OBSERVATION OF QUADRUPOLAR NMR SIGNALS OF ^{7}Li AND ^{23}Na IN HYDRATED ORIENTED DNA

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SUMMARY: The NMR spectra of ^{7}Li and ^{23}Na in oriented DNA fibers show a splitting into three lines, due to quadrupolar interactions. The magnitude of this splitting depends on the water and salt content, and on the crystal form of the DNA. The spectra indicate that the alkali metal ions are in rapid motion around the fiber axis. These observations are relevant for an alternative interpretation of sodium NMR signals in biological tissues, which was recently proposed by Shporer and Civan, and states that all the sodium present yields a signal of which only 40% is easily detected due to quadrupolar effects.

The ^{23}Na nuclear magnetic resonance (NMR) in biological tissues has been studied on many occasions during recent years. A short review is given by Shporer and Civan [1]. In nearly all cases it has been observed that the intensity of the ^{23}Na signal was 30 - 40% of the expected value. Cope suggested that the sodium not observed in these experiments (60 - 70% of the total) is in some way bound to the tissue, thus producing a signal which is too broad to be detected in a conventional way [2,3]. In a pulsed NMR experiment the broad signal could be observed [3]. With the interpretation of these experiments in terms of a "free" and a "bound" fraction of sodium, the ^{23}Na measurements on tissue supported the "association - induction hypothesis" [4] for ion specificity in cells.

Very recently, Shporer and Civan pushed forward an alternative interpretation of ^{23}Na spectra of biological tissue [1]. They suggest that the

sodium nuclei in tissue experience electric field gradients. According to theory [5] this will cause a quadrupolar splitting of the ^{23}Na signal into three lines; the spectrum will consist of a central line with two satellites on each side of it. The distance between each of the outer lines and the central one is given by $\frac{1}{2}B \cdot (3\cos^2\theta - 1)$, where θ is the angle between the electric field gradient and the magnetic field H_0, and B is a measure for the magnitude of the quadrupolar interaction. In isotropic samples all angles θ occur, and the distribution of the angles θ produces a superposition of the outer lines, resulting in a broad line which is difficult to detect, in particular when there is also a spread in B. So, in many cases, only the central line, accounting for 40% of the total intensity, will be observable. Shporer and Civan demonstrated the existence of this quadrupolar effect in an isotropic sample of sodium linoleate in water; the satellites were indeed difficult to detect.

We want to report results from ^{7}Li and ^{23}Na NMR measurements on hydrated samples of oriented DNA, which clearly demonstrate a quadrupolar effect. Both these nuclei have a spin 3/2, and qualitatively should show the same behavior.

The DNA samples were prepared by the use of a wet spinning method [6], which gives a film of highly oriented NaDNA or LiDNA, containing any desired amount of NaCl or LiCl [7]. By folding an 8 mm. wide film of oriented calf-thymus DNA (Worthington Biochemical Corporation) several times perpendicular to the orientation, oriented DNA samples with the approximate dimensions 8 x 8 x 2 mm. were prepared. The water content of the samples was adjusted by storage at a suitable relative humidity (R.H.). Similar samples were used earlier in NMR studies of the hydration of DNA [8,9].

The NMR experiments were performed on a Varian Wide Line Spectrometer using a Variable RF Frequency Unit V-4210A. Both the ^{7}Li and the ^{23}Na derivative signals were studied at a frequency of 16 MHz.

^{23}Na spectra of several NaDNA samples in the A form were studied.

The observed spectra all gave a well resolved triplet. The splitting of the outer lines is proportional to $(3\cos^2\epsilon - 1)$, where ϵ is the angle between the fiber axis and the magnetic field. The maximum splittings observed are 2 to 50 Gauss, depending on the amount of NaCl and H_2O present

