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SLOW NEUTRON SCATTERING FROM ORIENTED DNA FILMS

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Abstract.

Three different experimental techniques of neutron scattering has been used to study the nature of hydration in highly oriented DNA. It was found that the water molecules are ralatively strongly bound in the system but the structure of hydration does not seem to be strongly dependent on the crystalline form of DNA. It is conjectured that water molecules interact both between different segments in the same helix as well as between helices. The inelastic spectrum from hydrated LiDNA clearly displays a peak similar to the one assigned to the hindered rotational motion in bulk water. No attempts to derive a hydration structure is made.

Introduction.

The interaction between water and a biopolymer has been extensively studied by several experimental techniques. These studies have recently been reviewed by Cooke and Kuntz[1] and by Berendesen[2].

Cooke and Kuntz conclude that the experimental data give a reasonable consistent picture of the mobility and organization of water molecules near macromolecular surfaces. Three different types of water molecules can be identified:

I) "bulk water" which has a relaxation time τ in the range 10^{-11} to 10^{-12} sec.

II) "bound water" with $\tau \approx 10^{-9}$ sec.

III) "irrotationally bound water" with $\tau > 10^{-7}$ sec.

Thus type I water does not interact to any appreciable extent with the macromolecule while the motions of type II water molecules are significantly hindered. The type III water molecules are essentially bound to certain specific sites at the macromolecule. Berendsen uses the term "specific hydration" for this case.

During the last years slow neutron scattering has been used to obtain information on the hydration phenomenon[3,4]. Dahlborg and Rupprecht[5] report a preliminary study on an oriented sample of NaDNA. Spectra of scattered neutrons were recorded with the momentum transfer vector directed parallel to and perpendicular to the molecular axis. The water molecules in the samples were found to be arranged in a more or less regular way. Thus the water molecules could be considered as "bound" to hydrogen bonding sites at the surface or in the interior of the DNA molecule. From the width of the measured spectra it was also concluded that the mobility of the water molecules in the NaDNA film was considerably smaller than in bulk water. In the terminology of Cooke and Kuntz[1] this corresponds to the presence of type II and of type III water in the samples. However, the interpretation of the data was found to be very complicated as the spectra are obtained from two intercoupled systems; the DNA molecule with counter-ion and the water.

Samples.

The oriented DNA samples were prepared from films of highly oriented DNA obtained with the wet spinning method[11]. The NaDNA sample was similar to those described in the first paper[5] while for LiDNA a doublestrip sample with the approximate dimensions 70 x 70 X 0.4 mm was used. Each strip was formed from two LiDNA films (approximate dimension 35 x 290 x 0.05 mm) with a folding and pressing procedure [6,5] giving, in total, eight layers of film. On each short-side of the 35 mm wide strip a piece of DNA film was left and used for holding the two strips together side-by-side with a piece of adhesive tape and for fastening the sample in a flat sample container.

Diffraction experiments.

In order to study the hydration properties of DNA samples of different crystalline forms ordinary diffraction measurements were performed at a two-axis spectrometer at the TRIGA reactor in Ljubljana . The wave length of the incident neutrons was 1.11 Å. LiDNA as well as NaDNA hydrated with D_2O was studied at several contents.

In all samples hydrated with D_2O a peak at $Q = 1.9$ Å^{-1} was seen in the angular distribution of scattered neutrons. The peak which from the discussion above must be of coherent origin was riding on a high incoherent backhround originating from the protons in the DNA molecule. The integrated peak intensity $I(\alpha)$ is given in fig. 1 as function of the angle α between the helix and the momentum transfer vector at two different relative humidities. The sample was LiDNA with very low content of LiCl. Earlier a similar peak was reported in NaDNA hydrated with D_2O[5] (see also below). The LiDNA sample is known to have the crystalline C form while NaDNA has the A form.

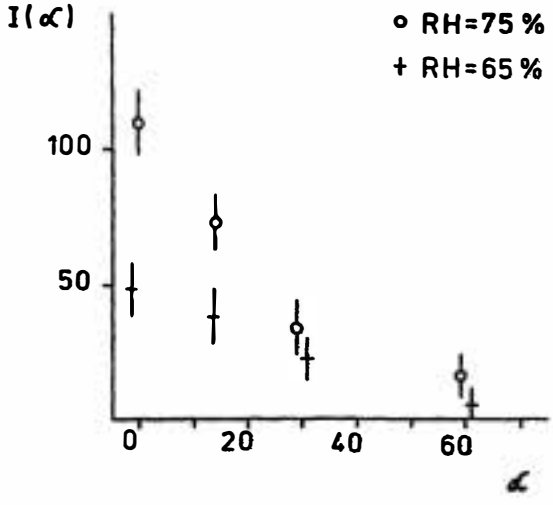

Fig. 1: Total intensity of coherent peak at $Q = 1.9$ Å^{-1} as function of the angel, α, between Q and the helix at two relative humidities.

