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Brillouin Scattering in Systems of Biological Significance [and Discussion]

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Phil. Trans. R. Soc. Lond. A 1979 **293**, 341-348
doi: 10.1098/rsta.1979.0101

Phil. Trans. R. Soc. Lond. A **293**, 341–348 (1979)
Printed in Great Britain

Brillouin scattering in systems of biological significance

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Brillouin scattering spectra, with a single mode argon-ion laser and triple-pass Fabry–Perot interferometer, have been measured for natural biological fibres and for films of synthetic polypeptides. For wet unstretched rat tail collagen, with the scattering vector parallel and perpendicular to the fibre axis, single Brillouin shifts of ± 7.9 GHz and ± 6.3 GHz respectively were observed. With the scattering vector at 45° to the fibre axis, two peaks (± 6.35 GHz and ± 7.7 GHz) were found. For horse hair keratin the shifts for the scattering vector parallel and perpendicular to the fibre axis were ± 8.7 GHz and ± 7.7 GHz respectively. A single peak only was found with the vector at 40° to the fibre axis. Two synthetic polypeptides poly(DL-caprylic acid) and poly(γ-ethyl-L-glutamate), known to have the α-helix conformation, gave shifts of ± 6.36 and ± 6.1 GHz. The results are discussed.

1. Introduction

Within a year or two of the discovery of X-ray diffraction, Brillouin realized that light could be scattered by the periodic fluctuations in density that occur in solids or liquids as a result of thermally excited sound waves. The scattered light is shifted in frequency from that of the incident light by, typically, 1–10 GHz for 90° scattering because of the interaction of the incident light waves with the coherent longitudinal sound waves. The simplest Brillouin spectrum of an isotropic substance therefore consists of the central Rayleigh line together with two satellites of lower and higher frequency respectively. Anisotropic systems give rise to more complex spectra. Many measurements of Brillouin spectra of low molecular mass solids, liquids and solutions have been made, but comparatively few on high molecular mass materials or their solutions.

Biological systems are distinguished from other forms of matter by their content of high molecular mass substances, such as proteins and nucleic acids, and from synthetic polymers, by the fact that they are, for the most part, bathed in an aqueous buffer containing many other molecular species of both low and high molecular mass. Large numbers of proteins have now been isolated in pure form and some crystallized and analysed by X-ray diffraction. In the living plant or animal very few structurally important macromolecules exist singly; of these, collagen, the highly important protein of animal connective tissues, and the cellulose of plants are two. The Brillouin spectra of collagen have recently been examined by Harley *et al.* (1977). (We return to it in this paper because of new features observed in our experiments.) However, even vertebrate tendons that consist largely of collagen may contain small quantities of other molecules; cartilage, for example, contains polysaccharides as well as collagen. Muscle consists essentially of two proteins, myosin (itself a complex) and actin; the exo-skeleton of arthropods contains chitin and proteins, as do tendons of such insects as locusts.

There is a number of consequences of this natural situation. The ultimate object of observing Brillouin spectra of biological systems is to relate the measurements to their intrinsic molecular

properties. This can, in general, only be done if the biological sample is maintained in surroundings approximating to those found within the organism. Proteins, if allowed to dry, may be denatured and not recover their original properties on rewetting. In the experiments which follow, the collagen specimens have been kept in saline, although this is probably one of the few proteins that withstand dehydration without serious damage.

In addition, some tissues such as insect tendons may be observed in both the natural state and after removal of the protein. One may thus examine the whole tendon or its chitin moiety, so that, together with those proteins that form glasses and crystals, the opportunity arises to study materials of biological origin in a number of forms. In some instances the optical properties may be related to the elastic ones and ultimately to the force constants of the molecules involved, as in the work of Harley *et al.* (1977). The mechanical properties of biological materials measured by imposing known stresses are of practical value, but do not normally relate to fundamental molecular characteristics because of natural faults and uneven distribution of stress. In this contribution we have confined our attention to collagen, to keratin in the form of unstretched and stretched horse hair, and to two synthetic polypeptides, poly(DL-caprylic acid) [R = —$(CH_2)_5\,CH_3$] and poly(γ-ethyl-L-glutamate) [R = —$CH_2COOC_2H_5$]. While the first two were in their natural fibrous form, the polypeptides were examined as thin films.

