HYDRATION OF NaDNA BY NEUTRON QUASI-ELASTIC SCATTERING

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ABSTRACT Preliminary results of neutron quasi-elastic scattering experiments are reported for hydrated paracrystals of sodium deoxyribonucleic acid (NaDNA). The samples were investigated at two water contents: 3.5 ± 1.0 and 9.5 ± 1.5 mol H_2O per mole nucleotide. The results of the scattering experiments were almost independent of whether the NaDNA fibers were oriented parallel or perpendicular to the momentum transfer. The data indicate that at the lower hydration the water molecules do not diffuse appreciably on the time scale of the neutron measurements ($\sim 3 \times 10^{-10}$ s). At the higher hydration the water molecules diffuse isotropically in a sphere of 9 Å in diameter with a diffusion coefficient of $(5 \pm 2) \times 10^{-6}$ $cm^2 s^{-1}$.

INTRODUCTION

The investigation of water in living and model biological systems, which has spurred much interest for quite some time, is still an area of great activity due to the primary role of water in sustentation (1, 2). Hydrates of macromolecules in the solid state have an advantage over aqueous solutions in this work since the large molecules are essentially rigid. This has led to much research on hydrated powders, crystals, and fibers. In particular, the powerful technique of neutron quasi-elastic scattering (NQES) had its first biological application in the study of hydrated oriented DNA prepared by Rupprecht's wet spinning technique (3). In this communication the results of higher resolution NQES experiments are reported. The increase in the resolution of the scattering experiments has enabled a more detailed analysis of the water dynamics in the hydrated DNA than was previously possible.

MATERIALS AND METHODS

The samples of highly oriented sodium deoxyribonucleic acid (NaDNA) were prepared by wet spinning (4) calf-thymus DNA (Worthington Industries, Inc. Columbus, OH). The samples contained 1% NaCl by weight and, therefore, were in the A form at both water contents studied (5). A single paracrystal of NaDNA was cleaved into seven slices, each

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with area 5×8 mm^2 and thickness from ~0.5 to 1.0 mm, and the samples were oriented in an aluminum sample holder so that their fiber axes were aligned parallel. The water contents of the samples were established by the method of Falk et al (6). The high hydration sample was exposed in the 86% relative humidity (r.h.) atmosphere from saturated aqueous potassium chromate, while the low hydration NaDNA was equilibrated in a 33% r.h. atmosphere over aqueous magnesium chloride. All samples were equilibrated for a period of 4 d. The water contents of the low and high hydration NaDNA were determined by gravimetric measurements and neutron counting rate observations during the TOF experiments; the gravimetric measurements were confirmed by Nuclear Magnetic Resonance Free Induction Decay (NMR FID) measurements (7). This was to account for any possible water loss during the transfer of the samples to the aluminum sample holders used for the NQES studies and during the actual scattering experiments. The low hydration NaDNA contained 3.5 ± 1 mol H_2O per mole nucleotide; the high hydration sample contained 9.5 ± 1.5 mol H_2O per mole nucleotide.

The NQES experiments were performed on the time of flight (TOF) spectrometer IN5 at the Institut Laue-Langevin (ILL) using a wavelength 10.05 Å to maximize the resolution (~20 μeV full-width half-maximum). Experiments were performed with the NaDNA samples at two orientations: with their fiber axes perpendicular and parallel to the neutron momentum transfer. (In fact, the parallel orientation was exact only for the scattering angle of 90°.) The sample holder was loaded into the sample chamber at an angle of 135° to the incident neutron beam. The scattered neutrons were detected at angles from 23° to 104° corresponding to momentum transfers of 0.25 to 0.98 Å^{-1}. The scattered neutrons were detected over a period of 8–10 h to give sufficient signal-to-noise of the TOF spectra. The resolution of the spectrometer was determined experimentally by taking spectra of a vanadium standard before and after the experiments on the NaDNA. The spectrum for an empty container was measured to correct the NaDNA data for scattering from the aluminum container. All measurements were made at 21°C.

The scattering laws, $S_{inc}(Q, \omega)$, for the samples were obtained from the TOF spectra using the standard programs available at the ILL (8). The

$S_{inc}(Q, \omega)$ were analyzed as the sum of elastic and broadened terms:

$$S_{inc}(Q, \omega) = [p + (1 - p)A_0(Q)]\delta(\omega)$$

$$+ (1 - p)[1 - A_0(Q)]\frac{1}{\pi}\frac{\Gamma_{1/2}^2}{\Gamma_{1/2}^2 + \omega^2}. \quad (1)$$

The first term on the right-hand side of Eq. 1 is the elastic term weighted by the elastic incoherent structure (EISF), $A_0(Q)$, while the second term was considered to be a Lorentzian with half-width at half-maximum of $\Gamma_{1/2}$. Vanadium spectra were used to normalize the NaDNA spectra and to account for the convolution of the spectra due to the resolution of the instrument. Since it was known that some protons on the oriented NaDNA fibers do not rotate or diffuse (7), the fixed proton fraction, p, was introduced into the data analysis. The fraction p was well determined from the sample stoichiometry since the water contents of the samples were known.