It is known that is a considerable difference in the translation per residue in the c-direction and also in the turn angle per residue in the two cases. The possibility that the peak originates from the DNA structure can thus be excluded. The results instead indicate that the water molecules form a sort of internal structure in the samples. The structure does not seem to be strongly correlated to the surface of the DNA molecule. This point will be further discussed below. At the lower humidity (RH = 65 %) which corresponds to 6.1 mole D_2O/mole nucleotide[7] $I(\alpha)$ is rather broad while at RH = 75 % corresponding to 8.4 mole D_2O/mole nucleotide $I(\alpha)$ is considerably sharper. The area under $I(\alpha)$ is definitely larger at RH = 75 % than expected from the number of hydrated molecules. This indicates that the degree of orientational order in the direction of the helix is larger at the higher water content.

The water molecules hydrated to DNA may perform several types of motion. Falk et al.[8] concluded from an infrared study that there exists an inner hydration layer of about 10 water molecules per nucleotide in which the water molecules are incapable of crystallization upon cooling. An additional layer of about 3 water molecules per nucleotide crystallizes with difficulty. The NaDNA sample used in this measurement had a high water content (86.5 % relative humidity which corresponds to about 14 water molecules per nucleotide[7]). According to the definitions of Cooke and Kuntz[1] this indicates the presence of not only type II and type III water molecules in the sample but also the presence of water molecules of type I. In order to describe the water dynamics retational as well as translational motions should accordingly be taken into account.

It is probable that the D_2O molecules perform translational as well as rotational motions. In order to see if these might be seen in the measured intensity distributions the simplified theoretical calculation has shown that the intensity measured at small Q is not of dynamical origin. Instead it is conjectured that the water molecules stabilize the molecular structure of DNA and introduce a long range order. Thus the intensity at small Q should be of coherent nature. This is also corroborated by the fact that the small Q region corresponds to distances larger than the distance between second nearest

neighbours in D_2O. The intensity for $Q < 1.2$ Å^{-1} in fig. 2 varies with the angle between Q and the helix axis which indicates that the water molecules interact both between different segments in the same chain molecule as well as between different molecules.

The peak around $Q = 1.9$ Å^{-1} in fig. 2 was seen earlier by Dahlborg and Rupprecht[5]. The width of the peak for $\alpha = 0$ is about what is expected from the known mosaicity of the NaDNA film. Thus is can be considered as equivalent to a Bragg peak, the intensity of which can be used for determination of the hydration structure.

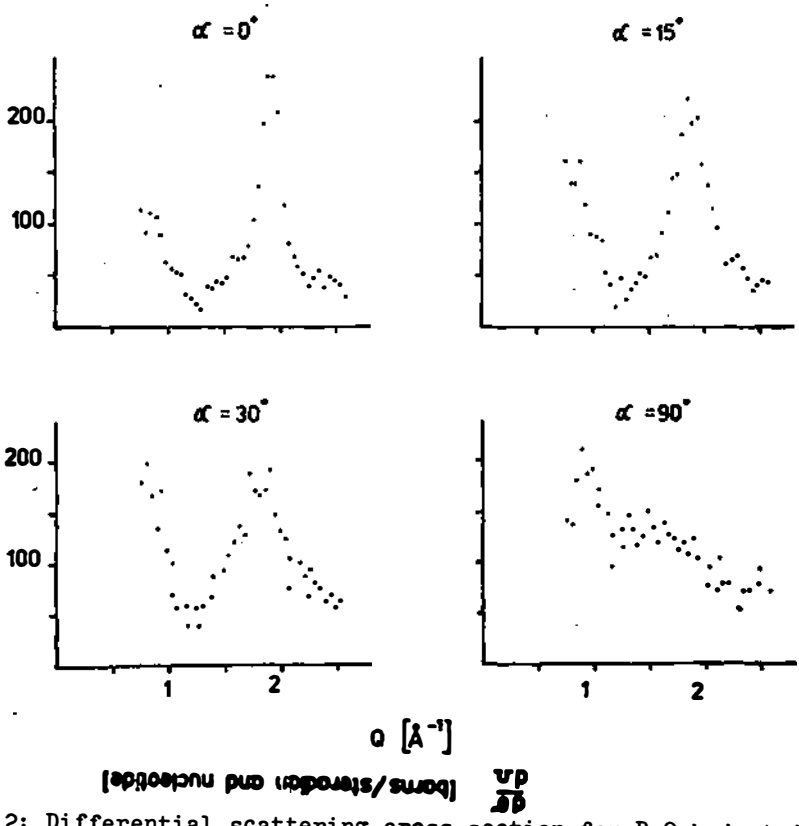

Fig. 2: Differential scattering cross section for D_2O hydrated to DNA obtained under the assumption that the scattering from the DNA skeleton is not depending on sample humidity.

