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Velocity of high frequency sound waves in oriented DNA fibres and films determined by Brillouin scattering

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With 4 figures and 1 table

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During the last few years the study of low frequency excitations of various biological macromolecules has found an increasing interest, because such vibrations have been predicted (1) to play an important role for biological functions.

Recent theoretical calculations (2) on the vibrational spectrum of nucleic acids indicate that these double helical macromolecules might have low frequency optical modes in the microwave frequency region. According to ref. 2, the lowest frequency optical mode corresponds to a relative axial motion of one helix with respect to the other. This low frequency optical mode is expected to soften further when the DNA molecule – at increasing humidity – transforms from the socalled A- to the B-conformation.

In search for this low frequency mode we have studied DNA-fibres and DNA-films in the dry and hydrated state by means of light scattering observing the frequency shifts between 5 and 120 GHz. By using a multipass Fabry-Pérot interferometer of high sensitivity we observed, for the first time, Brillouin scattering by microwave *acoustic* phonons in oriented solid DNA samples and measured directly their velocity in different directions. We were, however, not able to detect any *optical* modes in this frequency regime.

Our experimental arrangement was similar to the one used previously by *Harley* et al. (3) for the study of collagen fibres. Radiation from a single frequency argon or krypton laser was focused onto a DNA fibre or film mounted in a sealed sample holder, which could be rotated. The scattered light was analysed at various scattering angles by a fivepass Fabry-Pérot interferometer having a frequency resolution of about 0.5 GHz. Due to the high contrast ratio, the Brillouin-lines could be clearly seen besides strong back ground noise.

Oriented semi-crystalline *DNA-fibres* of diameter from 20 μm to 200 μm and of lengths up to 1 cm were prepared by stretching a concentrated gel during the drying process similar

to procedures described earlier (4). The starting material we used was highly polymerized calf thymus DNA (type I, Sigma Chemical Co.). The very highly oriented *DNA-films* (size typically $20 \times 5 \times 0.05$ mm^3) have been prepared and characterized (5, 6) by one of the authors (A. R.).

Fig. 1 shows a typical Brillouin spectrum obtained from a dry DNA-fibre – kept over silica gel – for the scattering geometry indicated in the insert. Here the wavevector of the participating phonon is parallel to the fibre axis and is given by

$$k_{\parallel} = 2k_0 \sin\theta = 2\pi\nu_a/v_{\parallel}$$

where k_0 and 2θ are the wavevector and scattering angle, of the external light beams; ν_a and $v_{\parallel}$ are the frequency and phase velocity of the emitted (or adsorbed) phonons, respectively. Note that in this geometry the phonon momentum is independent of the refractive index of the fibre. The central line in figure 1 corresponds to the strong elastic scattering of the fibre. The two satellites (a) and (b) on each side of the central peak are caused by inelastic light scattering and were always observed together. Dry DNA-films give similar spectra for the scattering geometry shown in figure 1.

Let us first discuss the *doublet (a)*. Its frequency shift ν_a, observed for various laser frequencies and scattering angles, varied always linearly with the magnitude of wavevector $k_{\parallel}$ as shown in figure 2. We therefore conclude that the lines (a) correspond to *acoustic* phonons travelling parallel to the DNA

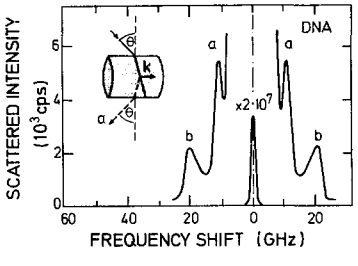

Fig. 1. Typical Brillouin spectrum of a dry semi-crystalline fibre of DNA recorded with a Fabry-Pérot interferometer using a free spectral range of 51 GHz in the scattering geometry ($\theta = 45°$) as shown. The frequency shifts of the two doublets (a) and (b) were unambiguously determined by varying the free spectral range

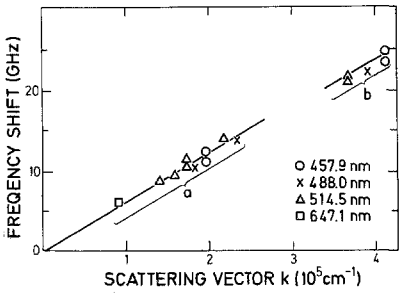

Fig. 2. Dispersion relation of longitudinal sound waves of a dry fibre with k parallel to the fibre axis as determined from doublet (a) of figure 1. Also the frequency shifts of doublet (b), arising from backscattering are shown. Since all observations fall roughly on the same line the velocity of longitudinal sound waves seems to be independent of frequency and direction of propagation

orientation axis. Since furthermore the lines (a) show the same polarization as the incident laser beam, we attribute them to the scattering from *longitudinal* phonons (7). The resulting velocities $v_{\parallel}$ of these phonons are given in the first column of table 1. It is interesting that

Table 1. Sound velocities of DNA-fibres and DNA-films
(The values have been derived either from the doublet (a) or (b) respectively. Values for DNA-films are representative for three types of films (Ref. 14) showing no significant differences.)

sample	$v_{\parallel}$ (msec^{-1})	$v_{\perp}$ (msec^{-1})
Dry fibre	3800 ± 200 (a)	3700 ± 250 (b)
Dry film I (14)	3550 ± 150 (a)	3750 ± 150 (a)
Film II (14) (at 66% rel. humidity)	2700 ± 150 (a)	3600 ± 400 (a)

these values compare rather well with the sound velocity derived from recent inelastic neutron scattering data (8) on three-dimensional crystals of the pure bases of 1-methylthymine.