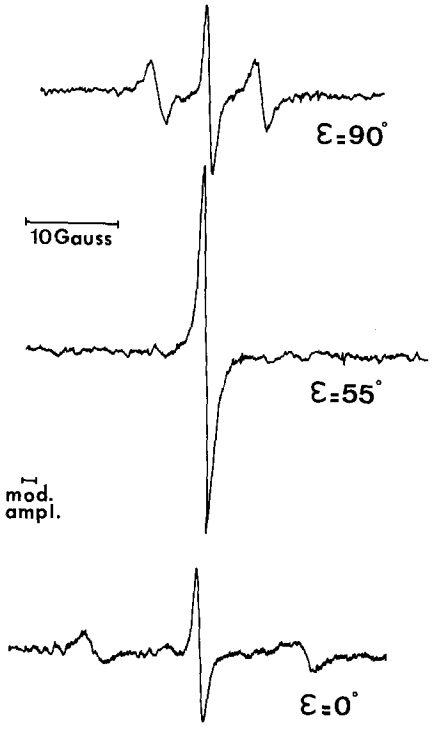

FIG. 1. ^{23}Na NMR spectra of oriented NaDNA at different angles ϵ between fiber axis and magnetic field. Resonance frequency 16 MHz., magnetic field 14.2 kGauss. The NaDNA sample contained about 6 g. NaCl and 65 g. H_2O (84% R.H.) per 100 g. dry weight.

in the samples. Representative spectra for one sample are shown in fig. 1.

Similar splittings were observed for ^7Li in LiDNA in the B form, as is shown in fig. 2. Here the maximum splittings (some 300 mGauss) are much less than in the case of sodium.

The angular dependence with $(3\cos^2\epsilon - 1)$ is expected for an uniaxial anisotropic system. The fact, that for $\epsilon = 90^\circ$ a well resolved splitting is observed, indicates that motational averaging occurs perpendicular to the fiber axis in such a way, that the resulting average field gradient seen by the ^{23}Na and ^7Li nuclei becomes axially symmetric.

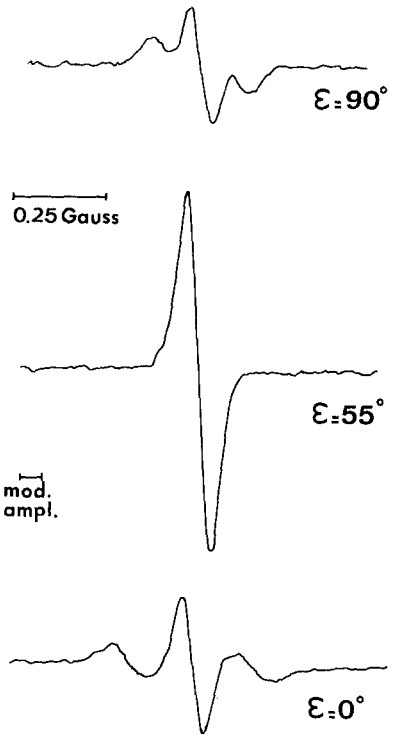

FIG. 2. ^{7}Li NMR spectra of oriented LiDNA at different angles ϵ between fiber axis and magnetic field. Resonance frequency 16 MHz., magnetic field 9.7 kGauss. The LiDNA sample contained about 10 g. LiCl and 60 g. D_2O (75% R.H.) per 100 g. dry weight.

Contrary to the former observations, only the central line could be observed for ^{7}Li in LiDNA in the C form. This can be due to the lower contents of LiCl and D_2O [7], which possibly restricts the mobility of the lithium ions.

DNA being a constituent of biological tissues, the quadrupolar splittings observed in the ^{7}Li and ^{23}Na NMR spectra of oriented DNA strongly support the interpretation by Shporer and Civan that the observed ^{23}Na NMR spectra in biological tissues are affected by quadrupolar interactions. This interpretation implies, that all sodium nuclei present contribute to an easily detectable signal with 40% of the total intensity. The remaining 60% of the intensity is smeared out by quadrupolar effects and is much more difficult to detect. This interpretation is in

contradiction to the conclusion by Cope, that the two fractions of the intensity correspond to "free" and "bound" sodium ions.

The use of oriented samples of DNA with various contents of electrolyte and water offers special advantages in physico-chemical studies. The observations presented here are an example, because it is very difficult to detect the quadrupolar interaction in nonoriented samples. We hope that continued measurements, as well by wide line as by pulsed NMR, will help to elucidate the quadrupolar effect on alkali metal ions occuring in DNA.

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