netropsin on the structural arrangement of the DNA bases and on the B-A transition. The results of these studies clearly demonstrate a strong suppression of the formation of A-DNA at relative humidities (RHs) down to about 50%. The suppression was complete in the NaDNA-netropsin complex studied with ^{2}H NMR which had a netropsin input ratio, r, of 0.22 drug/base pair. The sample used for UV linear dichroism had a similar input ratio while the X-ray diffraction samples had input ratios between 0.033 and 0.39 drug/base pair. Together, the results of these studies are in agreement with previous infrared (IR) linear dichroism studies of the conformation of the sugar-phosphate backbone in NaDNA-netropsin complexes, which showed that the B-A transition is suppressed for r-values down to approximately 0.1 drug/base pair (Fritzsche, H., Rupprecht, A. and Richter, M., Nucleic Acids Res. 12 (1984) 9165 – 9177).

INTRODUCTION

DNA is conformationally flexible, i.e., depending on its surrounding media, it can adopt different conformations (B, A, Z, and others) (1). Historically, the existence of the B and A form was described in the early days of DNA fiber X-ray diffraction studies (2–5). Hydrated films and fibers of NaDNA exist in a fairly crystalline A form at relative humidities (RHs) below 85–90% with optimal crystallinity at about 75% RH, while the more amorphous, semi-crystalline B-DNA exists at higher RHs. The B-A transition is cooperative and reversible and is regulated, among other factors, by the water activity. In solution, increasing replacement of water by organic solvents (e.g. ethanol) converts DNA from the B into the A form (6,7). This conformational flexibility may be of importance for the correct biological functioning of DNA. For example, during transcription the existence of a transient DNA-RNA hybrid is assumed. Since RNA exists exclusively in the A form, this DNA-RNA hybrid is also supposed to be in the A form (8,9). The mechanism of the Zn 'fingers' of the transcription factor might also involve A-DNA (10,11). Recently it was shown that binding of small acid-soluble spore proteins promote the B-A conformation change that underlies the UV resistance of spores of Gram-positive bacteria (12). Any suppression of the B-A transition restricts the conformational flexibility of DNA and may therefore interfere with many important biological processes.

Infrared (IR) linear dichroism studies have shown the potency of several drugs to suppress the B-A transition of DNA stretches much longer than the binding site of the drug on DNA (13–17). Depending on the relative drug concentration, a substantial fraction of DNA was shown to lose its conformational flexibility and to remain in the B form even at RHs below 75%. Netropsin (Fig. 1) is a DNA-binding agent with antibacterial, antiviral and antineoplastic properties (18). Although too toxic for clinical use, it has been studied extensively as the prototype of a non-intercalative DNA-binding drug. It is one of the most potent drugs to suppress the formation of A-DNA ; at 50–75% RH 24 base pairs of DNA are blocked by one netropsin molecule (15,19,20). Netropsin is known to bind preferentially to AT stretches of DNA in the minor groove, but exclusively to B-DNA (18,19,21–23). Netropsin even converts A-DNA back to B-DNA in alcoholic solutions (24). In a cocrystal of netropsin and the dodecamer duplex $[d(CGCGAATTCGCG)]_2$ it was shown by X-ray diffraction that the spine of hydration in the minor groove of B-DNA was replaced by the drug (25,26). Additional information

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about the structure was obtained in the recent X-ray diffraction study of a single crystal of the d(CGCGATATCGCG)-netropsin complex (27). The results suggested that the drug seems to bind equally well in two different orientations in the crystal lattice by use of single hydrogen bonds rather than bifurcating bonds. Other stabilizing forces are provided by ionic and van der Waals interactions.

These findings should be important for the elucidation of some biological processes directed by the DNA as well as for an understanding of the action of drugs at a molecular level. Therefore, we have studied the conformation of NaDNA in films of oriented NaDNA-netropsin complex by deuterium nuclear magnetic resonance (^{2}H NMR), X-ray diffraction and ultraviolet (UV) linear dichroism. Of these methods, the first and last reflect, in particular, changes of the base inclination with respect to the helix axis, which specifies the orientation direction of the samples. The results obtained clearly demonstrate that NaDNA complexed with netropsin is in the B form even under conditions of low water activity (RHs down to 50%). Accordingly, the results of this study complement previous findings about the B-A transition (15) which were based on the conformational changes of the sugar-phosphate backbone observed with IR linear dichroism (28,29). It was found that the suppression of the A form in the RH interval down to 54% was complete for r-values of approximately 0.1 drug/base pair (15).

