Self-Diffusion and Association of Li^+, Cs^+, and H_2O in Oriented DNA Fibers. An NMR and MD Simulation Study

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The diffusional mobility of water, Cs, and Li counterions in oriented DNA fibers have been investigated by means of NMR self-diffusion measurements as a function of varying water content and salt concentration. In addition, ^1H relaxation times T_1 of water in the systems have been measured. To interpret these data and gain insight into the dynamics and structure of the DNA samples, molecular dynamics (MD) computer simulations have been carried out for corresponding systems at high water content. Data from MD simulations have been used to calculate the anisotropic self-diffusion coefficients and orientational distribution functions to reveal the most probable coordination sites for the counterions. The mutual agreement between the simulated and experimental self-diffusion coefficients is very good. In both the experiments and the simulations, it is found that Li ion diffusion is considerably slower than the Cs ion diffusion. This is found to be due to loss of water of hydration for Li^+, giving strong and long-lived interactions with the negatively charged DNA phosphate groups.

Introduction

In biological systems, DNA is commonly found in compact and ordered forms, e.g., in sperm heads, virus capsoids, chromosomes, and bacterial nucleoids.[1,2] The stability of these ordered forms of DNA is also related to the presence of multivalent counterions such as polyamines, e.g., spermine and spermidine.[3−5] Furthermore, the secondary structure of DNA is largely dependent on the specific hydration and water accessibility and the explicit coordination of counterions.[3] To understand the DNA−counterion interactions and the hydration of DNA, NMR self-diffusion and relaxation studies on ordered DNA systems have previously been conducted in our laboratory.[6−9]

Along with increasing power of modern computers, larger fragments of biological systems, dissolved in a natural aqueous solvent medium, can be studied using more realistic molecular models and force fields. To make computer simulations of complex systems more meaningful, they should be carried out in connection with experiments. Close connection between computer simulations and experiment has been utilized, for example, in the determination of three-dimensional protein structures. A standard method has been to add the measured NOE interproton distance intervals and populations of torsional angles calculated from *J* coupling constants through Karplus-type analysis to the force fields used in simulations.[10] Also, because of the matching time scales, the combined use of NMR relaxation and MD simulations has turned out to be a fruitful approach for the study of intermolecular solute−solvent interactions. This latter approach has been successfully applied in a number of studies from this laboratory.[11]

In this study we combine, for the first time, NMR diffusion measurements and MD simulations to investigate the self-diffusion of counterions and water in ordered DNA systems. We have measured the diffusion of water as well as Cs and Li counterions in macroscopically oriented fibers of DNA under different conditions of water and salt content. The self-diffusion coefficient is a parameter that is very useful to study in this kind of approach. It is a well-defined property that is sensitive to molecular interactions and to the structural properties of the system and that is also reasonably easy to obtain from simulations. This is in contrast to, for example, measurements of spin relaxation parameters, which may often be hard to interpret and that are difficult to obtain from simulations. An important motivation for our approach is that if the experimental and simulated results agree well with each other, it can be assumed that other properties not accessible by experimental methods are also well described in the computer simulation of the corresponding system. The hydration structure and the counterion coordination have therefore been studied in detail on the basis of the MD simulation trajectories. Although the diffusion is the major property of interest in this work, we have also measured the spin−lattice relaxation of water protons in the sample.

Materials and Methods

Samples. Highly oriented fibers from calf thymus DNA were prepared as described previously[6,12] using the wet-spinning method.[13,14] Salt-free oriented fiber samples were bathed several times in an ion-free solution of doubly distilled water/ethanol. The samples containing excess salt were bathed in solutions containing CsCl and LiCl, respectively, of the desired concentration (0.20 and 0.36 M). To achieve different relative humidities (RH) in the samples, they were equilibrated for at least 10 days in a desiccator containing a saturated solution of the appropriate salt ($NaNO_2$ = 66%RH, NaCl = 75%RH, KCl = 85%RH, K_2CrO_4 = 88%RH and Na_2SO_4 = 95%RH (see ref 15 for details)). The CsDNA samples containing added salt are predominantly in the A form at relative humidities below 85%[16] and in the B form above 85% RH, while all the studied LiDNA samples are mainly in the B form except for the salt-

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free LiDNA sample, which is expected to partly be in the C form at humidities below 85%.[12,17]

NMR Measurements. A Chemagnetics CMX Infinity system with a 4.7 T superconducting magnet (200 MHz proton frequency) was used for the NMR measurements. The probe was a gradient probe equipped with actively shielded coils (Cryomagnet Systems Inc.) and a home-built gradient driver system. Sample holders were made of hydrogen-free KEL-F and positioned in 10 mm NMR tubes. No field/frequency lock was used. All measurements were made at 25 ± 0.5 °C. Diffusion measurements were made using the PGSE,[18] LED,[19] and MQLED[20] pulse sequences, and the collected data were fitted nonlinearly to the Stejskal−Tanner equation:[18]

$$\frac{A}{A_0} = \exp\left[-n^2\gamma^2G^2\delta^2\left(\Delta - \frac{\delta}{3}\right)D\right] \qquad (1)$$

Here, n is the quantum coherence order, G is the gradient strength, γ is the gyromagnetic ratio of the studied nucleus, δ is the gradient duration, Δ is the diffusion time, and D is the diffusion coefficient that is to be extracted. The diffusion experiments were performed by keeping Δ (50−150 ms), G (0.05−0.5 T/m), and pulse timings (5−50 μs) constant while varying δ (1−15 ms). (Limits of the parameters are given in brackets.) The gradient strengths were calibrated against a 2.1 M LiCl solution at 25 °C, for which $D_{Li} = 9.14 \times 10^{-10}$ m^2/s.[21] T_1 relaxation measurements were made using the ordinary inversion−recovery experiment.