2. Experimental methods and results

The spectral analysis of light scattered from biological materials is made difficult by several factors. The first of these is the very intense elastic scattering which necessitates the use of tandem or multipass interferometers. As is well known, the extinction (that is the ratio of instrumental peak height to background) is greatly increased in such instruments compared with a single pass device. In our work we have used a triple pass Fabry–Perot interferometer of extinction approximately 5×10^6 and a finesse (ratio of free spectral range to instrumental half-value width) of *ca.* 45. The light source was a single mode argon ion laser operated in the power range 10–40 mW at 0.488 μm.

In many biological materials the rather poor optical surfaces provide a second difficulty, and in addition the samples often require mechanical support for precise positioning in the optical arrangement. For quantitative work it is necessary to establish a well defined scattering vector by ensuring good optical paths through the sample. A technique we have used widely is shown in figure 1; the sample is held between microscope slides and is surrounded by a suitable liquid which provides some degree of index matching, reduces surface scattering and in many cases gives a suitable environment for the specimen. An additional advantage of this arrangement is that, as noted by Vaughan (1976), with the scattering vector aligned in the plane of the sample the Brillouin shift is given by

$$\Omega = \pm 2n_1(U/\lambda_0)\sin\beta \text{ Hz},$$

where n_1 is the refractive index of air, λ_0 is the wavelength *in vacuo* and 2β the measured scattering angle in air. Knowledge of the refractive index of the sample is not required and the hypersonic speed in the sample (U) may be simply related to the measured Brillouin shift. In our work the scattering angle was $90 \pm 1°$.

As an example of the technique, figure 2 (*a*) shows the Brillouin spectrum of a 63 μm film of poly(DL-caprylic acid) sandwiched with water between slides. The surface of the sample was

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optically poor and a thin layer of water (in which it is insoluble) greatly reduced the surface scattering. Scattered light was collected from a thickness of about 80 μm; thus, Brillouin spectra of both water and sample are evident. The lower spectrum, figure 2(*b*), shows the Brillouin scattering of water alone.

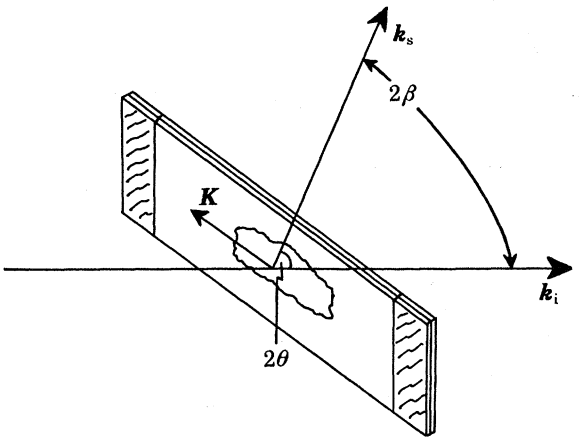

FIGURE 1. The optical arrangement. The scattering vector K (equal to $k_s - k_i$) lies in the plane of the sample which is supported in a suitable medium between cover slides. The scattering angle within the sample is 2θ, and as measured in air 2β.