MATERIALS AND METHODS

Materials

Highly polymerized calf-thymus NaDNA was used: Pharmacia (lot # 534562) for the ^{2}H NMR and X-ray diffraction studies and Worthington (lot # 35S749) for the UV linear dichroism study. Netropsin hydrochloride (MW= 466.9) was a purified research grade product (ϵ_{max}= 23500 l cm^{-1} mol^{-1} at 296 nm) of the Institute of Microbiology and Experimental Therapy, Jena, FRG (commercially available through SERVA, Heidelberg, FRG).

^{2}H NMR

Protons at the 8-position of guanine and adenine were exchanged with deuterons (30) by incubating a NaDNA-D_2O solution for 10 days at 65°C (31). The so ^{2}H labelled NaDNA was oriented with the wet spinning method (32–34). The spun deposit on the cylinder was bathed for three weeks in 60 ml 75% ethanol/25% water (v/v) with 0.03 M NaCl and 1.429 mM netropsin at neutral pH. From the 8 mm wide oriented film obtained after the drying procedure (containing about 1% NaCl by dry weight (34)), a parallelepipedic sample of oriented NaDNA-netropsin complex was prepared (33) with the approximate dimensions 2.5×2.5×8 mm. This approach of loading the drug was chosen rather than the simpler method of directly depositing it onto the sample surface because of the problem in obtaining a homogeneous distribution in this thick DNA film (~70 μm as compared to ~0.45 μm for the films used for UV linear dichroism). The uptake of netropsin was determined by measuring the optical density at 300.9 nm of netropsin in the ethanolic bath before and after the bathing, and using a pre-calibrated curve of absorbance as a function of netropsin concentration in ethanolic baths. The drug input ratio, r, of this NaDNA-netropsin sample used for ^{2}H NMR was found to be 0.22 drug/base pair. A drug-free oriented NaDNA sample of the same dimensions was also prepared from the same DNA lot using an identical protocol but without netropsin, and was used as a reference. Both the NaDNA-netropsin and the reference sample, equilibrated at 75% RH, were placed in sealed 5 mm (o.d.) NMR tubes.

The water content of these samples at 75% RH was determined gravimetrically at the end of the experiment. The total weight was first measured, and the samples were thereafter dried over silica gel for five weeks. During this drying procedure the complex and the reference lost approximately the same amount of water per nucleotide. Thus, the total water content of the NaDNA-netropsin complex was found to be approximately the same as for drug-free NaDNA, amounting to about 8 H_2O/nucleotide at 75% RH (34).

The DNA-D_2O interaction was assessed using 'high resolution ^{2}H NMR'. For this experiment, H_2O was partly exchanged with D_2O by keeping the NaDNA-netropsin sample above a saturated D_2O-NaCl solution (75% RH) for ~24 hours. Solid state ^{2}H NMR spectra were obtained on a homebuilt spectrometer operating at 38.4 MHz (35), and the water ^{2}H spectrum was recorded on a NT200 spectrometer (Nicolet Scientific Corp.) operating at 30.7 MHz (36). The spectra were acquired at 25 ± 1°C using π/2 pulses (2.0 μs at 38.4 MHz and 24.5 μs at 30.7 MHz) in a phase cycled quadrupolar echo sequence (37). In all these experiments the samples were oriented with the DNA helix direction along the magnetic field.

X-ray diffraction

0.109 mmoles nucleotide in the form of spun NaDNA fibers (MW per nucleotide = 331) were deposited with the wet spinning method (32–34) onto each of four spinning cylinders. The cylinders were thereafter bathed for 17 days at +5°C in four 35 ml 75% ethanolic baths at neutral pH with the following netropsin concentrations: 0.00 (reference), 0.057, 0.286, and 1.429 mM netropsin. The NaCl concentration was 0.03 M to give about 1% NaCl by dry weight in the final film (34). The four oriented films obtained after the drying procedure (thickness ~40 μm) had the following drug input ratios: r = 0.00 (reference), 0.033, 0.13, and 0.39 drug/base pair; see ^{2}H NMR above for the procedure of determing the uptake of netropsin from the ethanolic baths.