In the interpretation and discussion of our results we implicitly assume that the interaction of counterions and water molecules with DNA can be treated with a two-state model under conditions of fast chemical exchange. This means that the ions or water molecules are assumed to reside in a state associated with the DNA (the "bound" state) or outside the domain of the DNA molecule in a "free" bulk state, characterized by fractional populations p_B and p_F, as well as relaxation times or diffusion coefficients for the two states, respectively. For relaxation, the condition of fast chemical exchange means that the lifetimes in either states are short compared to the intrinsic relaxation times $T_{1,B}$ and $T_{1,F}$. The relaxation rate (the inverse of the relaxation time) is then a population-weighted average of these relaxation rates:[22]

$$\frac{1}{T_1} = p_F\frac{1}{T_{1,F}} + p_B\frac{1}{T_{1,B}} \qquad (2)$$

For the diffusion, a completely analogous relation with D_B and D_F, the diffusion coefficients of the two states, holds instead of the relaxation times,[23] with the condition of fast exchange now being that the lifetimes are shorter than the diffusion time Δ in the experiments. Fast exchange should certainly be valid, since the diffusion time as well as the relaxation times are on the order of 100 ms (see below), while the lifetimes of ions and water molecules are expected to be on the order of milliseconds or shorter[24−26] (see also the residence times from the simulations reported below). In addition, it should be noted that the discussion and interpretation of our diffusion results are largely performed at a quantitative level by explicit comparison with calculated diffusion coefficients, obtained from MD simulations that are independent of the two-state model.

Molecular Dynamics Simulations. Simulations have been performed for an all-atom DNA model consisting of a complete turn of double-stranded B-DNA [d(ATGCAGTCAG)]$_2$ (10 base pairs, 635 atoms). To provide a natural environment for the DNA system, it was placed in a rectangular simulation cell together with 480 water molecules and 20 counterions. By application of periodic boundary conditions in all box directions, the model represents an infinite array of parallel-packed DNA double helices immersed in an aqueous solvent. After adjustment of the dimensions of the simulation cell to the geometry of the double helix and the normal density of the solution, an average distance of about 24.5 Å was obtained between the nearest helix axes.

To describe the bonded and nonbonded interactions for DNA, we have used the latest all-atom CHARMM force field (version 2.5).[27] As a solvent, the flexible SPC water model by Rahman and Toukan[28] has been used. This water model, which contains anharmonic O−H bond stretch potentials, gives very good results for the self-diffusion coefficient of bulk water and also a correct dielectric constant for water at room temperature. These two properties should be very important in studies of counterion self-diffusion and the hydration of DNA. The counterions are described using rigid ion models with the following Lennard-Jones parameters: $\sigma = 1.506$ Å and $\epsilon = 0.69$ kJ/mol[29] for Li^+ and $\sigma = 3.92$ Å and $\epsilon = 2.132$ kJ/mol for Cs^+.[30] All the nonbonded cross interactions are constructed using the simple Lorentz−Berthelot combination rules.[31]

We have employed the constant temperature, constant pressure (*NPT*) MD algorithm by Nosé and Hoover,[32] and simulations have been carried out at $T = 298$ K and $P = 1$ atm. The Ewald summation technique[31] has been applied to take into account the long-range electrostatics interactions. The double time step algorithm by Tuckerman et al.[33] was used to integrate the equations of motion, with the short time step being 0.2 fs for the fast fluctuating degrees of freedom, such as the covalent bond stretching and angle bending, and also for the nearest nonbonded interactions. A longer time step of 2.0 fs was used to cover the slower motions.

Molecular dynamics simulations on molecular systems of the present size require extensive use of computer resources. In addition, because of the complexity of the whole DNA system and the fact that some dynamic processes and internal motions are very slow on the time scale of MD simulations, long simulations are required to cover large enough regions of the phase space. Two separate simulations of DNA with Li^+ and Cs^+ counterions were carried out. In both cases the systems were first equilibrated for 200 ps, whereafter averages were collected for 2 ns for LiDNA and 1 ns for CsDNA. The starting configuration of DNA was a canonical B form, and it remained generally in the B form during the simulations. Note that possible transitions to other forms were partially hindered because of topological reasons imposed by the periodic boundary conditions along the helix axis, which effectively prevents any winding or unwinding type of motion of the helix.

In addition we have carried out MD simulations for ionic solutions of Li^+ and Cs^+ (without DNA) with the same models and physical conditions to have reference diffusion data to compare with the corresponding results from the full simulations of the DNA systems. The bulk simulations were done for 500 water molecules and 4 ion pairs (Cl^- ions were used as anions), which corresponds to a 0.4 M concentration.

All the calculations of the self-diffusion coefficients were obtained from the slope of the time dependence of the mean-square displacements. In this paper we present the results of the simulation that are of interest for the interpretation of our NMR experiments. Other aspects of the simulations of the ordered Li- and CsDNA systems will be presented elsewhere.[34]

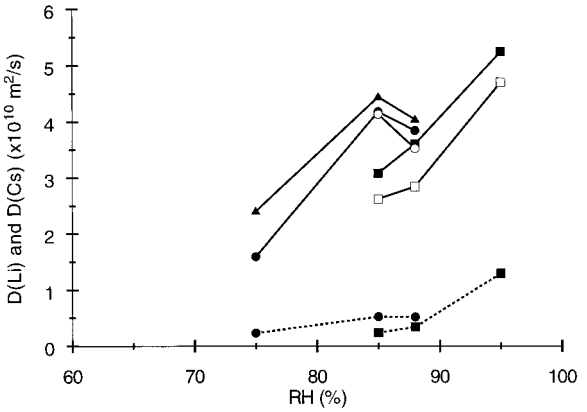

Figure 1. Experimentally found Li (dashed line) and Cs (solid line) diffusion coefficients. Squares are for salt-free samples, circles for 0.20 M XCl samples (X = Li, Cs) and triangles for 0.36 M CsCl sample. Filled symbols are for diffusion coefficients parallel to the DNA fiber axis and open symbols for those perpendicular to the DNA fiber axis.