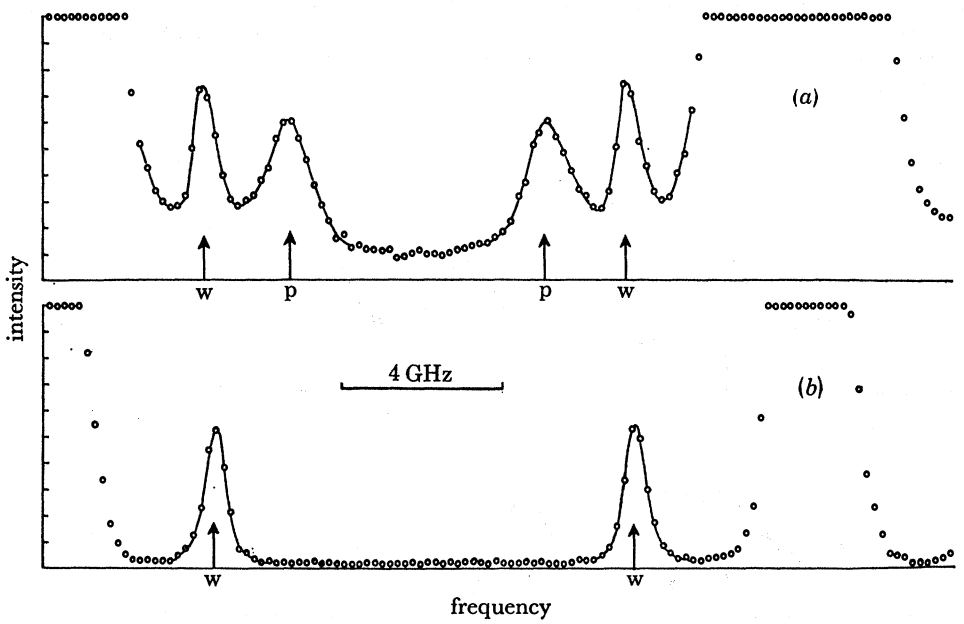

FIGURE 2. Brillouin spectra of synthetic polypeptide poly(DL-caprylic acid) and water. One order of interference is shown; the Brillouin peaks are shifted respectively from the adjacent elastic peaks. The measured shifts are for the polypeptide ±6.36 GHz, and for water ±4.26 GHz.

Collagen

Figure 3 shows spectra of rat tail collagen. The collagen was unstretched and maintained in physiological saline solution between slides; the fibre axis was aligned parallel with the scattering vector. The measurement with two etalon spacers as shown established the Brillouin shift without ambiguity. Figure 4 shows the effect of rotating the sample about an axis normal to the cover slides so as to change the angle between the fibre axis and the scattering vector.

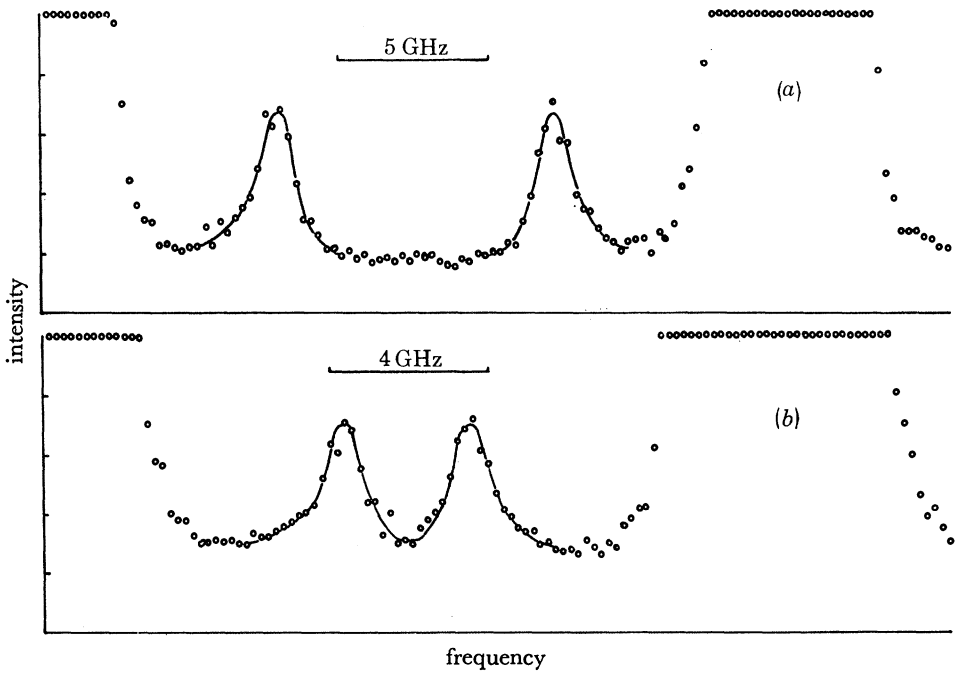

FIGURE 3. Brillouin spectra of wet collagen with two different interferometer spacings: (*a*) 6.19 mm ≡ 24.25 GHz free spectral range, (*b*) 7.90 mm ≡ 19.00 GHz free spectral range. The measured Brillouin shifts were (*a*) ±7.78 GHz and (*b*) ±7.87 GHz, in good agreement with one another. The scattering vector is parallel with the fibre axis.