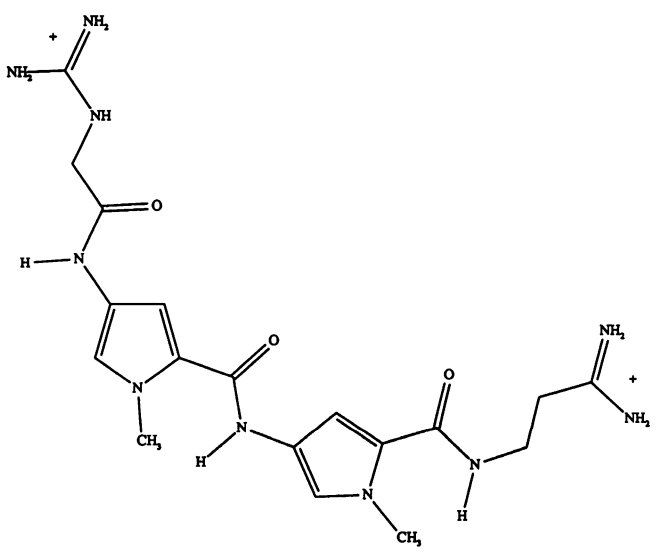

Figure 1. The structural formula of netropsin.

For the X-ray diffraction study, these films were folded into flat concertina-like packs (total thickness ~0.3 mm) which were mounted in holders as described earlier (34). A universal flat X-ray diffraction camera, Type PW 1030, was used with Nickel-filtered CuK_α radiation and a collimator of 500 μm. The specimen-to-film distance was 52.2 mm and the exposure time about 3 hours. Exposures were made after equilibration of the samples for several weeks at 52, 75 and 86% RH.

UV linear dichroism

Thin oriented NaDNA films (~0.45 μm), obtained with the wet-spinning method (38), were deposited on thin quartz plates. Netropsin was dissolved in 80% ethanol and an appropriate amount of this solution was placed directly onto the films. The drug input ratio, r, calculated stoichiometrically was approximately 0.25 drug/base pair, assuming that the drug is homogeneously absorbed by the film.