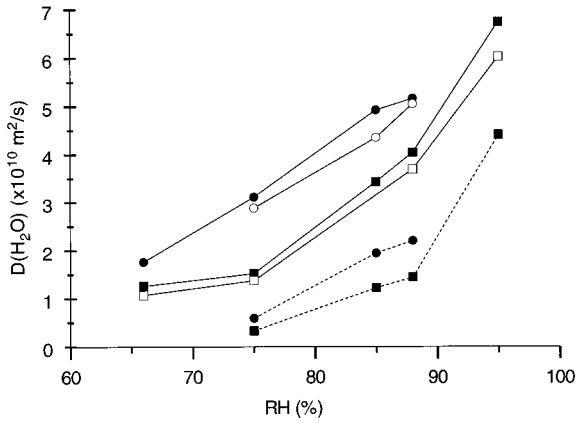

Figure 2. Experimentally found water diffusion coefficients for the LiDNA samples (dashed line) and the CsDNA samples (solid line). Squares are for salt-free samples and circles for 0.20 M XCl samples (X = Li, Cs). Filled symbols are for diffusion coefficients parallel to the DNA fiber axis and open symbols for those perpendicular to the DNA fiber axis.

Results

NMR Self-Diffusion Experiments. Figure 1 displays the diffusional behavior of Cs^+ and Li^+ as a function of RH for all the samples under investigation. The diffusion parallel to the fiber axis is consistently somewhat faster than that in the perpendicular direction, this being the case for both counterions (we did not actually measure the diffusion perpendicular to the fiber axis for Li, since this has been done before in our lab; see ref 6). This is to be expected from the anisotropy of the samples.[6] The diffusion coefficients of both Li and Cs counterions increase as a function of RH except for Cs^+ in the region between 85 and 88% RH for the samples containing CsCl, where the diffusion coefficients decrease slightly. This change in the trend can be traced to the structural transition of DNA from the A to the B form (see below).

The most obvious and interesting feature of the experimental counterion diffusion coefficients is the consistently faster diffusion of Cs^+ compared to that of Li^+ at the same RH. The ratio of diffusion coefficients, D_{Cs}/D_{Li}, is changing from approximately 12 at low RH (85%) to about 4 at high RH (95%) for salt-free samples, whereas in bulk solution at infinite dilution this ratio is only 2.[35]

Figure 2 shows the experimental diffusion coefficient of the water molecules, measured using ^{1}H NMR. We have assumed

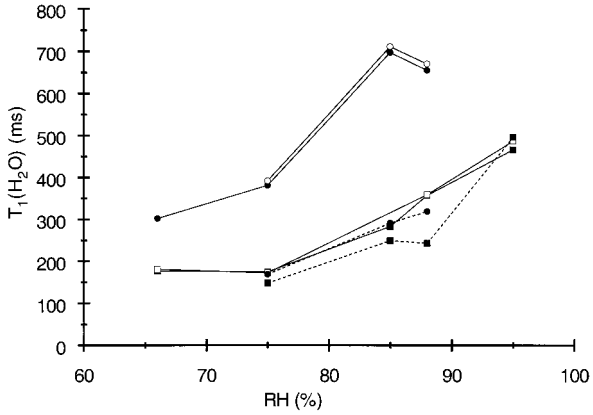

Figure 3. Experimentally found T_1s for water protons in LiDNA and CsDNA samples. Symbols are the same as for Figure 2.

that the proton signals from DNA will not contribute to the echo decay in the diffusion measurements, since they are not mobile. The water diffusion coefficient seems to increase with increasing water content (increased RH), which reflects a higher mobility upon an increase of the amount of bulk water. It should be noted that the water diffusion coefficients for the LiDNA samples are lower than those of the CsDNA samples. For the salt-free samples at 75% RH, the diffusion coefficient of water when measured parallel to the DNA fiber axis is about 4 times higher for the CsDNA sample than for the LiDNA sample, the actual numbers being 1.26×10^{-10} and 0.33×10^{-10} m^2/s, respectively. At 95% RH the water diffusion coefficients for the two samples have approached each other and the difference is a factor 1.4.

From Figure 2 we can also see that the diffusion coefficients of water are higher for the salt-containing samples than for their corresponding salt-free samples. This is a natural consequence of the higher water content for the salt-containing samples. We can note, however, that at lower humidities this difference is much more pronounced for the CsDNA samples than for the LiDNA samples, even though the salt-containing samples contain equal numbers of extra Cs and Li ions, respectively. This may be explained by the fact that Li has a well-defined hydration shell, which is not the case for Cs (see Discussion below). Furthermore, it can be seen that, for both Cs samples investigated, the diffusion coefficients parallel and perpendicular to the DNA fiber axis are almost equal.

^{1}H NMR Relaxation of Water. The T_1 values for ^{1}H in the samples as a function of RH are displayed in Figure 3. The signal from the protons in the inversion−recovery experiment will, of course, be a weighted average from all the different protons in the sample. The proton signals associated with the DNA molecule will, however, be very broad because of the slow motion of DNA. This broad contribution will therefore vanish under the baseline. In addition, owing to the expected short relaxation time of these DNA protons, they will not contribute to the signal buildup in the inversion recovery experiment.

