

## Hydration of DNA, a Wide Line NMR Study of Oriented DNA

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The role of water in the functions and structures of biological macromolecules is considered as important.<sup>1</sup> Proposals concerning the existence of icelike structure in the water of hydration<sup>2-4</sup> have stimulated much work. Nuclear magnetic resonance (NMR)<sup>5</sup> is a very suitable method for studying water structure, especially if molecularly oriented samples are used. A most interesting wide line NMR investigation has been performed by Berendsen<sup>6,7</sup> with oriented collagen fibres. Among other things, it was found that the proton magnetic resonance (PMR) signal from wet collagen gave a single line the width of which (less than 25 mG) showed a strong dependence of the angle between the fibre direction and the external field. On the basis of second moment calculations, Berendsen suggested that the collagen macromolecules stabilize the existence of chain-like structures in the water of hydration along the fibre direction by the formation of hydrogen bonds at appropriate sites.

Recently Berendsen and Migchelsen<sup>8</sup> have extended this investigation to oriented fibres of silk fibroin, keratin, and salmon sperm DNA. It was found that none of these exhibited a hydration structure similar to that of collagen. A single water line was obtained which in some cases showed an angular dependence. The inverse peak-to-peak amplitudes of the PMR signal derivative curves were plotted as a function of the angle between the fibre direction and the field as a qualitative indication of the angular dependence of the second moment. Contrary to the case of collagen the resulting curves indicated an anisotropy in a direction almost perpendicular to the fibre direction.

In this communication some preliminary results are reported from wide line NMR measurements on oriented thymus NaDNA (Worthington) prepared by a new wet

spinning method.<sup>9</sup> NaDNA films, 8.5 mm broad, were folded back and forth to form concertina-like packs which were slightly compressed into square pieces of oriented DNA with a thickness of about 2 mm. Several such pieces were then placed on top of each other with the same direction of molecular orientation and slightly compressed into a big package with the approximate dimensions  $8.5 \times 8.5 \times 17.5$  mm. After further storage for a week at 75 % r.h. the package of oriented DNA was mounted in a precision ground 12 mm glass tube which was then closed and supplied with an angular scale. The derivative of the PMR signal band at 14.8 Mc/sec was recorded on a Varian V-4200 wide line NMR spectrometer at various angles between the direction of molecular orientation and the field. A narrow (less than 40 mG) intense line was obtained which showed a strong angular dependence. This

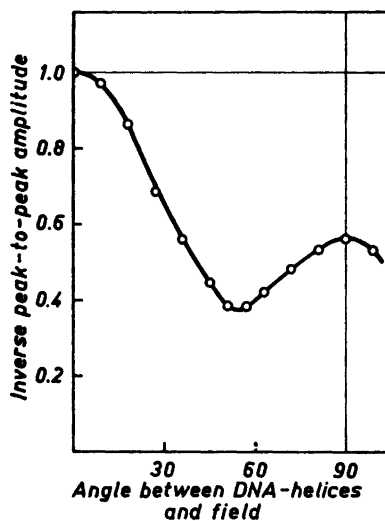


Fig. 1. Inverse peak-to-peak amplitude of the PMR signal derivative of oriented NaDNA at 75 % r.h. as a function of angle between direction of molecular orientation and field.

is illustrated in Fig. 1 where the inverse peak-to-peak amplitude of the recorded signal derivative has been plotted as a function of the angle between the direction of molecular orientation and the field. The resulting curve has quite another angular

dependence than the DNA curve obtained by Berendsen and Migchelsen, but agrees in its general appearance with the collagen curve,<sup>9</sup> having a minimum at an angle of about 55° and a maximum at 0°. This result indicates a hydration structure in the DNA sample studied which is similar to that occurring in wet collagen. For any discussion concerning the disagreement between the two investigations, it is necessary to know details of the fibre drawing method used by Berendsen and Migchelsen.

The present work will be extended to wide line NMR measurements on oriented Na- and LiDNA samples at various relative humidities and temperatures. The spectra will be subjected to a complete band shape analysis to elucidate the coupling between the water and the adsorbant. The influence of electrolyte content will also be investigated.

Other nuclei than hydrogen might give valuable information.<sup>10</sup> Preliminary measurements of the <sup>31</sup>P NMR signal, however, gave a broad weak line which showed no angular dependence.

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1. Szent-Györgyi, A. *Bioenergetics*, Academic Press, New York 1957.
2. Forslind, E. *A theory of water, Proc. Swed. Cement and Concrete Res. Inst. No. 16* (1952).
3. Jacobson, B. *Nature* **172** (1953) 666.
4. Klotz, I. M. *Science* **128** (1958) 815.
5. Andrew, E. R. *Nuclear Magnetic Resonance*, Cambridge University Press, Cambridge 1955.
6. Berendsen, H. J. C. *An NMR study of collagen hydration* (Diss.) Rijksuniversiteit te Groningen 1962.
7. Berendsen, H. J. C. *J. Chem. Phys.* **36** (1962) 3297.
8. Berendsen, H. J. C. and Migchelsen, C. *Ann. N. Y. Acad. Sci.* **125** (1965) 365.
9. Rupprecht, A. *Acta Chem. Scand.* **20** (1966) 494.
10. Brot, C. and Szwarc, H. *J. Chim. Phys.* **60** (1963) 671.

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## Semiconductivity of Dried Oriented DNA

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Szent-Györgyi's idea of comparing the gigantic macromolecules of biology with semiconductors has inspired many investigations.<sup>1</sup> In the case of DNA quantum-mechanical calculations by Hoffmann and Ladik<sup>2</sup> have shown that in the B form of DNA, with the base-pairs stacked perpendicular to the axis of the helix, the orbital overlap of the bases along the axis is sufficient to promote conductivity, and on this basis a mechanism for DNA duplication and cancer was proposed.

The experimental *d.c.* conductivity measurements have mainly been performed on dried DNA.<sup>3-5</sup> It has been found that the conductivity  $\sigma$  as a function of absolute temperature  $T$  follows the standard equation for a semiconductor  $\sigma = \sigma_0 \exp(-\Delta E/kT)$ . The activation energy  $\Delta E$  and the pre-exponential factor  $\sigma_0$  have been found to depend on the degree of dryness.

Samples of molecularly oriented DNA should be of great value for electrical measurements, a fact which has not passed unnoticed by others.<sup>4</sup> This inspired the development of a new wet spinning method whereby films of oriented DNA can be prepared in large amounts.<sup>9</sup>

In this communication some preliminary results concerning *d.c.* conductivity measurements on a dried film of oriented LiDNA are reported. The conductivity cell consisted of a piece of Teflon with two sprung-clips, 1 mm apart, serving both as holders for the film and as electrodes. Platinum was used as electrode material. The device was enclosed in a thermostatically controlled brass container. The DNA film was dried under vacuum at about 70°C and its conductivity was measured, still under vacuum, as a function of the temperature at various times using an EKCO N 616 B electrometer. The conductivity was ohmic. Measurements were also performed after drying in vacuum at about 125°C. The results for conduction perpendicular to the direction of molecular orientation are