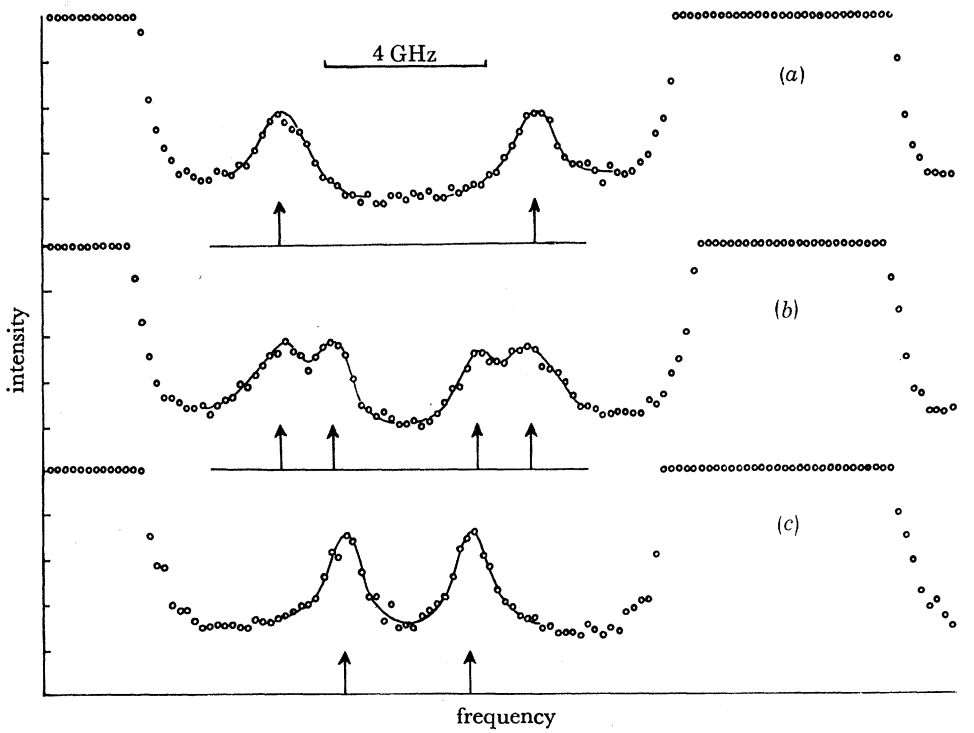

FIGURE 4. Brillouin spectra of wet collagen at different angles between the fibre axis and scattering vector: (*a*) vector perpendicular to axis, shift ±6.3 GHz, (*b*) vector at 45° to axis, shifts ±6.35 and ±7.7 GHz, (*c*) vector parallel to axis, shift ±7.87 GHz. The interferometer spacing was 7.90 mm.

The result shown in figure 4(*c*) is much as might be expected; the Brillouin shift and corresponding hypersonic speed are reduced by *ca.* 20% in comparison with propagation along the fibre. No observation of Ω of comparable quality with $\boldsymbol{K}$ perpendicular to the fibre axis were reported by Harley *et al.* (1977), (cf. their figure 1 with our figure 4), nor were any attenuation figures reported.

The most remarkable spectrum from collagen, however, is shown in figure 4(*b*), with the scattering vector at 45° to the fibre axis. Two distinct Brillouin modes are apparent, whereas one might have expected a single mode of frequency shift intermediate between the results at 0 and 90°. Such spectra were obtained at three out of four positions examined on the sample (the fourth gave a broad ill-defined spectrum). The close correspondence of the doublet peaks with the separate individual peaks at 0° and 90° might suggest that, at these positions, several different domains with local fibre structure parallel and perpendicular to the scattering vector are being examined. However in this case it is hard to see why similar broadened or double peak spectra were not observed in any of the dozen or more measurements made at 0° or 90°.