Qualitatively, the ^{1}H-relaxation rate $R_1(=1/T_1)$ is an average of the interaction with all nearby spins and will depend on a product of two factors: the inverse sixth power of the distance to the nearby spin that it is interacting with, and an effective correlation time describing the time scale of the molecular motions that determines the fluctuation of this dipolar interaction. The other spin-carrying isotopes in our samples, neglecting those of low abundance, are ^{14}N and the counterions ^{6}Li, ^{7}Li, and ^{133}Cs. Owing to the large number of water molecules

TABLE 1: Self-diffusion Coefficients (Units of 10^{10} m^2/s) for Li^+, Cs^+, and H_2O in the Presence of DNA and in Bulk Solution from Molecular Dynamics Simulations[a]

	Li^+	Cs^+	H_2O
		LiDNA	
total	1.5 ± 0.2		10.4 ± 0.2
$D_\perp$	1.6		10.6
$D_\parallel$	1.4		10.2
		CsDNA	
total		6.4 ± 0.5	10.1 ± 0.3
$D_\perp$		5.6	10.3
$D_\parallel$		7.8	9.8
		0.4 M Bulk Solution	
	9.5 ± 0.5	16 ± 0.5	21.5 ± 0.5
		Experiment at Infinite Dilution	
	10.3	20.6	23.5

[a] In the case of DNA solution, shown are the total self-diffusion coefficients and projections on the plane perpendicular to the DNA axes ($D_\perp$) and on the DNA axes ($D_\parallel$). The last line contains experimental self-diffusion coefficients as a reference.

compared to counterions at all RH studied, the large abundance of ^1H isotopes (almost 100%), and their large gyromagnetic ratio, we will assume the relaxation of ^1H to be caused primarily by other ^1H nuclei. We will discuss our water proton spin relaxation results within this qualitative level of description.

It can be expected that as humidity increases, the relaxation time of the water protons should increase because of faster molecular motions, which means that the water molecules are more free to average out dipolar couplings. This should also lead to larger T_1 values for the salt-containing than for the salt-free samples, since the salt-containing samples also contain somewhat more water molecules. These predictions are well borne out by the data in Figure 3. There is, however, one exception to this, which is a jump in the trend between 85 and 88% RH for the salt-containing Cs sample, where DNA goes from the A form to the B form. This will be further discussed below.

Diffusion Coefficients from MD Simulations. The calculated molecular dynamics diffusion data for Li^+ and Cs^+ ions and water molecules in the DNA system and in the bulk salt solutions are quoted in Table 1. As in the experiments, the MD simulations also show a stronger retardation of Li ions compared to Cs ions in the DNA system. The ratio of D_{Cs}/D_{Li}, obtained from the DNA simulations, is close to 4, whereas in the bulk solution at 0.4 M salt concentration, it is approximately 1.7. There is no anisotropy in the diffusion for Li ions, while there is a clear effect for Cs ions.

The simulation shows essentially no difference at all between the two samples for the diffusion coefficient of water, which has a value of $(10.0 \pm 0.3) \times 10^{-10}$ m^2/s (cf. Table 1). This is in contrast to the experimental result where there is a clear difference between the Li and Cs systems, respectively. The experimental water diffusion is also somewhat slower than that found from the MD simulations. The simulated diffusion coefficients for the bulk systems show good resemblance to the experimental literature data obtained from dilute salt solutions. This gives evidence that the force field used in our work is reliable. Comparison of the experimental and calculated diffusion coefficients for all species will be discussed in more detail in the next section.

Discussion

Counterion Diffusion and Association to DNA. It is not trivial to define an exact RH in the simulations. A good estimate can, however, be obtained from the water content in the sample. Lee et al.[16] found that an RH of 95% for samples of LiDNA with Li/P = 1.3 corresponds to 25 water molecules per phosphate for the same sample. In a study by Lavalle et al.[36] it was found that Na-, K-, Rb-, and CsDNA contain the same amount of water at a counterion/P ratio of 1.1 and that 25 water molecules per phosphate corresponds to about 95% RH for the CsDNA sample. Since the amount of water in the samples and in the simulations should be the most important parameter for the diffusional behavior, we believe this is a relevant parameter for estimating the relative humidity in the simulations. In a salt-free sample, 25 water molecules/phosphate must correspond to a somewhat higher humidity. Therefore, a value of 95% RH or somewhat higher, is a reasonable estimate of the relative humidity in the simulations. The water content in oriented DNA samples will be further discussed below.

When the calculated counterion diffusion values for Cs and Li (Table 1) are compared with the experimental results at the highest RH, 95% (Figure 1), it can be seen that there is good agreement between the data, even at a quantitative level. For diffusion parallel to the DNA fiber axis, the experimental values are 5.2×10^{-10} and 1.3×10^{-10} m^2/s for Cs and Li ions, respectively. This should be compared with the calculated values of 7.8×10^{-10} and 1.4×10^{-10} m^2/s, respectively. The relative difference in diffusion between the two ions in the DNA system, measured by the quotient D_{Cs}/D_{Li}, has the values 4.1 and 4.3 from the experiments and the simulations, respectively. This shows that experimentally there is a considerably larger retardation of Li ions compared to Cs ions, which is also well predicted by the simulations. It should also be noted that this larger retardation effect is even more pronounced in the experiments at lower water content, with D_{Cs}/D_{Li} having a value of 13 at 85% RH. In conclusion, the good agreement between simulated and experimental diffusion coefficients means that we can confidently use our simulation results for further interpretation and understanding of the experiments by investigating in more detail the behavior of the ions and water molecules around DNA. In particular we can try to clarify the reasons why the Li ions seem to be more strongly attached to DNA than Cs ions.

This result on the difference between the two ions in the DNA system can be compared with the diffusion coefficients at "infinite dilution", 20.6×10^{-10} and 10.3×10^{-10} m^2/s for Cs^+ and Li^+, respectively,[35] i.e., a factor 2.0 faster diffusion of Cs compared to Li. The difference in diffusion coefficients at infinite dilution can be fairly well explained by the different sizes of the hydration shell of the ions. Reference 35 reports several hydration numbers for the alkali ions; 2.8 from the concentration dependence of the diffusion coefficient (Robinson and Stokes, 1959) and 4 from activity coefficients (Hinton and Amis 1971) for Li. The corresponding numbers for Cs are 0.5 and 0, respectively. Our MD simulations of bulk salt solution show that Li^+ ions have a rather stable hydration shell of radius 4.2 Å consisting of four water molecules. The Cs^+ ions, on the other hand, do not form any well-defined hydration shell, and it can be assumed that its effective hydration radius is equal to the "bare" ion radius of 2.0 Å.

