Interaction of water with oriented DNA in the *A*- and *B*-form conformations

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ABSTRACT High resolution ^2H nuclear magnetic resonance (NMR) was used to investigate the interaction of D_2O with solid samples of uniaxially oriented Li-DNA (*B*-form DNA) and Na-DNA (*A*- and *B*-form DNA). At low levels of hydration, 0 ~ 4 D_2O/nucleotide, the ^2H spectra shows a very weak (due to short T_2) broad single resonance, suggestive of unrestricted rotational diffusion of the water. At ~5 or more D_2O/nucleotide, the Li-DNA (*B*-form) spectra suddenly exhibit a large doublet splitting, characteristic of partially ordered water. With increasing hydration, the general trend is a decrease of this splitting. From our analysis we show that the DNA water structure reorganizes as the DNA is progressively hydrated. The D_2O interaction with Na-DNA is rather different than with Li-DNA. Below 10 D_2O/nucleotide Na-DNA is normally expected to be in the *A*-form, and a small, or negligible splitting is observed. In the range 9–19 D_2O/nucleotide, the splitting increases with increasing hydration. Above ~20 D_2O/nucleotide Na-DNA converts entirely to the *B*-form and the D_2O splittings are then similar to those found in Li-DNA. We show that the complex Na-DNA results obtained in the range 0–20 D_2O/nucleotide are caused by a mixture of *A*- and *B*-DNA in those samples.

INTRODUCTION

Water plays a crucial role in modulating the properties of DNA. It is known that the conformational state of DNA is sensitive to the activity of water (Texter, 1978; Leslie et al., 1980; Zimmerman and Pheiffer, 1980) and the role water plays in the interconversion of DNA between *B*, *A*, and *Z* forms has been discussed in some detail in an analysis of crystallographic data on DNA (Kennard et al., 1986; Saenger et al., 1986). The binding of proteins, or other macromolecules, to DNA necessarily induces a rearrangement of the water at the protein-DNA interface. While the role of water is often neglected when considering DNA-protein recognition, it must be an important part of the process (Texter, 1978; Helene and Lancelot, 1982). There is, therefore, considerable interest in evaluating the effect of DNA sequence and conformation on the DNA-water interactions. Sometime ago Migchelsen et al. (1968) used deuterium nuclear magnetic resonance (NMR) to study the interaction of D_2O with samples of oriented Na- and Li-DNA. In a recent paper (Brandes et al., 1988*c*) we used ^2H NMR to study the interaction of water with an assembly of DNA molecules, and showed that this method could be used to monitor the DNA organization in different types of sample preparations (random powders, uniaxially oriented DNA, and cholesteric films). Here, we use ^2H NMR to study the interaction of D_2O with samples of solid, uniaxially oriented Na- and Li-DNA in the *A*- and *B*-form. As we will show, the variation of the observed splitting of the ^2H doublet spectra with hydration, cannot be interpreted in terms of a simple model in which there is sequential binding of water to DNA sites of decreasing binding energy, without any change in the nature of the previously occupied sites. To account for the variation in splitting with hydration we require reorganization of the water structure during hydration.

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MATERIAL AND METHODS

Solid samples of uniaxially oriented Na- and Li-DNA were prepared from high molecular weight calf thymus DNA (product number 27-4562; Pharmacia Fine Chemicals, Piscataway, NJ) by the wet-spinning method (Rupprecht, 1966, 1970). Three sample preparations of Na-DNA, denoted I (lot #514562), II (lot #514562), and III (lot #534562), were made at different occasions. These preparations were equilibrated in 80% EtOH and 0.03 M NaCl, to obtain an excess NaCl concentration of 1.2% by dry weight (Rupprecht and Forslind, 1970), and showed identical x-ray diffraction patterns when equilibrated at 75% relative humidity (RH). A higher salt concentration was obtained in the three Li-DNA preparations: I (lot #514562), II (lot #534562), and III (lot #534562), by equilibration in 80% EtOH and 0.25 M LiCl, which produces an excess LiCl concentration of 5.9% by dry weight (Rupprecht and Forslind, 1970). The water content of the samples were determined as described before (Brandes et al., 1988*a*), assuming a

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water absorption for the Li-DNA samples of 39.4% (mg D_2O/mg dry DNA) at 66% RH, and 49.9% (mg D_2O/mg dry DNA) for the Na-DNA samples at 75% RH. The water absorption, determined gravimetrically as a function of different relative humidities, is shown in Table 1. Deuterium NMR spectra of Na-DNA I and II, and Li-DNA I were recorded at 38.4 MHz with quadrature phase detection at 25 ± 0.5°C using a broadband spectrometer (constructed by R. R. Vold and R. L. Vold), interfaced to a 2090 oscilloscope, and a 1280 computer system (Nicolet Scientific Corp., Northvale, NJ). A NT 200 spectrometer (Nicolet Scientific Corp.) operating at 30.7 MHz was used to record spectra from Na-DNA III and Li-DNA II and III. Solid state ^2H spectra were obtained on a GN 500 spectrometer operating at 76.8 MHz equipped with a 2090 oscilloscope (Nicolet Scientific Corp.) and an external 1 kW power amplifier (model LPI-10, ENI Inc., Rochester, NY). A phase cycled $(\pi/2)_x - \tau_1 - (\pi/2)_y - \tau_2 -$ Acq quadrupole echo sequence (Davis et al., 1976) was used with 2.0 μs $(\pi/2)$ – pulses at 38.4 MHz, 3.8 μs at 76.8 MHz, and 24.5 μs at 30.7 MHz. The water spectra were accumulated using pulse spacings, $\tau_1 = \tau_2 = 100$ μs, and ~100 scans, while $\tau_1 \sim \tau_2 \sim 50$ μs and 10,000 scans were used to obtain the "solid-state" spectra of DNA bases, which were labeled with deuterium at the purine 8 position (see Results and Discussion). The splitting between the two peaks in the water spectra (see Theory section) were determined by lineshape simulations whenever the two resonances significantly overlapped.