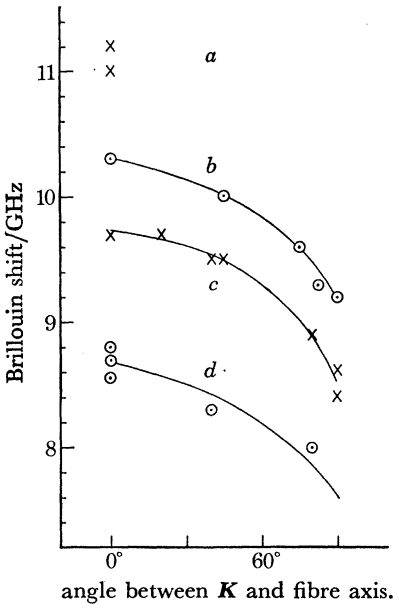

FIGURE 5. Measurements of Brillouin shift in hard keratin at different angles between scattering vector and fibre axis, and under different conditions: (*a*) unstretched in air, (*b*) stretched × 1.7 and immersed in liquid paraffin, (*c*) unstretched and immersed in liquid paraffin, (*d*) unstretched in water.

Keratin

Hair and wool are complexes of proteins containing sulphur and are stabilized by disulphide bonds. They are examples of tissues containing keratin that normally do not react appreciably with the natural environment. Moreover, they are mechanically strong: hence their commercial importance. As with Fraser *et al.* (1972) and Fraser & MacRae (1973), to whose publications reference should be made for comprehensive details, the term keratin is used here to denote an insoluble complex of sulphur-containing proteins found in certain epidermal tissues. Thus so-called soft keratin is found in *stratum corneum* and the eponychium of nails; and hard keratin, which is our immediate concern, is found in hair, nails, claws and beaks. Keratins differ chemically and in their high-angle X-ray diffraction patterns. Soft keratins contain less

sulphur than their hard counterparts. Mammalian hard keratins, the Brillouin spectra of which we have examined in the form of horse hair, give an α-type pattern when in an undeformed state. When suitably stretched the α-keratin X-ray pattern changes to the β-form. Exposure to steam allows hair to be extended to about double its original length.

Our Brillouin scattering measurements on horse hair, using the same technique as described for collagen, are summarized in figure 5 and table 1. The samples were carefully chosen to be colourless and without pigment. No sign was found of the doublet spectra observed in collagen and, as indicated, the Brillouin shift changed smoothly as the angle between the scattering vector and the fibre axis was changed, both for dry and wet fibres, stretched and unstretched. The result in air represents the value for nearly completely dried material as a consequence of the laser heating and drying. The results in liquid paraffin correspond to intermediate humidities where loss of moisture from the fibre is inhibited by the paraffin.

TABLE 1. HYPERSONIC SPEED AND ATTENUATION IN COLLAGEN AND KERATIN

material	scattering vector, $\boldsymbol{K}$	Brillouin shift, Ω/GHz	speed, U/km s^{-1}	attenuation, α/cm^{-1}
dry collagen	parallel to fibre axis	± 11.3	3.8_8	12×10^3
wet collagen	parallel to fibre axis	± 7.9	2.7_2	13×10^3
wet collagen	perpendicular to fibre axis	$\pm 6.^9$	2.1_6	18×10^3
dry keratin	parallel to fibre axis	± 11.2	3.8_4	7.5×10^3
wet keratin	parallel to fibre axis	± 8.7	2.9_8	10.5×10^3
wet keratin	perpendicular to fibre axis	± 7.7	2.6_6	15×10^3

The results are for a scattering vector of magnitude 1.82×10^5 cm^{-1}. Estimated error limits on the Brillouin shift and speed are $\pm 3\%$ and on the attenuation $\pm 12\%$ due to uncertainties inherent in deconvolution analysis.