The much slower diffusion of Li ions in the case of the DNA systems under investigation cannot therefore be explained in terms of the size of the ordinary hydration shells. The smaller effective radius of Cs means a stronger electrostatic attraction of the counterions to the negatively charged phosphate groups on DNA, and from this point of view one would expect a stronger retardation for the Cs ions, which does not appear to be the case. Other explanations have to be sought in order to

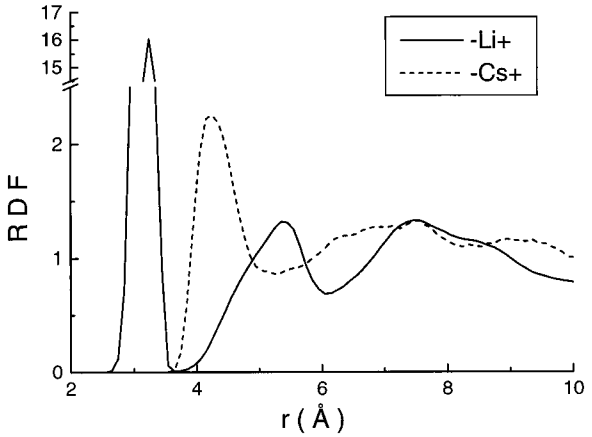

Figure 4. Phosphor−counterion RDF for Li (solid line) and Cs (dashed line).

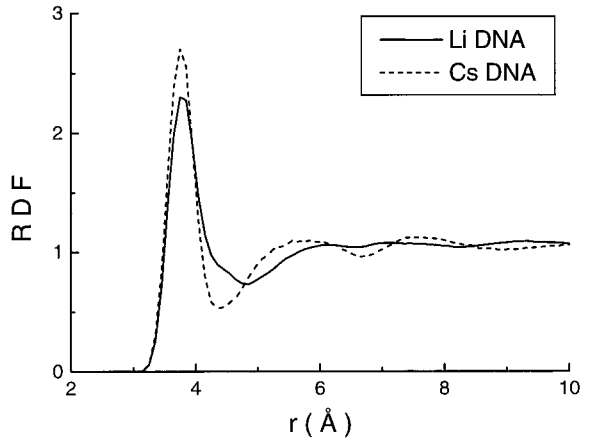

Figure 5. Phosphor−water oxygen RDF in LiDNA (solid line) and CsDNA (dashed line).

find the origin of the observed behavior for the two counterions in the DNA system.

Another possible hurdle for the ion diffusion might be steric hindrance due to the tightly packed DNA molecules. Still, this does not seem to be the cause of the difference between Li and Cs (although it certainly plays a role in slowing the overall diffusion). If the diffusion of Li ions is more hindered than that of the Cs ions, because of steric effects, then this should inevitably affect the diffusion of water molecules because Li ions with their large hydration shells stuck between DNA would create additional obstacles to water diffusion. The results from our MD simulations show that diffusion coefficients of water are the same in Li- and CsDNA (see Table 1), and in the experiment at the highest water content, the observed difference is rather small. The experimental data seem to approach each other quickly in this region, and it is probable that at a somewhat higher RH the difference would, as in the simulation, be negligible.

The most probable reason for the considerably slower diffusion of the Li ions is therefore a more tight association of Li ions to DNA. That this is also the case can, in fact, be clearly seen from the MD data, where the Li counterions are observed to associate more strongly with the phosphate groups of DNA. In Figure 4, we have displayed the radial distribution functions (RDF) between the DNA phosphates and the counterions. A very high first peak of the Li−P RDF, corresponding to a distance of 3.2 Å between the P and Li atoms, can be observed. This distance corresponds to a direct contact between an Li ion and one of the oxygens of the phosphate group. From the simulation data it can be evaluated that about 60% of the Li counterions on the average are positioned in such bound states. The minimum between the first peak of the Li−P RDF and the rest of this RDF is vanishingly low, its magnitude being as small as 0.003, which implies a very slow exchange between associated and free Li ions. The RDF between Cs counterions and the phosphates has a much lower and broader first maximum followed by an almost indistinguishable minimum. This is an indication of a typical weak electrostatic attraction of Cs counterions to the phosphate groups on DNA, creating no serious obstacles to the counterion motion. Still, we can define a state corresponding to the distances under the first maximum of the RDF (less than 5 Å for the Cs−P RDF) as "bound". With this definition, only 16% of the Cs counterions can be considered bound.

We have also calculated "residence times" for the bound state of counterions. This time may be determined from the exponential decay of the probability for an atom to leave the bound state after a time t (a more strict definition of the residence time and the computation algorithm is given elsewhere[37,38]). The residence time for Li^+ ions turned out to be about 1 ns or even longer (it is difficult to define the residence time exactly if it is comparable with the simulation time). In fact, we observed three Li ions that were bound to one of the phosphates during the whole simulation period. The residence time of Cs ions in the vicinity of the phosphate was determined to be 20 ps. One can see from this analysis that more Li ions than Cs ions are strongly associated with the phosphates on DNA, and they stay attached much longer. This is the primary reason for the much slower Li ion diffusion.

In Figure 5 we display also the phosphate−water oxygen RDF in both Li- and CsDNA. It can be seen that this RDF is more structured in the case of CsDNA; it has a higher first maximum and a lower first minimum compared to LiDNA. This means that the hydration shell of the phosphates on DNA is nearly unperturbed (or very little perturbed) by the Cs ions. Li ions, on the other hand, bind directly to the phosphates, destroying the phosphate hydration shell, and a new hydration shell is built up around the phosphate−Li complex.