THEORY

In a preceeding study (Brandes et al., 1988*c*), we discussed how the interaction of water (D_2O) with a DNA-molecule could be studied by ^2H NMR. In analyzing the spectra, it was assumed that the water molecules transiently occupied several different binding sites, and that at each site *i* the water orientational distribution function was characterized by two second rank order parameters, S_{0i}^2 and S_{2i}^2 (Halle and Wennerström, 1981). The simplest model of restricted reorientational water diffusion is the "diffusion-in-a-cone model" (Warchol and Vaughan, 1978), in which the water C_2-axis is

TABLE 1 Measured ^2H NMR doublet splitting of D_2O for the DNA axis oriented parallel to the magnetic field ($\theta_{AL} = 0°$)

Sample type	RH*	Preparation I		Preparation II		Preparation III	
		N‡	$\Delta\nu$§	N‡	$\Delta\nu$§	N	$\Delta\nu$§¶
	%	*D_2O/nucleotide*	*Hz*	*D_2O/nucleotide*	*Hz*	*D_2O/nucleotide*	*Hz*
Li-DNA	0	0.5	no signal observed				
	6						
	20	2.5	~0‖				
	35	5.2	2,600		3,896		2,278
	47	5.6	2,300		3,076		2,134
	52	6.7	1,970	(not measured)	2,435	(not measured)	1,740
	66	7.3	1,950		2,236		1,340
	75	9.6	1,780		2,200		1,084
	79	11.8	1,870		2,100		980
	84	16.8	1,046		1,110		700
	88	21.7	375		390		444
	92	24.2	207		370		231
	97	24.4	165		200		
Na-DNA	0	1.0	~0	0.3	~0		
	6			1.3	~0		
	20	2.8	~0	2.8	~0		~0
	35	5.7	0	5.1	370		0
	47	6.0	0			(not measured)	0
	52	7.2	0	6.7	675		50
	66	7.7	0	7.3	536		238
	75	9.2	0	8.5	265		148
	79	10.0	187	8.7	240		0
	84	12.3	440	11.0	210		204
	88	19.4	900	18.4	906		560
	92	21.2	710	20.0	730		480
	97	21.8	630	20.8	668		320

*Relative humidity values were obtained from table (Weast, 1971). The temperature (room temperature) was not controled during equilibration. Estimated accuracy: ±4%.

‡Estimated accuracy of the measurement: ±0.4 D_2O/nucleotide.

§Estimated accuracy of the measurement: ±100 Hz.

¶Measured at $\theta_{AL} = 90°$. To compare with the splittings measured at $\theta_{AL} = 0°$, multiply the splittings in this column by 2 (see Eqs. 7a and 7b).

‖Only a broad poorly resolved (due to bad signal-to-noise ratio) resonance was observed.

allowed to diffuse freely within a cone of half angle θ_0, and spinning about this axis is unrestricted. For this model, the orientational distribution function is characterized by a single order parameter, S_0^2 since $S_2^2 = 0$. This motional model is probably not valid in our case since bound water molecules would more likely execute flips and, in general $S_2^2 \neq 0$.

Spectral effects of exchange

Because of rapid exchange of water molecules (or deuterons) between binding sites, the averaged order parameters $\langle S_0^2 \rangle$ and $\langle S_2^2 \rangle$ were introduced. The observed spectral splitting, $\Delta\nu$, is then related to the averaged reduction factor, $\langle A \rangle_N$, as follows (Halle and Wennerström, 1981; Brandes et al., 1988*c*):

$$\Delta\nu = \frac{1}{2}\nu_Q |(3\cos^2\theta_{AL} - 1)\langle A \rangle_N|, \tag{1}$$

where
$$\langle A \rangle_N = \frac{1}{2}\{3\cos^2\alpha - 1 + \eta\sin^2\alpha\}\{\langle S_0^2 \rangle_N\} - \frac{1}{2\sqrt{6}}\{3\sin^2\alpha + \eta(\cos^2\alpha + 1)\}\langle S_2^2 \rangle_N$$
$$\approx 0.098\,\langle S_0^2 \rangle_N - 0.413\,\langle S_2^2 \rangle_N, \tag{2}$$

and
$$\langle S_m^2 \rangle_N = \sum_{i=1}^{N} p_i S_{mi}^2 \qquad m = 0, 2. \tag{3}$$

Here $\frac{4}{3}\nu_Q = (e^2qQ/h) \sim 213$ kHz is the deuterium quadrupole interaction constant, $\eta \sim 0.11$ is the asymmetry parameter (Waldenstein et al., 1964), $\alpha \approx 52.2°$ is one-half of the DOD angle of water molecules, θ_{AL} is the angle between the DNA axis and the magnetic field, p_i is the fractional occupancy of the ith site, and N is the total number of available sites. Note that the averaged reduction factor, $\langle A \rangle_N$, can be positive or negative depending on the sign of the two averaged order parameters, and their relative magnitude. The observed splitting is, however, always positive, and the sign of $\langle A \rangle_N$ can therefore not be determined.