RESULTS

In all cases the spectra of the scattered neutrons contained an elastic part superimposed on a broadened component (see Fig 1). The dependence of the EISF and of the width of the broadened component on the momentum transfer, Q, are illustrated in Fig. 2, a and b, respectively. The low hydration samples show only a slight decrease of the EISF with increasing Q, i.e., $S_{inc}(Q, \omega)$ is essentially an elastic peak at all Q. The variation of the EISF with Q for the more hydrated sample is more pronounced. The $\Gamma_{1/2}$ for the more hydrated sample is constant up to $Q \sim 0.7$ Å after which it increases slightly with Q. The $\Gamma_{1/2}$ for the less hydrated sample is not considered quantitatively due to the too small intensity of the quasi-elastic part. In all cases the variations of both the EISF and the $\Gamma_{1/2}$ with Q are nearly independent of the orientation of the NaDNA fiber axis with respect to the momentum transfer.
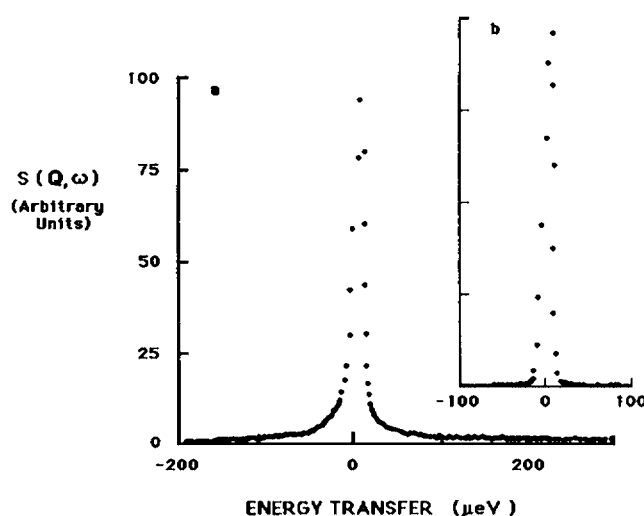
The measurements on IN5 are qualitatively supported

FIGURE 1   (a) A typical NQES spectrum for the hydrated NaDNA at the scattering angle of 104°. The particular spectrum is from the more hydrated NaDNA having ~9.5 mol H_2O per mole nucleotide. The NaDNA fiber axis was oriented perpendicular to Q. (b) A spectrum for vanadium showing the resolution of IN5.

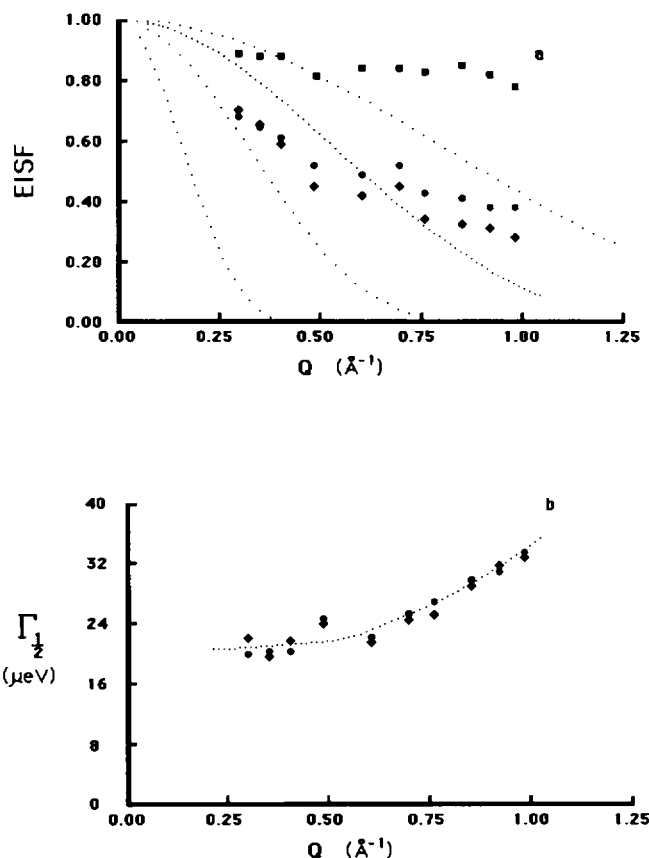FIGURE 2   (a) The momentum transfer dependence of the EISF, $A_0(Q)$, for the NaDNA at both high and low hydrations with ~3.5 and ~9.5 mol H_2O per mole nucleotide, respectively. The diamonds and circles are the data from the high hydration sample with the DNA fiber axis oriented perpendicular and parallel to Q, respectively. The squares are the data from the low hydration samples at both orientations of the fiber axis. The curves are Eq. 3, with $a = 3$ Å ($\cdots\cdots$) and with $a = 2, 5$, and 10 Å ($\cdot\cdot\cdot$, top to bottom, respectively). (b) The variation of the half-width at half-maximum, $\Gamma_{1/2}$, of the motionally broadened component for the high hydration NaDNA. The diamonds and the circles are the results from the experiments with the DNA fiber axes oriented perpendicular and parallel to Q, respectively. The curve is the behavior predicted by the model of reference 10 for $D_L = 5 \times 10^{-6}$ cm^2 s^{-1} and $a = 4$ Å.