On the basis of the above picture, we can predict the effect on the counterion diffusion of reducing the water content. For lower water content, a competition between DNA and Li ions for the remaining water molecules takes place. When a Li ion binds to a phosphate, it displaces several water molecules from the hydration sphere, which become available to hydrate other sites on the DNA. One can expect that by lowering the humidity, more and more Li ions are bound directly to phosphate groups, and as a result, they become quite immobile because of the strong electrostatic attraction caused by its small "bare" ion radius. This leads to a rapid fall of the diffusion coefficient upon decreasing RH. This mechanism, however, does not become effective for Cs ions, since these are not as strongly hydrated as the Li ions. This is also what is observed experimentally where the Li diffusion for the salt-free DNA system is reduced to 18% of its value upon decreasing the RH from 95% to 85%. The Cs diffusion, on the other hand, is only reduced to 60% of its value in the same region (see Figure 1).

Hydration in the DNA Samples and Effect on Counterion and Water Diffusion. The study by Lee et al.[16] on Na- and LiDNA mentioned above reports on DNA−DNA interhelical distances and water content as a function of RH. They have used an LiDNA sample with 4.5 g LiCl/100 g DNA, which amounts to a Li/P ratio of 1.3. Our salt-free samples, of course, have Li/P = 1.0, while the 0.20 M LiCl sample corresponds to 4.8 g LiCl/100 g DNA (ref 39), translating to an Li/P ratio of

1.4. Our 0.20 M LiDNA sample should therefore be well comparable to their LiDNA sample. The CsDNA sample used in the study of Lavalle et al.[36] had a Cs/P = 1.1, which would most closely correspond to our salt-free CsDNA sample. It can be seen from the NaDNA data from these two studies and a study by Falk et al.[40] that for an Na/P of 1.0 (Falk et al.), 1.05 (Lee et al.), and 1.1 (Lavalle et al.), the number of water molecules at a given RH does not differ very much within this salt range, although the salt-containing sample of Lavalle et al. does seem to attract a couple of water molecules, maybe one or two, more than the salt-free sample of Falk et al. at a given RH (Lavalle et al. show these data together). This was also found in a study by Rupprecht and Forslind,[39] where 0.20 M LiDNA and NaDNA samples were found to contain about 1.5 water molecules more than the corresponding salt-free samples at 64 and 72% RH for Li- and NaDNA samples, respectively. There is no reason to believe that this will also not be qualitatively the case for CsDNA, so the salt-containing CsDNA samples will also contain one or a few more water molecules than the salt-free at a given RH.

In the study of Lee et al. it is found that at 75% RH 1 g LiDNA contains 0.42 g H_2O, which translates to 5.6 water molecules per Li ion (or 7.7 water molecules per nucleotide). As mentioned already, ref 35 reports several hydration numbers for the alkali ions: 2.8 from the concentration dependence of the diffusion coefficient (Robinson and Stokes, 1959) and 4 from activity coefficients (Hinton and Amis 1971) for Li. The corresponding numbers for Cs are 0.5 and 0, respectively. Judging from this and considering also that DNA needs to be hydrated, requiring about 20 water molecules in the primary hydration shell,[3] there seem to be too few water molecules in the 0.20 M LiCl sample at relative humidities of 75% or lower to accomplish full hydration of the Li cation. For the Cs ion, however, hydration seems to be no problem at all. At 75% RH it is seen from the data of Lee et al. that the interhelical distance for LiDNA is about 20 Å. LiDNA would be predominantly in the B form at humidities of 75% and above, giving a helical radius of about 10 Å. This would mean that the helices lie very tightly squeezed together, in turn yielding very strong electrostatic phosphate−phosphate interactions that must be overcome by placing a cation close to the phosphates. It seems that this would require the "binding" cations to be quite tied to the phosphates, and the nonbinding cations, i.e., excess salt, would be more free to move around. For the A form of DNA the DNA−DNA distance is probably smaller at a given RH than it is for the B form of DNA, since the A form has a larger diameter. The A−B transition will be discussed more in the next section.

It is interesting to compare the diffusion coefficients of the counterions to those of the water molecules in the same sample. In Figure 6 these are depicted for all the samples as a function of RH (only results parallel to the DNA fiber axis have been plotted to clarify the graph) in the same figure. It is clear that for the salt-free sample, the water diffusion coefficient follows closely that of the Cs ion diffusion at the lower humidities. Furthermore, at humidities above 88%, the water molecules become more mobile relative to the Cs ions. For the salt-containing CsDNA sample this difference between the water mobility and that of the Cs ions is larger but in the same direction. On the other hand, the difference between the diffusion coefficients of the counterion and the water seems to be more pronounced for the LiDNA samples, even though both the diffusion of Li and water in the LiDNA samples are slower than those of Cs and water in the CsDNA samples. At a relative

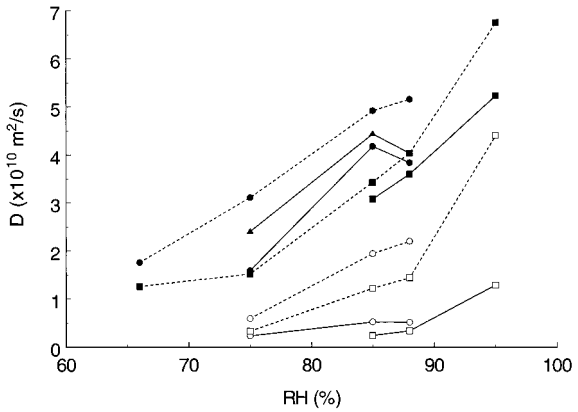

Figure 6. Experimentally found diffusion coefficients of the counterion X (solid line) and water (dashed line) in XDNA parallel to the DNA fiber axis. Squares are for salt-free samples, circles for 0.20 M XCl samples, and triangles for the 0.36 M CsCl sample. Open symbols are for X = Li and filled for X = Cs. The data are the same as shown in Figures 1 and 2.

humidity of 85−88%, the number of water molecules in the 0.20 M LiDNA sample is 12−15 water molecules per phosphate.[16] The number of Li ions is 1.3, and with a hydration shell of four water molecules per Li ion, five or six water molecules per phosphate are needed for Li hydration.