If an additional NMR spectrum is collected from another nuclei in the water, for example ^{1}H, a different dependence of $\langle A \rangle_N$, and consequently the splitting, on the two order parameters is expected (Halle and Wennerström, 1981). With this additional information it is, in principle, possible to solve for the magnitudes of both $\langle S_0^2 \rangle_N$ and $\langle S_2^2 \rangle_N$. However, because these order parameters would not improve our selection of appropriate hydration model (see below), we did not attempt to find them.

Models of DNA hydration

A theoretical model of DNA hydration can be tested by comparing its calculated order parameters with those obtained experimentally. In the following, two plausible hydration models will be presented, and the results presented in the next section will be used to select the most appropriate model.

(i) Sequential hydration model

The simplest hydration model considers the water molecules to bind sequentially to surface sites in order of decreasing binding energy. The binding sites are further assumed to have either unit or zero probability of being occupied, and the exchange of water molecules only takes place between the occupied sites (i.e., sites with unit probability). In this case, the total number of water molecules in the sample equals the number of occupied binding sites. (Note, because we use random sequence DNA, it is not possible to discriminate between preferential hydration of the nucleotides. The hydration, N, is therefore expressed as an average number of waters per nucleotide.) According to the sequential hydration model, the order parameters, when averaged with a total of $(N - 1)$ water molecules, $\langle S_m^2 \rangle_{N-1}$, are given by

$$\langle S_m^2 \rangle_{N-1} = \frac{1}{(N-1)}\sum_{i=1}^{N-1} S_{mi}^2, \qquad m = 0, 2. \tag{4a}$$

If we assume that the individual order parameters, S_{mi}^2, are independent of the level of hydration, then $\langle S_m^2 \rangle_N$ is related to $\langle S_m^2 \rangle_{N-1}$ as follows:

$$\langle S_m^2 \rangle_N = \frac{1}{N}\left\{\sum_{i=1}^{N-1} S_{mi}^2 + S_{mN}^2\right\}, \qquad m = 0, 2. \tag{4b}$$

Combining Eqs. 4a and b yields

$$\langle S_m^2 \rangle_N = \frac{1}{N}\{(N-1)\langle S_m^2 \rangle_{N-1} + S_{mN}^2\}, \qquad m = 0, 2. \tag{4c}$$

If the averaged order parameters are known for two hydration levels, then it is possible to calculate the individual order parameters, S_{mN}^2, at site N by rearranging Eq. 4c as follows;

$$S_{mN}^2 = N\langle S_m^2 \rangle_N - (N-1)\langle S_m^2 \rangle_{N-1}, \qquad m = 0, 2. \tag{5}$$

Since we only obtained spectra of the ^{2}H nucleus here, it is not possible to determine the averaged order parameters, $\langle S_m^2 \rangle_N$, needed to calculate the individual order parametes S_{mN}. However, if the averaged reduction factor, $\langle A \rangle_N$, can be found, then the individual reduction factors, A_N, can be calculated by combining Eqs. 2, 4a and b, according to;

$$A_N = N\langle A \rangle_N - (N-1)\langle A \rangle_{N-1}. \tag{6}$$

(ii) Reorganizational hydration model

In a second model of hydration, the sequential addition of water molecules is assumed to cause a reorganization of

the total water structure. The individual order parameters of water molecules in previously occupied sites then change in response to the filling of new sites. Alternatively, water molecules in previously occupied sites might move to previously unoccupied sites in response to the addition of a water molecule. In these cases the correlation between the averaged reduction factors $\langle A \rangle_{N-1}$ and $\langle A \rangle_N$ is unknown, and the individual reduction factors, A_N, cannot be determined.

RESULTS AND DISCUSSION

Deuterium NMR spectra of the DNA hydration water (D_2O) have been obtained for solid Li- and Na-DNA samples, as a function of hydration. A typical spectrum of a Li-DNA sample with an average of 7.3 water molecules/nucleotide is shown in Fig. 1, and the observed splitting is tabulated in Table 1. The inside trailing edges of the resonances in Fig. 1 are caused by a distribution of helix axis orientations about $\theta_{AL} = 0°$ (Brandes et al., 1988*c*). When the effects of this distribution are taken into account, the magnitude of the "true" averaged reduction factors, $|\langle A \rangle_N|$, computed from the observed splittings, $\Delta\nu$, are slightly larger than the ones calculated from Eq. 1 (Brandes et al., 1988*c*). However, since the difference is small (~7% at $N \sim 9$) (Brandes et al., 1988*c*), the effects of such a distribution have been neglected in the calculations of $|\langle A \rangle_N|$. Note, at $\theta_{AL} = 0°$, Eq. 1 simplifies to