The *doublet (b)* in figure 1, on the other hand, shows a very different behaviour. Its frequency shift is independent of the incident angle as expected for *optical* phonons. Since, however, the shift increases linearly with the laser frequency, the lines (b) cannot originate from light scattering by optical phonons. Instead we believe that lines (b) arise also from *acoustic*

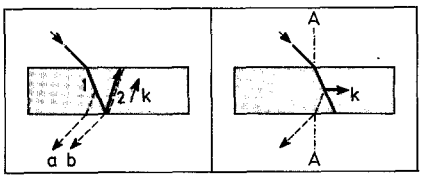

Fig. 3. Schematic representation of the scattering geometries

phonons by a scattering process indicated on the left part of figure 3: A fraction of the incident laser beam "1" propagating inside the fibre or film is reflected from the lower surface back into the material giving rise to beam "2". Part of this beam – backscattered by acoustic waves – is monitored as (b) under the same external angle as the scattered light (a). Since in this way line (b) originates – independent of the external angle θ – always from quasi-back-scattering, its frequency shift has for all external scattering angles θ the value

$$\nu_b = 2nk_0 v_\perp/2\pi$$

with n being the refractive index of the material and $v_\perp$ the velocity of the phonon travelling along the *direction* "2" in figure 3. Using $n = 1.5$ (9) we deduce – for fibres and films – from the observed shifts ν_b values $v_\perp$, which approximately agree with the values $v_\parallel$ determined from lines (a) for phonon propagation *parallel* to the DNA orientation axis (fig. 2) Our observations therefore imply that in dry DNA the velocity of longitudinal phonons is almost isotropic.

Evidence for a slightly anisotropic longitudinal sound velocity in DNA is provided by spectra of DNA-films taken in a scattering geometry (see fig. 3, right hand side) where the DNA orientation axis is varied stepwise from a position parallel to k to a position perpendicular to k by simply rotating the films around the axis A–A. For the dry film the resulting sound velocity $v_\perp$ is about 10% higher than $v_\parallel$. These results – an example is given in table 1 – are in good agreement with those obtained from lines (b). The interesting fact that the velocity perpendicular to the DNA-axis even slightly exceeds the value $v_\parallel$ – in strong contrast to the observation on simpler polymers like PE (12) – seems to become more pronounced in the hydrated state where the base stacking is known (10, 11) to be more perfect. For example, experiments with a humidified DNA-film in the B-form (see table 1) showed that the velocity parallel to the DNA-axis was at least 11% lower than $v_\perp$. The surprising fact that the parallel velocity $v_\parallel$ does not exceed $v_\perp$ is probably a consequence of the helical structure of the DNA-molecule: A stack of tightly packed oriented springs (as a very simplified model) would probably show qualitatively the same unusual "negative" anisotropy.

Our explanation for the origin of the lines (b) mentioned above is supported by another observation. We took, for comparison, also Brillouin spectra from thin homogeneous fibres of fused silica. These spectra again exhibited two doublets (a) and (b) (see fig. 4) both showing the same variation with laser frequency and external scattering angle as described above for the DNA-fibres. From both the lines (a) and (b) a longitudinal sound velocity in SiO_2-glass is deduced, which agrees excellently with the value reported in the literature. Obviously, optical modes are not necessary for the interpretation of the spectrum.

DNA is known to go from the A- to the B-conformation when the humidity is increased. At this phase transition a further softening of low lying optical modes has been predicted. We have therefore recorded Brillouin spectra of a DNA-fibre continuously for two hours after having filled the lower part of our sample chamber with distilled water. We observed a continuous decrease of the Brillouin shift corresponding to a decrease of the velocity from 3800 $msec^{-1}$ to about 1800 $msec^{-1}$. Thus the wet fibre (probable in the B-conformation) has a velocity of longi-

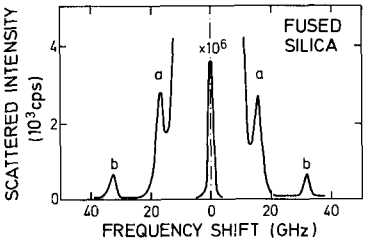

Fig. 4. Typical Brillouin spectrum of a fused silica fibre (diameter about 120 μ) using the experimental conditions described in figure 1 (but with a free spectral range of 76 GHz)

tudinal sound waves close to that of pure water. No evidence for low lying optical modes could be detected.

In all experiments described above – most clearly for the DNA-films – we noticed a surprisingly large dependence of the *intensity* of the Brillouin-scattered light on the propagation direction of the scattering phonon: Strong Brillouin scattering was only observed for phonons travelling parallel to the DNA-axis, whereas for phonon propagation perpendicular to this direction the light scattering was weaker by nearly one order of magnitude. This observation indicates a rather large anisotropy of the photoelastic response in oriented DNA fibres and films: Apparently the refractive index of DNA is modulated strongly only by dilatations (or compressions) parallel to the DNA-axis.

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Summary

We have measured the velocity of acoustic microwave phonons in fibres and films of oriented DNA. The sound velocity is found to be surprisingly isotropic and to decrease substantially during hydration of DNA. No optical modes have been detected in the frequency range covered from 5 to 120 GHz. The photoelastic constants are strongly anisotropic.

Zusammenfassung

Wir haben die Geschwindigkeit akustischer Mikrowellenphononen in Fasern und Filmen orientierter DNS bestimmt. Die Schallgeschwindigkeit ist überraschend isotrop und nimmt mit steigender DNS-Hydration wesentlich ab. Im Frequenzbereich zwischen 5 und 120 GHz konnten keine optischen Moden nachgewiesen werden. Die photoelastischen Konstanten sind stark anisotrop.

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14) Film I: 1 g NaCl/100 g dry DNA; Film II: 5 g LiCl/100 g dry DNA; Film III: 1 g LiCl/100 g dry DNA.

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