The experimentally found water diffusion coefficients displayed in Figures 2 and 6 show that the H_2O molecules in the LiDNA samples move more slowly than those in the CsDNA samples in the entire RH range investigated, but the effect is particularly large at lower RH. It is also true for both the salt-free samples and those containing extra salt. This difference is likely an effect of hydration of the free Li ions. This hydration of Li but not of Cs is also well reflected by the water T_1 relaxation times in Figure 3, where clearly there is no large salt dependence for the LiDNA samples, but where the water T_1s of the salt-containing CsDNA sample are very much larger than those for the salt-free CsDNA sample. All the water in both LiDNA samples at 88% and lower RH values is used for counterion hydration. Only at the highest RH, 95%, can we see a huge increase in the water T_1 for the salt-free LiDNA sample. Here, the number of water molecules in the sample is larger than the number of water molecules needed for DNA and counterion hydration. For the CsDNA samples, however, a large part of the additional water in the 0.20 M CsCl sample compared to the salt-free sample is bulk water, i.e., "free" water.

Structural Features of A- and B-DNA and the Manifestations of the A−B Transition in the NMR Experiments. The structures of A- and B-DNA are very different, the A form of DNA being wider and slightly shorter than the B form. This structural change occurs between 85% and 88% RH for the CsDNA samples containing added CsCl and is evident in ^{133}Cs quadrupolar splittings (data not shown) and the trend in the water spin−lattice relaxation time depicted in Figure 3, as well as in the Cs diffusion (Figures 1 and 6).

Hydration of A and B forms of DNA is also very different. Several studies have shown that B-DNA is more hydrated than is A-DNA (for references see, for example, ref 3). This could explain the sudden decrease of the water T_1 relaxation time in the salt-containing CsDNA samples. When DNA is in the A form, less water is needed to hydrate DNA and therefore more water can be considered to be bulk water. When the structural transition to B-DNA takes place, more water is needed for hydration of the macromolecule. The water bound to DNA can be expected to have a shorter T_1 than that in the bulk phase. Saenger[3] writes that the solvent accessibility for a water

molecule of radius 1.4 Å is equal for A- and B-DNA. If, however, the radius of the solvent is increased, as it would be if water, for example, hydrates a cation, the solvent accessibility is larger for B- than for A-DNA. The water diffusion coefficients in the salt-containing CsDNA samples, on the other hand, do not show this break in trend upon the A to B transition (cf. Figures 2 and 6). Here, the fact that the total water content has increased seems to be more important than the motional constraint that the increased hydration of DNA will lead to. In addition, the relaxation is a local property governed by local molecular dynamics on the nanosecond time scale and by the strength of the dipolar interaction with nearby spins. Diffusion, on the other hand, is a long-range property governed by molecular motions over longer time scales in the millisecond range. Therefore, it is somewhat surprising that the Cs diffusion in the salt-containing samples, contrary to that of water, decreases upon the A to B transition. It is not obvious to us what the molecular origin of this effect is. However, it has been concluded[36] that apart from the hydration, the ion specificity is important in the A to B transition, and some specific effects may also affect the diffusion of Cs ions in this case.

Conclusions

The experimental Li and Cs counterion NMR self-diffusion measurements have revealed that the Li diffusion is considerably slower than that of the Cs ions in the oriented DNA fibers. This effect is larger at lower water content, with D_{Cs}/D_{Li} varying from about 12 to about 4 in the range of relative humidities from 66% to 95%. Generally, the diffusion is faster the higher the content of water or added salt in the sample is. The same trend is also observed in the mobility of water as detected by self-diffusion and from the water-^{1}H spin−lattice relaxation times. The difference between Cs and Li cannot be explained by the difference in hydrated ion radius, which only gives a factor of 2 in the value of D_{Cs}/D_{Li} for diffusion of these ions in dilute salt solutions free of DNA. The MD simulations of Cs- and LiDNA at a water content comparable to around 95% RH confirm this result almost quantitatively. On the basis of this good agreement between experimental and simulated diffusion data, the MD simulations can be used to investigate the origin of this effect. It has been found that this difference can be traced to the fact that Cs as in a bulk salt solution is only weakly hydrated and therefore diffuses with the size of its "bare" ion radius, which is about 2.0 Å. Li, on the other hand, which is strongly hydrated in bulk salt solution, basically loses all its water of hydration in the DNA system. This then enables strong electrostatic association between the small (0.74 Å) Li ion and the negative phosphate charges, with one shared hydration shell around the Li−phosphate group complex. It has also been found that the A to B transition that takes place between 85 and 88% RH is sensitively detected by a break in trend of the Cs diffusion and water-^{1}H spin−lattice relaxation time that decreases upon this transition (which otherwise increases with increased water content).

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References and Notes

(1) Calladine, C. R.; Drew, H. R. *Understanding DNA*; Academic Press: London, 1992.

(2) Earnshaw, W. C.; Sherwood, R. C. *Cell* **1980**, *21*, 319−331.

(3) Saenger, W. *Principles of nucleic acid structure*; Springer-Verlag: New York, 1984.

(4) Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1976**, *45*, 285−306.

(5) Tabor, C. W.; Tabor, H. *Annu. Rev. Biochem.* **1984**, *53*, 749−790.

(6) Andreasson, B.; Nordenskiöld, L.; Eriksson, P. O.; Rupprecht, A. *Biopolymers* **1994**, *34*, 1605−1614.