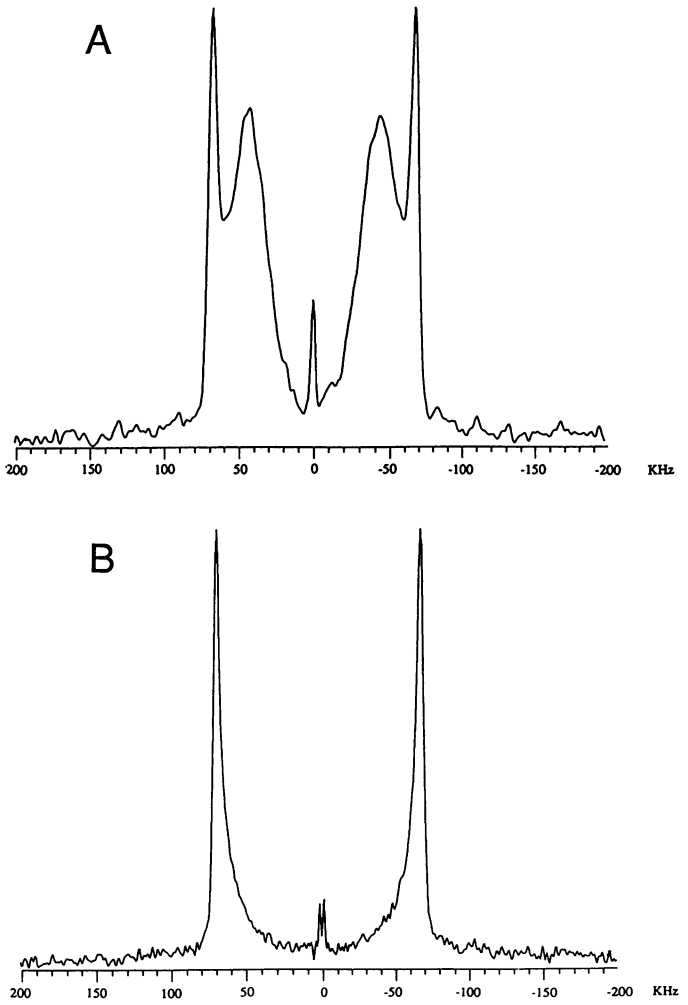

Figure 2. A) Solid state ^2H NMR spectrum of oriented NaDNA at 75% RH labelled with ^2H at the 8-position of guanine and adenine. The spectrum was acquired using 72000 scans, a line broadening of 3 kHz, and a pulse delay of 0.5 sec. The DNA helix direction is along the magnetic field. B) Solid state ^2H NMR spectrum of the oriented NaDNA-netropsin complex at 75% RH labelled with ^2H at the 8-position of guanine and adenine. The drug input ratio, r, is 0.22 drug/base pair. The spectrum was acquired using 93000 scans, a line broadening of 1 kHz, and a pulse dealy of 0.5 sec. The DNA helix direction is along the magnetic field.

The quartz plates were placed in cylindrical cells equipped with fluorite windows. The RHs inside the cells were maintained by saturated salt solutions (39). A polarizer with a transmission of 37% at 260 nm was used. The sample was arranged with an inclination of 45° between the entrance slit of the monochromator and the orientation direction of the DNA sample to suppress artifacts by polarization of the optical parts of the spectrometer. The spectra were recorded on a double-beam grating spectrophotometer Specord M40 (Carl Zeiss Jena, FRG) with parallel and perpendicular positions of the polarizer with respect to the orientation direction of the fixed DNA sample. The absorbance maxima of the two DNA spectra with parallel and perpendicular polarizations did not coincide. Depending on the RH, the spectra with parallel polarization had their maximum at 262–266 nm, while the maximum of the spectra with perpendicular polarization occurred at 258–259 nm. The dichroic ratio, R, was defined as the ratio of the absorbances at 260 nm of the spectra with parallel and perpendicular polarization, respectively:

$$R = \frac{A_{||}}{A_{\perp}} \qquad (1)$$

RESULTS AND DISCUSSION

^2H NMR

The solid state spectrum of the ^2H labelled NaDNA reference sample, equilibrated at 75% RH, is shown in Fig. 2A. As has been previously shown (40), NaDNA samples often contain a significant amount of B- or C-DNA (causing the outer peaks at ~ ±70 kHz) in addition to the expected A-DNA conformation (causing the inner peaks at ~ ±40 kHz). The particular shape of the spectrum in Fig. 2A can be simulated if a B-DNA content of 57±5% is assumed (40). In contrast, Fig. 2B shows that the NaDNA-netropsin complex has no A-DNA contribution at 75% RH, as is evident from the missing inner peaks.

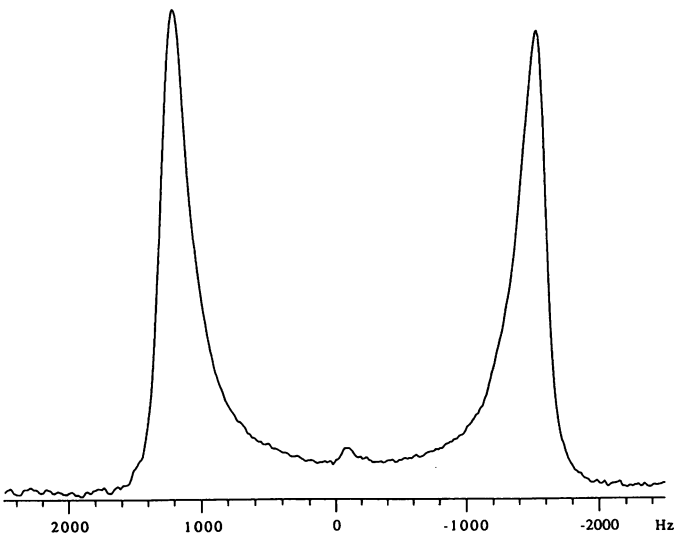

Figure 3. ^2H NMR spectrum of the hydration water (D_2O) in the oriented NaDNA-netropsin complex (r = 0.22 drug/base pair) at 75% RH. The spectrum was acquired using 640 scans, a 20 Hz line broadening, and a pulse delay of 0.1 sec. The DNA helix direction is along the magnetic field.