$$|\langle A \rangle_N| = \frac{\Delta\nu}{\nu_Q}, \tag{7a}$$

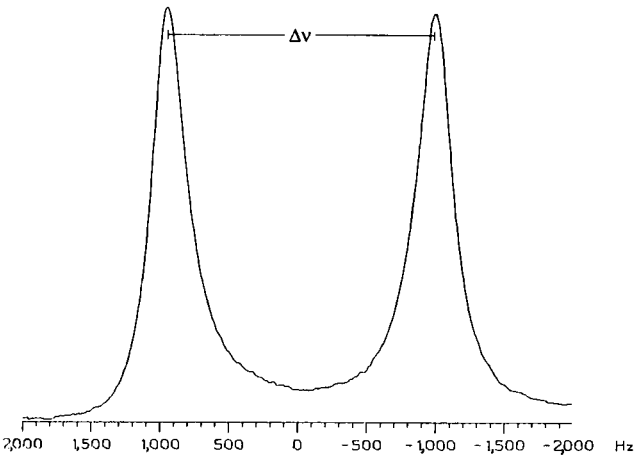

FIGURE 1 ^2H NMR spectrum of D_2O in solid Li-DNA at a hydration of 7.3 D_2O/nucleotide, and $\Theta_{AL} = 0°$ (preparation I).

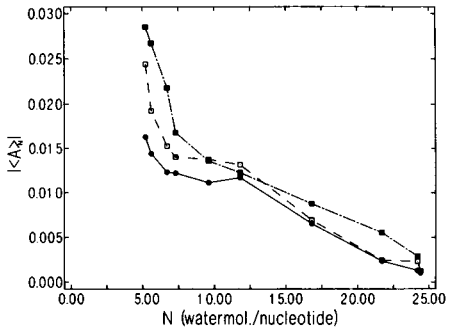

FIGURE 2 Measured magnitude of the averaged reduction factors, $|\langle A \rangle_N|$, of D_2O in Li-DNA. Preparation I (●, ——), preparation II (□, ------), preparation III (■, —— - ——). Because the water contents of preparations II and III were not measured, the values from preparation I were used to plot preparations II and III. The lines have been drawn as an aid for the eye.

and at $\theta_{AL} = 90°$

$$|\langle A \rangle_N| = 2\frac{\Delta\nu}{\nu_Q}. \tag{7b}$$

The calculations for the Li- and Na-DNA samples are shown in Figs. 2 and 3, respectively.

Analysis of the Li-DNA data

The variation in the magnitude of the averaged reduction factor, $|\langle A \rangle_N|$, with hydration is shown for three different Li-DNA preparations in Fig. 2. At the lowest hydration levels, $N < 5$, only the phosphate groups are believed to be hydrated (Saenger, 1984), and a low intensity broad resonance, caused by a short spin–spin relaxation time,

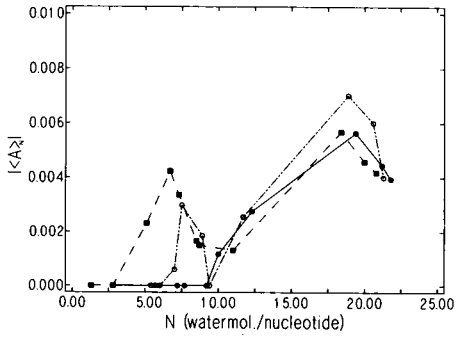

FIGURE 3. Measured magnitude of the averaged reduction factors, $|\langle A \rangle_N|$, of D_2O in Na-DNA. Preparation I (●, ——), preparation II (■, -----), preparation III (○, —— - ——). Because the water contents of preparation III were not measured, the average values of preparations I and II were used to plot preparation III. The lines have been drawn as an aid for the eye.

T_{2e}, is observed. At a hydration of $N = 5$ (5 water molecules are required to complete the hydration of the phosphodiester group [Saenger, 1984]) a splitting, $\Delta\nu \sim$ 3-4 kHz, is observed (Table 1). Within the framework of the sequential hydration model these results would require that the fifth water molecule added has a very large individual reduction factor. Alternatively, it may be that a minimum of ~5 water molecules are required to convert the DNA to a more ordered regular *B*-form (Corongiu and Clementi, 1981; Dickerson et al., 1982) with a concomitant ordering of water molecules. Evidence for a reorganization of the DNA structure at $N = 5$ has been previously obtained using solid state ^2H NMR measurements of ^2H labeled DNA (Brandes et al., 1988*a*).

In the hydration range $N = 6$ to 12, the added water molecules primarily bind to the bases (Saenger, 1984). This is reflected in a substantial reduction in the observed $|\langle A\rangle_N|$ (or D_2O splitting) in the range $N = 5$–7 followed by little change in the range $N = 7$–12 (Fig. 2). At $N = 12$, the primary hydration shell is filled and additional waters form a secondary hydration shell, until $N = 20$, at which point the DNA hydration shell is completed (Saenger, 1984). Over this hydration range ($N = 12$–20) $|\langle A\rangle_N|$ decreases at a lower rate compared with $N = 5$–7. With the addition of more water, the DNA sample swells (Saenger, 1984) and $|\langle A\rangle_N|$ continues to decrease as more "bulk water" is incorporated.