Time-of-flight measurements.

In order to study the molecular dynamics in the LiDNA-water system a number of time-of-flight measurements were performed at the cold neutron facilities in Ljubljana and in Studsvik. The spectrometer in Ljubljana being of rotating crystal type is placed at the cold moderator at the TRIGA reactor. The wave length of the incident neutrons is 4.03 Å and the time resolution at this wave length is about 5 %. At the Studsvik spectrometer the wave length of the incident neutrons is 4.73 Å and corresponding time resolution is 2.6 %[9]. All spectra presented below are corrected for the wave length variation of the detector efficiency and for multiple scattering events in the sample. Dahlborg and Rupprecht[5] observed that the measured time-of-flight spectra from water hydrated to Na DNA were rather similar to the spectra measured in bulk water and in ice.

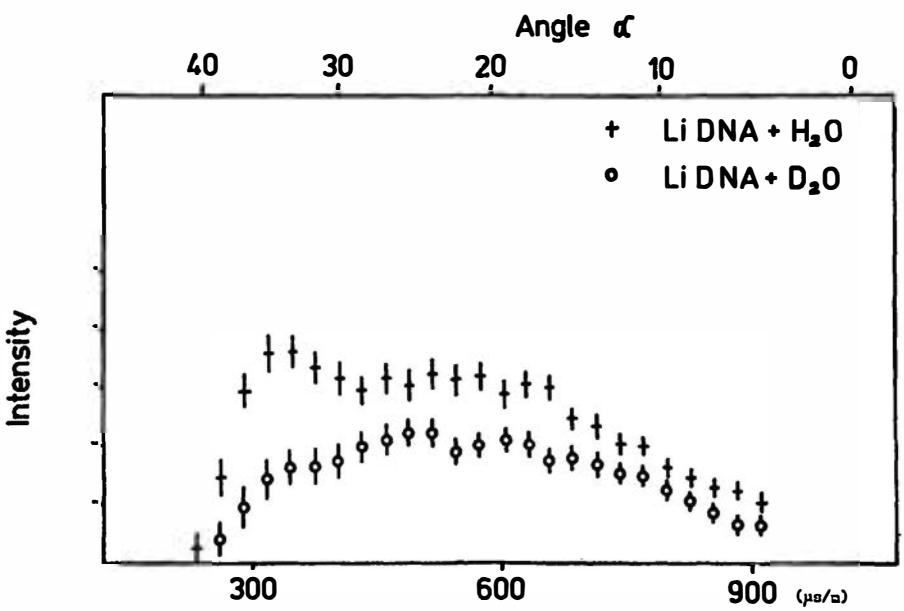

Fig. 3: Time-of-flight spectra from Li DNA hydrated to H_2O and D_2O. Relative humidity was 75 %.

From an inspection of fig. 3 it is obivious that when the water in the Li DNA sample is replaced by heavy water the intensity around 300 us/m, known to originate from the librational motion in the hydrogen boud in bulk water and in ice, is drastically reduced. Howver, when the hydrated water is replaced by heavy water some protons in the macromolecule will be replaced by deuterons due to exchange effects. Therefore the scattering from DNA backbone is different in the two spectra. However, out of 31 protons in a nucleotide only 8 will be exchanged by deuterons. Thus it can be concluded that the peak seen around 300 us/m is due to librational motions of water molecules and thus that there are hydrogen-bonded water molecules in the system.

References.

1. R.Cooke and I.D.Kuntz, Ann. Rev. Biophys. Bioengineering 3, 95 (1974).
2. H.J.C.Berendsen in Water, A Compresive Treatise, Ed. F.Franks, Vol. V. p. 293 (1975).
3. J.W.White, Proc. Roy. Soc., A 345, 119 (1975).
4. U.Dahlborg and E.Braun, Physica Scripta 14, 253 (1976).
5. U.Dahlborg and A.Rupprecht, Biopolymers 10, 849 (1971) and U.Dahlborg, Thesis 1978.
6. A.Rupprecht, Acta Chem. Scand. 20, 494 (1966); Biotech. Bioengineering 12, 93 (1970).
7. M.Falk, K.A.Hartman, Jr and R.C.Lord, J.Am.Chem.Soc. 84, 3843 (1962).
8. M.Falk, A.G.Poole and C.G.Goymour, Can. J. Chem. 48, 1536 (1970).
9. U.Dahlborg, M.Grönros, L.E.Karlsson, L.G.Olsson. T.Johnsson, D.Noreus and K.E.Larsson, to be published.