(7) Schultz, J.; Andreasson, B.; Nordenskiöld, L.; Rupprecht, A. *J. Phys. Chem.* **1994**, *98*, 8507−8518.

(8) Einarsson, L.; Eriksson, P.-O.; Nordenskiöld, L.; Rupprecht, A. *J. Phys. Chem.* **1990**, *94*, 2696−2702.

(9) Einarsson, L.; Nordenskiöld, L.; Rupprecht, A.; Furó, I.; Wong, T. C. *J. Magn. Res.* **1991**, *93*, 34−46.

(10) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11

(11) Odelius, M.; Laaksonen, A. In *Theoretical and Computational Chemistry: Molecular Dynamics*; Balbuena, P. B., Seminario, S., Eds.; Elsevier Science: Amsterdam, 1998.

(12) Schultz, J.; Nordenskiöld, L.; Rupprecht, A. *Biopolymers* **1992**, *32*, 1631−1642.

(13) Rupprecht, A. *Biochem. Biophys. Res. Commun.* **1963**, *12*, 163−168.

(14) Rupprecht, A. *Biotechnol. Bioeng.* **1970**, *12*, 93−121.

(15) These values have been taken from the following. (a) Spencer, H. M. In *International Critical Tables of Numerical Data, Physics, Chemistry and Technology*; McGraw-Hill: New York, 1972; Vol. 1, pp 67−68. (b) O'Brien, F. E. M. *J. Sci. Instrum.* **1948**, *25*, 73−76. These referrences report 93% RH for both KNO_3 and Na_2SO_4. Previous NMR measurements from this lab (refs 6 and 12) show that Na_2SO_4 actually gives a slightly higher RH than KNO_3 (the ^{23}Na-quadrupolar splitting trend in ref 12 and the trend in Li diffusion coefficient in ref 6). We have chosen (previously as presently) to trust the value of 93% RH for KNO_3 and to give Na_2SO_4 a slightly higher value than reported in the tables referred to above, i.e., 95% RH. It might, however, be the case that Na_2SO_4 gives 93% RH, as tabulated, and that KNO_3 actually gives a lower RH than tabulated, say 91%. The temperature dependence of the RH could be the cause for this deviation from tabulated values. That Na_2SO_4 gives a higher RH than KNO_3 is, however, clear.

(16) Lee, S. A.; Lindsay, S. M.; Powell, J. W.; Weidlich, T.; Tao, N. J.; Lewen, G. D.; Rupprecht, A. *Biopolymers* **1987**, *26*, 1637−1665.

(17) Marvin, D. A.; Spencer, M.; Wilkins, M. H. F.; Hamilton, L. D. *J. Mol. Biol.* **1961**, *3*, 547−565.

(18) Stejskal, E. O.; Tanner, J. E. *J. Chem. Phys.* **1965**, *42*, 288−292.

(19) Gibbs, S. J.; Johnson, C. S., Jr. *J. Magn. Reson.* **1991**, *93*, 395−402.

(20) van Dam, L.; Andreasson, B.; Nordenskiöld, L. *Chem. Phys. Lett.* **1996**, *262*, 737−743.

(21) Tanaka, K.; Nomura, M. J. *J. Chem. Soc., Faraday Trans. 1* **1987**, *83*, 1779.

(22) Wennerström, H. *Mol. Phys.* **1972**, *24*, 69−80.

(23) Stilbs, P.; Lindman, B. *J. Magn. Reson.* **1982**, *48*, 132−137.

(24) Bleam, M. L.; Anderson, C. F.; Record, M. T., Jr. *Biochemistry* **1983**, *22*, 5418−5425.

(25) Braunlin, W. H.; Nordenskiöld, L. *Eur. J. Biochem.* **1984**, *142*, 133−137.

(26) Sunnerhagen, M.; Denisov, V. P.; Venu, K.; Bonvin, A. M. J. J.; Carey, J.; Halle, B.; Otting, G. *J. Mol. Biol.* **1998**, *282*, 847−858.

(27) MacKerell, A. D.; Wiokiewicz-Kuczera, J.; Karplus, M. *J. Am. Chem. Soc.* **1995**, *117*, 11946−11975.

(28) Toukan, K.; Rahman, A. *Phys. Rev. B* **1985**, *31*, 2643.

(29) Dang, L. X. *J. Chem. Phys.* **1992**, *96*, 6970.

(30) Heinzinger, K. *Physica B+C* **1985**, *131*, 196.

(31) Allen, M. P.; Tildesley, D. J. *Computer Simulations of Liquids*; Clarendon Press: Oxford, 1987.

(32) Melchionna, S.; Ciccotti, G.; Holian, B. L. *Mol. Phys.* **1993**, *78*, 533.

(33) Tuckerman, M.; Berne, B. J.; Martyna, G. J. *J. Chem. Phys.* **1992**, *97*, 1990.

(34) Lyubartsev, A. P.; Laaksonen, A. *J. Biomol. Stuct. Dyn.*, in press.

(35) Cussler, E. L. *Diffusion-Mass Transfer in Fluid Systems*; Cambridge Universty Press: New York, 1984.

(36) Lavalle, N.; Lee, S. A.; Rupprecht, A. *Biopolymers* **1990**, *30*, 877−887.

(37) Impey, R. W.; Madden, P. A.; McDonald, I. R. *J. Chem. Phys.* **1983**, *87*, 5071.

(38) Lyubartsev, A. P.; Laaksonen, A. *J. Phys. Chem.* **1996**, *100*, 16410.

(39) Rupprecht, A.; Forslind, B. *Biochim. Biophys. Acta* **1970**, *204*, 304−316.

(40) Falk, M.; Hartman, K. A., Jr.; Lord, R. C. *J. Am. Chem. Soc.* **1962**, *84*, 3843−3846.