Test of the sequential hydration model

The magnitude of the averaged reduction factors, $|\langle A\rangle_N|$, shown in Fig. 2 together with a linear interpolation between data points may be used in conjunction with Eq. 6 to compute the individual site reduction factors, A_N, and these results are shown in Fig. 4 for the Li-DNA samples. Note that because the sign of $\langle A\rangle_N$ is unknown, the A_N values were calculated by arbitrarily assuming that $\langle A\rangle_N > 0$ in Li-DNA (*B*-DNA). To account for the large reduction in $|\langle A\rangle_N|$ that is observed in the range $N = 5$–7, we conclude, based on the sequential hydration model, that the averaged reduction factor associated with the first five water molecules, $\langle A\rangle_5$, is of opposite sign compared with the individual reduction factor, A_N, of the next few water molecules that are added. In the range $N = 8$–12 the A_N values are more or less constant, and of the same sign as $\langle A\rangle_N$. To account for the decrease in $|\langle A\rangle_N|$ that is observed in the range $N = 12$–24, the individual reduction factors must increase in magnitude for larger N, and have opposite signs compared with $\langle A\rangle_N$. To account for the decrease in $|\langle A\rangle_N|$ that is observed in the range $N = 12$–24, the individual reduction factors must increase in magnitude for larger N, and have opposite signs compared with $\langle A\rangle_N$. If this simplified model of sequential, independent binding were correct, it would imply that the largest $|\langle A\rangle_N|$ values are associated with water molecules that are free ($N = 20$–24). Because this is physically not realistic, we must conclude that the sequential model is obviously inadequate to explain our results for $N > 12$, and possibly at $N = 5$. To account for the large reduction in $|\langle A\rangle_N|$ with increasing N, for $N >$ 12, it is more realistic to assume that a reorganization of the water molecules results in the substantially reduced value of $|\langle A\rangle_N|$. Based upon these findings, we conclude that the reorganizational model best accounts for our data, but that the reorganization might be negligible in the range $N = 6$–12.

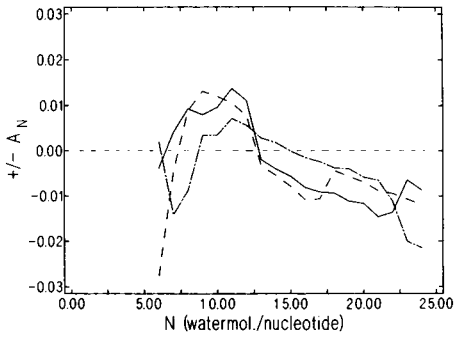

FIGURE 4 Calculated, individual reduction factors, A_N, of D_2O in Li-DNA. Preparation I (——), preparation II (---), preparation III (— - —). The lines have been drawn as an aid for the eye. For purpose of illustration, we have chosen to present the data, assuming that $\langle A\rangle_N > 0$ (see text).

Analysis of the Na-DNA data

As was observed for the Li-DNA samples, the Na-DNA samples show no splitting for $N < 5$, but T_{2e} is much shorter in Na-DNA than in Li-DNA. It is likely that the counterion, rather than the DNA conformation, is responsible for this difference since only the phosphates and counterions are hydrated at this hydration level (Falk et al., 1962, 1963; Saenger, 1984). Since accurate lineshapes could be obtained, we measured the effective spin–spin relaxation time, T_{2e}, at $\theta_{AL} = 0°$ and $\theta_{AL} = 90°$. For example at $N = 2.80$, T_{2e} (0°) = T_{2e} (90°) = 120 μs, which corresponds to a linewidth of 2,660 Hz. Because the measured linewidth is ~3,125 Hz, there is no large splitting "hidden" in the broad resonance. Evidently, these waters are poorly ordered and have short T_{2e} values. At $N \geq 5.1$, T_{2e} (0°) < T_{2e} (90°) which is indicative of ordered waters. In contrast to Li-DNA, preparation I of Na-DNA does not appear to develop an ordered water

structure at $N = 5$, since no splitting is observed for $N = 5$–9. However in this range of hydration, preparations II and III do exhibit splittings. Although these three samples exhibit different spectral properties, we note that they were prepared in an identical way, and showed identical x-ray patterns at $N = 9$. At this level of hydration, Na-DNA usually exhibits the "best" x-ray patterns (Lindsay et al., 1988; Lindsay, S., personal communication), presumably because the crystallinity is highest at this point. As we shall show, the different splittings observed for the three preparations arise from differences in the *B*-form DNA content of the Na-DNA samples.