by measurements on the TOF spectrometer IN6 at the ILL which operates with a higher neutron flux rate over a larger range of Q (7). On IN6 the low hydration sample also scatters the neutrons elastically, while, for the high hydration sample, a broadened quasi-elastic shoulder grows with Q. The results are again independent of the orientation of the DNA fiber axis with Q. These data are presently being analyzed with a model that extends to the higher Q range measured, in particular by taking into account the rotational motion of the water molecules, and are not presented here.

DISCUSSION

As seen from the strong elastic component of the scattered neutrons at low momentum transfers, the motion of the

water molecules adsorbed in the oriented NaDNA in the A form is a restricted "local" motion. The momentum transfer dependences of both the EISF and the broadening of the quasi-elastic peak (in particular, the constant value of the width at small Q) are consistent with such local motion. This is not unexpected as the NaDNA contains only A class water (9) at both hydrations, that is, all water molecules are hydrogen bonded to the DNA duplex.

The local motion is such that the water diffusion is equally free (on the timescale of the neutron measurements of $\sim 3 \times 10^{-10}$ s) in both the direction of the fiber axis and in the direction perpendicular to the fiber. This isotropy is apparent since the variation of both the EISF and the $\Gamma_{1/2}$ with Q are independent of the orientation of the NaDNA with respect to the momentum transfer. Particularly, if the diffusion along the fiber axis were less hindered (as one might expect, a priori), then the broadening of the quasi-elastic peak at low Q would be greater for the sample oriented perpendicular to Q than that of the sample oriented parallel. This difference has not been observed within the accuracy of the data ($\sim 10\%$), and so the diffusion is considered to be isotropic.

The scattering law for the NaDNA sample with the low water content (3.5 ± 1 mol H_2O per mole nucleotide) is essentially elastic at all Q irrespective of the orientation of the fiber axis to Q. This indicates that the water molecules do not diffuse in the DNA matrix on the time scale derived from the resolution of the instrument ($\sim 3 \times 10^{-10}$ s).

The EISF of the more hydrated samples (9.5 ± 1.5 mol H_2O per mole nucleotide) undergoes a greater decrease with Q, indicating that the motionally broadened component of the scattered neutrons is more intense. The local motion of the water in the more hydrated NaDNA is characterized further using the model of diffusion within a sphere (10). This model is suggested by the lack of evidence of any anisotropy of the water diffusion relative to the fiber axis and by its success in a hydrated polymer system at a similar hydration to the present study ($\sim 15\%$ water by weight) (11, 12). The model predicts a scattering law of the form

$$S_{inc}(Q, \omega) = \left(\frac{3j_1(Qa)}{Qa}\right)^2 \delta(\omega) + \frac{1}{\pi}\sum_n \left[L_n\left(\frac{D_L}{a^2}\right)\right], \quad (2)$$

where a is the radius of the sphere in which the water molecules are free to diffuse isotropically, and the $L_n(D_L/a^2)$ are Lorentzian functions with widths dependent on the radius of the sphere and the local diffusion constant, D_L, of the particles within the sphere. In a phenomenalogical approach, the infinite sum of the Lorentzians can be approximated by a single Lorentzian having a half-width at half-maximum, $\Gamma_{1/2}$, see Eq. 1. Also, from Eq. 2 the EISF is

$$A_0(Q) = \left(\frac{3j_1(Qa)}{Qa}\right)^2. \quad (3)$$

Calculations (10) show that, for $(Qa) \ll \pi$, the broadening of the quasi-elastic peak is constant at $\Gamma_{1/2} = 4.33(D_L/a^2)$. Thus, the model predicts that the $\Gamma_{1/2}$ of the quasi-elastic peak remains constant to a particular Q after which it will increase, and that the decrease of the EISF with increasing Q is related to the radius a.