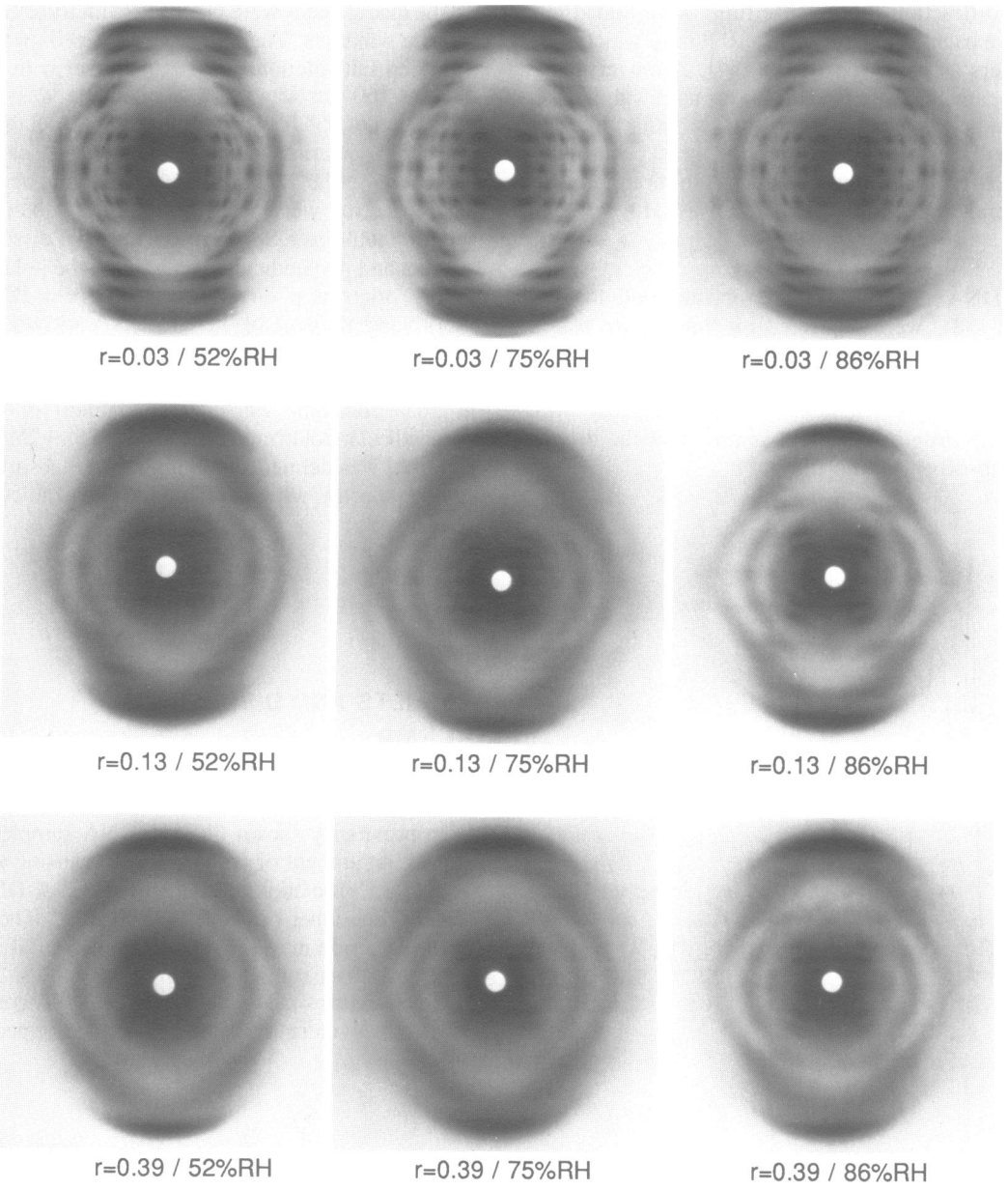

Figure 4. X-ray diffraction patterns of oriented NaDNA-netropsin complexes with r = 0.033, 0.13 and 0.39 drug/base pair, equilibrated at 52, 75 and 86% RH (relative humidity). The sample with r = 0.033 drug/base pair shows A form patterns while the B form prevails for r ≥ 0.13 drug/base pair. For the two lowest RHs some contribution of A form may be seen in the patterns with r = 0.13 drug/base pair.