Above $N = 9$, the spectral splittings are similar in all the Na-DNA preparations. At $N \sim 20$, the Na-DNA samples are expected to convert entirely from *A*- to *B*-DNA (Lindsay et al., 1988), and therefore above $N = 20$, the Li- and Na-DNA samples are both expected to be in the *B*-type conformation. In accordance with this expectation, the Na-DNA samples hydrated with $N \sim 21.7$ showed an average splitting, $(\Delta\nu)_{av} = 646 \pm 20$ Hz which is similar to that observed with the Li-DNA samples at $N \sim 21.8$, $(\Delta\nu)_{av} = 551 \pm 292$ Hz. In fact, our data suggest that the conversion occurs at $N \sim 19$ because the Na-DNA splittings decrease with hydration for $N \gtrsim 19$.

Determination of $\langle A \rangle_N$ in the *A*- and *B*-form of Na-DNA

Because we previously observed substantial amounts of *B*-DNA in samples of Na-DNA that, according to x-ray diffraction, only contained *A*-DNA, we suspected the difference between the Na-DNA preparations might be caused by different amounts of *B*-type DNA "contamination" (Brandes et al., 1988*b*). However, since *B*-form DNA in Na-DNA is usually not detected by x-ray diffraction below $N = 20$, any *B*-form present must, in this case, be in a somewhat disordered state. In fact, in an earlier study (Brandes et al., 1988*b*) we found that Na-DNA typically contained between 30 and 57% *B*-form DNA. Although the *A*-form was highly crystalline with a distribution width of helix axis orientations of $\lesssim 4°$, the *B*-form was more disordered, having a distribution width of $\lesssim 20°$. Here, we found that in the range $N = 10$–19 the observed water splitting increases from zero (or some small value) to ~1 kHz (at $\theta_{AL} = 0°$) in all three Na-DNA preparations. If the *A*- and *B*-forms are associated with different averaged reduction factors, we conclude that the water is exchanging rapidly between the *A*- and *B*-form contributions since only one spectral splitting was observed. At 75% RH both Li-DNA (B-form) and Na-DNA (*A*- and *B*-form) adsorb ~9 waters/nucleotide (see Table 1). It will, therefore, be assumed that N is identical for the *A*- and *B*-form in Na-DNA samples hydrated at 75% RH. In this case, the averaged order parameter in Na-DNA, $|\langle A \rangle_N|$ (Na-DNA), is expected to be given by

$$|\langle A \rangle_N|(\text{Na} - \text{DNA}) = b\langle A \rangle_N(B) + (1 - b)\langle A \rangle_N(A), \quad (8)$$

where b is the fraction of *B*-DNA, and $\langle A \rangle_N$ (A), and $\langle A \rangle_N$ (B) are the averaged reduction factors in *A*- and *B*-DNA, respectively. To establish the amount of *A*- and *B*-type conformation present in the Na-DNA, we used solid-state ^{2}H NMR (Brandes et al., 1988*b*) on Na-DNA samples that were deuterated in the purine 8-position. In this present series of measurements we found that aging of the samples caused a reduction of the *B*-DNA content. Samples that previously (Brandes et al., 1988*b*) consisted of ~57% *B*-DNA were, after aging ~19 months at constant relative humidity, observed to contain only ~40% *B*-DNA (Table 2, Fig. 5 *B*). We confirmed this finding by preparing a new batch of samples which were observed to contain ~57% *B*-DNA (Table 2, Fig. 5 *C*). The lowest *B*-DNA content, ~25% (Table 2, Fig. 5 *A*), was found in the ethanol treated (Brandes et al., 1988*b*) Na-DNA samples which were measured ~19 months after the treatment. The time dependent reduction of the *B*-DNA fraction is consistent with the suggestion of a slow crystallization of somewhat disordered parts of the sample in which *B*-form DNA is converted to *A*-form

TABLE 2 Relationship between *B*-DNA content and spectral splitting at $\theta_{AL} = 0°$, in ^{2}H labeled DNA (13 samples) at ~9 D_2O/nucleotide (75% RH)

Sample	*B*-DNA	ΔV
	%	Hz
	25 ± 5	1,000 ± 100
Na-DNA*	25 ± 5	800 ± 100
	37 ± 5	300 ± 100
Na-DNA‡	40 ± 5	140 ± 100
	41 ± 5	95 ± 100
	52 ± 5	392 ± 100
Na-DNA§	52 ± 5	451 ± 100
	55 ± 5	239 ± 100
	57 ± 5	556 ± 100
Li-DNA, prep I	100‖	1,780 ± 100
Li-DNA, prep II	100‖	2,200 ± 100
	100‖	2,168 ± 100
Li-DNA, prep III	100‖	1,084 ± 100¶

*EtOH treated. Measured 19 months after treatment.
‡Measured 20 months after preparation.
§Measured 3 weeks after preparation.
‖It is assumed that all Li-DNA samples contain 100% *B*-DNA (Brandes et al., 1988*a*).
¶Measured at $\theta_{AL} = 90°$. To compare with the splittings measured at $\theta_{AL} = 0°$, multiply this splitting by 2.