Synthetic polypeptides

Synthetic polypeptides in the form of poly(DL-caprylic acid) and poly(γ-ethyl-L-glutamate) were included in our study because they have for many years provided valuable model systems of protein-like character. Both our examples are known to contain a high proportion of chain in α-helical configuration, and both are highly insoluble in water. Both specimens were in the form of film. The Brillouin shift for poly(DL-caprylic acid) was 6.36 GHz and that for poly-(γ-ethyl-L-glutamate) was 6.1 GHz. The spectrum of the former was shown in figure 1.

Other materials at present under investigation include the flexor and apodeme tendons of locusts, wild (Tussah) silk, pure cellulose, other polysaccharides and bovine serum albumin.

3. DISCUSSION

Our observations on collagen, horse hair (keratin) and the synthetic polypeptides poly(DL-caprylic acid) and poly(γ-ethyl-L-glutamate) do not warrant an extensive discussion until they have been extended to cover a wider range of physical conditions. However, it is clear that the enclosure of the specimen in a medium which is likely to reduce surface scatter and simultaneously preserve the native or imposed molecular configuration is a useful procedure. The observations of the Brillouin shift (± 7.9 GHz) and corresponding hypersonic speed ($U = 2.72$ km s^{-1}) for collagen, with the scattering vector parallel to the fibre axis, compare with the value of Harley *et al.* (1977) of $U = 2.62$ km s^{-1} under similar conditions of environment

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and momentum transfer. The lower shift (± 6.3 GHz) observed with the scattering vector at 90° to the fibre axis gives a correspondingly lower hypersonic speed, which is to be expected from the morphology of the system. In addition, table 1 shows values of attenuation calculated from the usual equation $\alpha = \Delta\nu\pi/U$ cm^{-1} where $\Delta\nu$ is the full width at half height (f.w.h.h.) of the Brillouin peaks after allowance for instrumental broadening. As expected the hypersonic attenuation for propagation across the fibre is greater than along the fibre axis. It is also worth commenting that the attenuation is generally larger by about a factor of two than that found recently in cyanobiphenyl liquid crystals (see, for example, Vaughan 1976; Bradberry & Vaughan 1976).

The unusual observation of two peaks when the scattering vector is set at 45° to the fibre axis merits further investigation. The possibility that transverse waves could be involved adds interest to experiments on fibres under tension, already an obvious probe.

The keratin (horse hair) observations suggest that the Brillouin shifts may be related in a comparatively simple way to the water content of the fibre and to the degree of helical extension present. Since, however, the fibre as a whole is a complex of proteins, the possibility of more complicated elastic phenomena must be kept in mind. Clearly, a more extensive series of observations could provide valuable information. We have hesitated to quote values of the bulk modulus (and hence compressibility) of our materials because of minor unresolved uncertainties of density.

This work was in part supported by the Medical Research Council and carried out at the Royal Signals and Radar Establishment, Great Malvern.

We thank Dr B. R. Malcolm, University of Edinburgh, for the preparation and gift of the films of synthetic polypeptides and Miss K. M. Bogle, also of Edinburgh, for much assistance with biological specimens.

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Discussion

S. Cusak (*European Molecular Biology Laboratory, Grenoble, France*). I would like to add to the results of Dr Vaughan and Sir John Randall the observation of the transversely polarized acoustic phonon propagating along the fibre axis of rat-tail tendon collagen fibres (at 45% relative humidity) which has been made recently at the European Molecular Biology Laboratory, Grenoble. This was detected by examining the light scattered with a change of polarization (i.e. the v.h. spectrum) by using the Brillouin scattering apparatus described by Harley *et al.* (1977).

The transverse hypersound velocity is found to be 2.3 times smaller than the longitudinal hypersound velocity. Furthermore, the intensity of the transverse mode was a factor of 40 lower than that of the longitudinal mode. Attempts are being made to measure the variation

of the transverse velocity with humidity and angle of propagation relative to the fibre axis. With the new results of Dr Vaughan and Sir John Randall it is hoped soon to establish a complete picture of the low frequency excitations and elastic constants of collagen.

Reference

Harley, R., James, D., Miller, A. & White, J. W. 1977 *Nature, Lond.* **267**, 285.

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