DNA has previously been modelled using both static and dynamic parameters for the bases (35,40). In the simplest B-DNA model, the planes of the bases are, on the average, oriented at ~ 90° with respect to the DNA helix axis (sample axis) with a distribution width, σ. In addition, the bases are free to move within the limits $\pm\theta_0$. Typically, a distribution width $\sigma = 9\pm1°$ and a motion amplitude $\theta_0 = 12\pm2°$ was found in B-DNA (LiDNA) at 75% RH (10 H_2O/nucleotide) (35). However, the netropsin induced B-DNA (Fig. 2B) can best be simulated with $\sigma = 11\pm1°$ and $\theta_0 = 8\pm2°$. Apparently, the NaDNA-netropsin sample does not have as well ordered bases as a typical B-DNA sample, and seems to have a more restricted base motion. This difference may be explained by interference of the netropsin molecules with the packing of the DNA helices yielding a slight disorder in the helix axis orientations. Alternatively, the netropsin could interact directly with the bases/backbone to distort the base orientation and perhaps also reduce the base motion.

To assess if the water interaction with the NaDNA-netropsin complex differed from that with drug-free LiDNA, 'high resolution ^2H NMR' was used to study D_2O interacting with the complex (36). The NMR spectrum is shown in Fig. 3 (see also the central component in Figs. 2A and 2B). The observed splitting of 2.7 kHz is somewhat larger than expected since a splitting of $\sim1.8\pm0.6$ kHz is expected at a water content of ~8 D_2O/nucleotide (36). (The observed splitting is more similar to that found in LiDNA samples containing ~ 5 D_2O/nucleotide, 2.9 ± 0.9 kHz (36)).

Generally, a large spectral splitting is indicative of water molecules that, on the average, are oriented anisotropically. At high hydration levels the exchange of molecules between

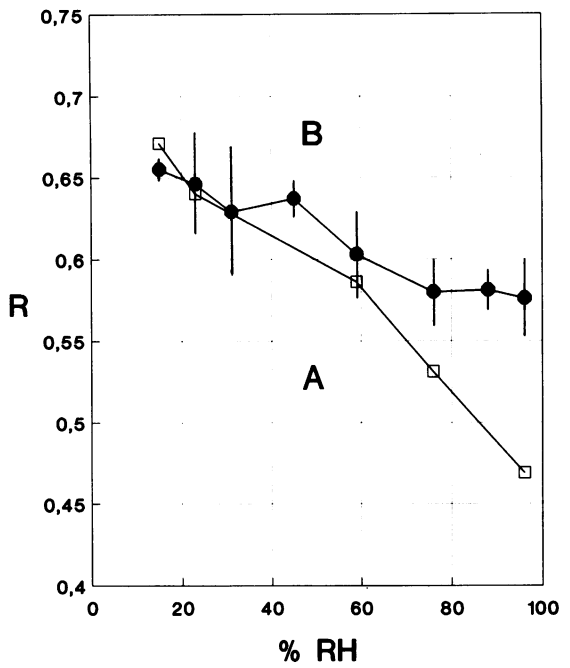

Figure 5. The dichroic ratio, R, at 260 nm of oriented films of NaDNA (squares, A) and NaDNA-netropsin complex with r = 0.25 drug/base pair (filled circles, B), as functions of the relative humidity (RH) of the ambient atmosphere. The error bars of curve B are typical for both curves.

anisotropic and isotropic sites cause the splitting to decrease (36). The splitting observed for the water in the NaDNA-netropsin complex was larger than in LiDNA, despite similar hydration. The water molecules therefore seem, on the average, to be more restricted in their reorientation in NaDNA that is complexed with netropsin than in drug-free LiDNA.