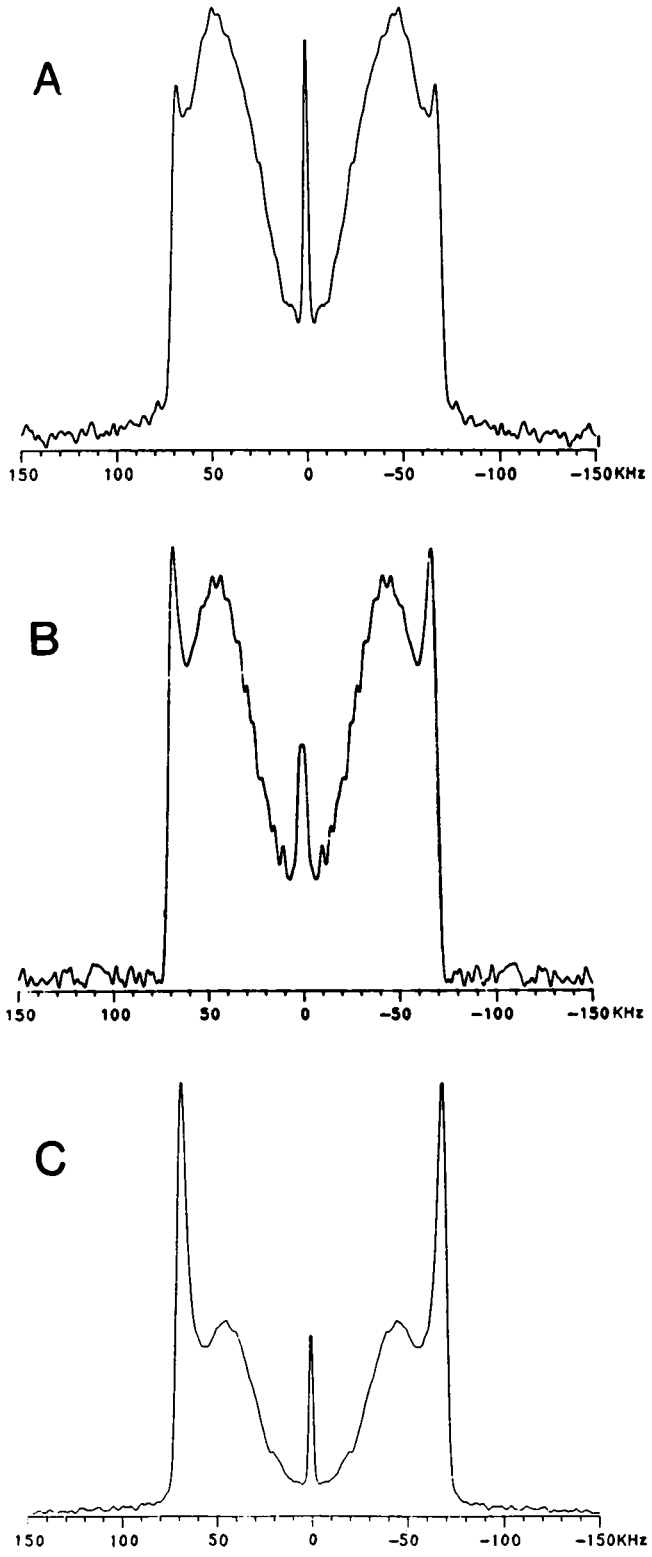

FIGURE 5 Spectra of different ^2H labeled Na-DNA samples showing a mixture of *A*- and *B*-DNA. The central component is caused by residual HOD. Lineshape analysis (Brandes et al., 1988*b*) yields; (*A*) 25% B-DNA, (*B*) 40% B-DNA, (*C*) 57% *B*-DNA.

DNA (Brandes et al., 1988*b*; Lindsay et al., 1988). After determination of the *B*-DNA content, the samples were subsequently hydrated with D_2O to measure the splitting of the water according to the procedures described above. The relationship between the *B*-DNA content and the water splittings, obtained at $N \sim 9$, are shown in Table 2 together with the splittings observed in Li-DNA. Because the sign of $\langle A \rangle_N$ cannot be determined (the splittings only give the magnitude), we could, on one hand, assume that $\langle A \rangle_N$ has the opposite signs in *A*- and *B*-DNA, and we would then find a linear relationship (Fig. 6) between the observed magnitude of the averaged reduction factor and the fractional *B*-DNA content as predicted by Eq. 8. If we, on the other hand, assume that they have the same signs, then a V-shaped relationship is found. In this case the largest splittings would be observed for 0% and 100% *B*-DNA, while no splitting would be expected for ~46% *B*-DNA. This is clearly physically unrealistic, and we therefore conclude that $\langle A \rangle_N$ has the opposite signs for *A*- and *B*-DNA. The calculated values (linear least square fit) thus found are; $\langle A \rangle_9(A) \approx \mp 0.011$ while $\langle A \rangle_9(B) \approx \pm 0.013$.