The $\Gamma_{1/2}$ for quasi-elastic peak for the neutrons scattered from the more hydrated NaDNA is constant up to $Q = 0.7 \pm 0.1$ Å^{-1}; the data from IN6 support this limit. From this increase in the broadening one obtains a preliminary estimate of $a = 4.0 \pm 1.0$ Å for the sphere in which the water molecules are localized. The Q dependence of the EISF supports this estimate for a; the EISF predicted by the model (Eq. 3) nearly agrees with that observed if the radius of the sphere is taken to be 3.0 ± 0.5 Å (see Fig. 2 a).

It is apparent from the EISF data that these estimates for a are preliminary, whereas the model predicts $EISF_{max} = 1$ at $Q = 0$, the measurement gives $EISF_{max} \sim 0.8$. It is a known effect that the EISF deviates from the form predicted by model dynamics when multiple scattering occurs, i.e., when the neutrons are scattered by more than one center within the sample (13). In particular, at low Q the EISF is <1.0 and at $Q \geq 1$ the EISF is greater than that predicted by the model. Since the samples studied had thicknesses of up to 1 mm, it is certain that some multiple scattering occurred. On the other hand, our observed $\Gamma_{1/2}$ are within a factor of two of those derived from the relaxation times of the hydrated DNA primary shell, as determined by both Brillouin scattering experiments (14) and computer simulations of molecular dynamics (15). Therefore, not only have our NQES results clearly demonstrated restricted motion of the water molecules in the hydrated NaDNA, but also they enable us to estimate a and D_L (see below).

The local diffusion coefficient for the water in the more hydrated NaDNA is estimated from the low Q value of the broadening, $\Gamma_{1/2} = 20 \pm 3$ μeV, to be $D_L = (5 \pm 2) \times 10^{-6}$ cm^2 s^{-1}. Thus, within a sphere of 9 Å in diameter (taking into account the Van der Waals radius of the proton) the water moleclues diffuse approximately one quarter as quickly as they do in bulk water (2), $D_{bulk} = 2.3 \times 10^{-5}$ cm^2 s^{-1} at 21°C. Also, D_L is larger by a factor of five than the diffusion coefficient for water in equally hydrated NaDNA as determined by NMR pulsed gradient spin-echo measurements (16), $D_{NMR} = (1.0 \pm 0.2) \times 10^{-6}$ cm^2 s^{-1}. This difference reflects the diffusion distance, $\Delta = (2 Dt)^{1/2}$, to which each technique is sensitive. The timescale, t, of the measurement is 3×10^{-10} s on the TOF spectrometer IN5 and typically 10 ms in the NMR pulsed gradient spin-echo measurements; thus, the diffusion distances probed by the NQES and NMR techniques are ~ 10 and $\sim 10^4$ Å, respectively. In a homogeneous medium with molecules undergoing unrestricted isotropic translational diffusion (such as bulk water), the diffusion coefficients

measured by the two techniques are equal (17). However, in the hydrated NaDNA, NQES probes the free diffusion of the water molecules on the small local distance scale (10 Å), while NMR measures the slower diffusion between these local sites (restricted by structural constraints) over the long range (10^4 Å); therefore, $D_L > D_{NMR}$.

CONCLUSIONS

The model of the water dynamics in the hydrated NaDNA inferred from the NQES measurements compliments the view of water in DNA presented in the literature. At the low hydration (3.5 ± 1.0 water molecules per nucleotide) the water molecules are bonded to the NaDNA matrix at the oxygen atoms at the ionic phosphate group of the helix backbone (6, 9). These water molecules do not diffuse freely on the timescale of 10^{-10} s. The incoherent scattering law from the more hydrated samples (9.5 ± 1.5 mol H_2O/mol nucleotide) is well modelled by considering the water to be free to diffuse isotropically within a sphere of radius 3.5 ± 1 Å. When compared with the dimension of the helix rise per base pair in the A form of DNA, 2.9 ± 0.4 Å (18), it is apparent that the water molecules exchange readily between the hydration sites of each nucleotide (6, 9).

In the more hydrated NaDNA the diffusion coefficient of the water molecules within the sphere of free isotropic diffusion ($D_L \approx 0.5 \times 10^{-5}$ cm^2 s^{-1}) is ~25% that of bulk water, but still much faster than the value determined by NMR which is sensitive to the long range diffusion behavior.

The dynamics of the water molecules inferred by this preliminary NQES study has been incorporated into a model of the NMR proton relaxation observed for NaDNA at low hydrations (7). Extension of the NQES studies to thinner samples, and to spectrometers operating to greater momentum transfers and at greater resolution, are expected to increase further the contribution of neutron scattering to the knowledge of the behavior of water in biological systems.

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