X-ray diffraction

As may be seen from the X-ray diffraction patterns in Fig. 4, the NaDNA-netropsin complex with r = 0.033 drug/base pair is in the A form (2,5,34,41) at all the RHs considered (like the reference patterns, not displayed). With $r \geq 0.13$ drug/base pair the samples display B form patterns (2,3,41) while reflexes characterizing the C form (4,42) are absent . Only at the lowest RHs (52% and 75%) does the sample with r = 0.13 drug/ base pair show some A form contribution in the patterns. The result of this X-ray diffraction study clearly shows that the A form is suppressed by netropsin in favour of the B form.

UV linear dichroism

Fig. 5, curve A, shows that the UV dichroic ratio, R, of drug-free NaDNA increases monotonously with decreasing RH. This increase in R reflects the change in inclination of the bases as the DNA conformation changes from B to A in the region 85–65% RH (43). On further drying the bases become more randomly oriented due to a partial (but reversible) disordering of the DNA structure (35,43) possibly involving loss of base stacking and probably also base pairing (43). Fig. 3 of Ref. (43) shows that R has a sharper transition than in Fig. 5, curve A, indicating that the structural changes in the present NaDNA film occured over a wider RH range. This may be due to different degrees of crystallinity in the these films (41) and to the restriction which the carrier quartz plate imposes on the thin, wet-spun NaDNA film, preventing the contraction which accompanies the B-A transition (38,43).

The relationship between R versus RH is different in the NaDNA-netropsin complex (Fig. 5, curve B) compared with the drug-free DNA (curve A). The dichroic ratio, R, of the complex film does not change significantly for RH values between 96% and 54% and it is higher than for the drug-free DNA. This higher level may be due to some contributions from netropsin to the absorbances in spite of the fact that netropsin has a minimum in the absorbance around 260 nm (18) where DNA has its maximum (38,43). Contributions to both $A_{||}$ and $A_{\perp}$ are to be expected because the netropsin molecules are located in the minor grove, which is inclined about 45° with respect to the helix axis; cf. Ref. (44). Further dehydration of the complex below 54% RH is connected with an increase of R, and at very low RH (<30%) both the complex and the drug-free DNA show similar R values. This increase of R (synonymous to a reduced dichroism) is probably due to the partial disordering of the DNA structure (43). It is, however, somewhat surprising, at first, that similar ^{2}H NMR studies of DNA at low humidities have only shown a small disorder of the bases (35). This might be explained by the fact that ^{2}H NMR and UV dichroism are monitoring different orientation angles of the bases. With ^{2}H NMR the relevant orientation vector lies along the long axis in a base pair, while the optical transition moments are roughly perpendicular to that axis and located in the base planes (45). Therefore, even if the base tilt only changes marginally, a large disorder could still be observed by UV dichroism if the base roll would be large.

This UV linear dichroism study by itself hardly permits definite conclusions about the DNA conformation in the NaDNA-netropsin film. However, in view of the ^{2}H NMR and X-ray diffraction results it is suggested that the nearly constant R value displayed by the complex between 96% and 54% RH (Fig. 5, curve B) could be interpreted as supportive of the occurrence of the B form in this RH region. Independent support for this interpretation is provided by earlier IR linear dichroism studies of the NaDNA-netropsin complex (15).

CONCLUSIONS

Oriented films of NaDNA complexed with netropsin have been studied by ^{2}H NMR, X-ray diffraction and UV linear dichroism to obtain information about the influence of netropsin on the structural arrangement of the bases and on the B-A transition of DNA. The suppression of A-DNA was complete in the sample with r = 0.22 drug/base pair that was studied with ^{2}H NMR at 75% RH, and the X-ray diffraction study showed a nearly complete suppression for r = 0.13 drug/base pair. It was also suggested that the nearly constant dichroic ratio level observed with UV linear dichroism for a NaDNA-netropsin film with r = 0.25 drug/base pair between 54% and 96% RH could be interpreted in favour of B-DNA in this RH region. Together, the results support previous IR linear dichroism studies of the conformation of the sugar-phosphate backbone in NaDNA-netropsin complexes, indicating that the B-A transition is suppressed by the ligand netropsin for r-values down to approximately 0.1 drug/base pair.

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