It is reassuring to notice that $|\langle A \rangle_9|$ of the *B*-form are identical in Na- and Li-DNA (Fig. 2). Substituting the averaged reduction factors for *A*- and *B*-DNA into Eq. 8, we find that a *B*-DNA content of ~46% is consistent with the observed, unsplit resonance of Na-DNA preparation I ($N = 9.2$). In contrast, preparation II ($N = 8.7$) exhibits a splitting corresponding to $|\langle A \rangle_9| = 0.0015$, which would require a *B*-DNA content of either 40% or 52%. We therefore cannot determine if the splittings observed for preparations II and III, in the range $N = 5$–9, are caused by a larger (>46%) or smaller (<46%) *B*-DNA content in these preparations as compared with preparation I. Note that the *B*-type (*C*-form) has previously been observed in Na-DNA samples at low relative humidities (Rhodes et

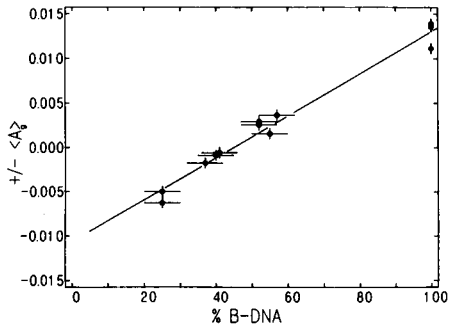

FIGURE 6. Relationship between *B*-DNA content and averaged reduction factor for $N = 9$, $\langle A \rangle_9$, in Na-DNA and Li-DNA (100% *B*-DNA). For purpose of illustration, we have chosen to present the data, assuming that $\langle A \rangle_N > 0$ in *B*-DNA (see text). The line was calculated from a linear least squares fit of the data.

al., 1982). Large variations in the splittings of Na-DNA samples at $N \sim 8.0$ were also reported by Migchelsen et al. (1968) who found a range $\Delta\nu = 0.52$–1.11 kHz in close correspondence with our values. Their Li-DNA (*B*-form) at $N \sim 7.6$ values were also similar to ours, $\Delta\nu = 1.96 - 2.61$ kHz.

After this work was completed, four different samples of Na-DNA fibers were obtained from Dr. Shindo. These samples were hydrated with D_2O (66% RH), and spectra were recorded with a JEOL FX-200 spectrometer, operating at 30.7 MHz (Tokyo College of Pharmacy). From two of these samples, spectra at $\theta_{AL} = 0°$ with spectral splittings of 992 and 1197 Hz, respectively, was observed whereas the other two samples exhibited a negligible splitting. Although these samples contained different amounts of NaCl, no relationship was found between salt content and spectral splittings. In view of our observations on films of oriented DNA (Brandes et al., 1988*b*) we suspect that the Na-DNA fibers prepared by Shindo also contained variable amounts of *A*- and *B*-DNA.

FINAL REMARKS

It should be noted that the model used here to account for the NMR splittings is considerably oversimplified. For example, the importance of the DNA organization within a sample has been neglected, although it has been shown (Brandes et al., 1988*c*) that the DNA organization can influence the splitting. In particular, if some of the binding sites are interhelical, sample morphology could affect the observed splittings. However, at $N \sim 9$, the magnitude of the averaged reduction factor in solid cholesteric-like Li-DNA, $|\langle A \rangle_{\sim 9}| = 0.012$ (Brandes et al., 1988*c*) is identical (within error) to our present uniaxially organized Li-DNA samples, $|\langle A \rangle_{\sim 9}| = 0.013$. In addition, our results obtained from DNA liquid crystals (Brandes and Kearns, 1986) suggest that interhelical DNA water interactions does not play an important part since the splitting observed at a water content of ~60 water/nucleotide (corresponding to $|\langle A \rangle_{60}| \approx 0.002$) is about three times smaller than the value observed in fibers at ~20 waters/nucleotide. When the liquid crystal was frozen ($T = -11°C$), ~13.5 water/nucleotide remained mobile, and $|\langle A \rangle_{13.5}| \approx 0.010$ was observed. This reduction factor is identical to what was observed in the present study of solid DNA samples. The influence of the DNA counterion on the spectra have also not been included in the model. However, in *B*-form DNA at $N = 9$ and $N = 21.7$, the magnitude of the averaged reduction factors were identical, independent of counterion, which suggests that the counterion is unimportant for the water order. Future studies of NMR spectra of the counterions in solid DNA might nevertheless improve the interpretation of the ^2H NMR spectra presented here, particularly at the lower hydration levels where no splittings were observed, and the counterion (Na versus Li) seemed to affect the spin–spin relaxation rate.

With the qualifications noted above, our observations show that the ordering of D_2O in solid DNA samples depends on the DNA conformation since the water order ($\langle A \rangle_N$) in *A*-DNA has opposite sign to that in *B*-DNA. The complex relation between the spectral splittings in Na-DNA on hydration is consistent with a mixture of *A*- and *B*-DNA in these samples. The relation between DNA helix geometry, sequence and hydration has been discussed by Dickerson et al. (1982) and others (Anderson et al., 1981; Saenger et al., 1986). Based on the results of x-ray diffraction studies on a DNA dodecamer, it was suggested there was a disordered "hydration spine" in *A*-DNA in contrast to an ordered "hydration spine" in *B*-DNA. In principle, it should be possible to use the x-ray coordinates of the ordered water molecules to calculate the $\langle A \rangle_N$ values, and consequently, the splittings expected in NMR measurements. The calculations of Corongiu and Clementi (1981) could also be used to predict the splittings. In future studies we plan to study the ordering of D_2O in oriented DNA at low temperatures in order to directly measure individual order parameters for individual binding sites. We will also investigate the effects of counterion and sequence on the DNA-water interactions.

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