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Observation of Protoplasmic Streaming by Laser-Light Scattering*

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Data are presented which demonstrate that the laser light scattered from the protoplasm of living algae cells is Doppler shifted by the streaming motion of the macromolecules and particles in the protoplasm. The laser-light–scattering spectra are used to determine the velocities and velocity distributions inside the cells.

Laser inelastic light scattering has become a common and useful technique for the observation of the hydrodynamics of biological macromolecules.[1-3] If random diffusion of the macromolecules is the only motion in the solution, the scattered light is diffusion broadened about the incident frequency, and the extent of the broadening can be measured to determine the magnitude of the diffusion coefficients of the macromolecules in the solution. If the macromolecules are in directed motion, the scattered light is Doppler shifted, and the magnitude of the shift in frequency is a direct measure of the velocity of the directed motion. This principle has been used to measure the velocities of flowing solutions[4] and to determine electrophoretic velocities of charged macromolecules in solutions under the influence of electric fields.[5-7] In this Letter we report the first observation of light-scattering spectra which have been Doppler shifted by the flow of protoplasmic components inside a living cell.

Our experiments have been performed on the common alga *Nitella flexilis* (species identification was very kindly performed by Professor Richard D. Wood of the University of Rhode Island). Nitella samples were obtained from Carolina Biological Supply Company and were grown in sterile aqueous media at room temperature. Nitella is one of the many lower plants which exhibit pronounced cytoplasmic streaming (for an extensive review of this phenomenon, see Kamiya[8]). The streaming is observable under a light microscope by the motion of large particles which are carried with the flow. However, the determination of velocity and velocity distribution by this procedure is very tedious and is necessarily confined to the motion of particles which are large enough to be seen under a microscope. We have found that the measurement of velocities and velocity distributions in Nitella by laser-light scattering is very precise, perfectly objective, and extremely rapid.

The light-scattering spectrometer is of conventional design.[1-7] The laser is a Spectra Physics Model 125A helium-neon laser and the spectrum of the photocurrent is measured by a SAICOR Model SAI-51B time-compression spectrum analyzer. The Nitella cells are positioned in the apparatus in a special holder which holds them firmly in place but does not bind them. The holder can then be rotated to select the desired spatial component of the velocity. (The magnitude of the spectral shift is proportional to $\vec{K}\cdot\vec{v}$ where $\vec{v}$ is the velocity and $\vec{K}$ is the scattering vector, the vector difference of the incident- and scattered-light wave vectors, a coordinate which is determined by the geometry of the experiment.[4,6]) All cells were immersed in aqueous solution at all times. In order to avoid damage to the Nitella, it was essential to attenuate the incident laser

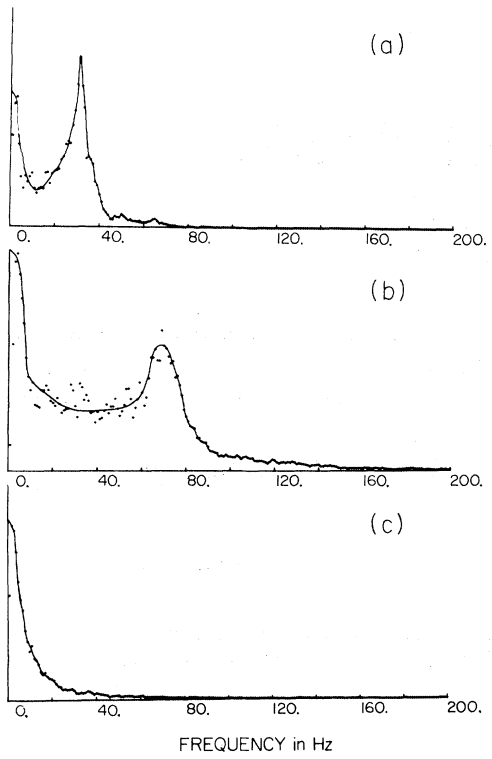

FIG. 1. Spectra of light scattered from the protoplasm of Nitella. The horizontal axis is frequency in hertz and the vertical axis is relative intensity. Spectrum (a) was taken at a scattering angle of 19.5 deg. Spectra (b) and (c) were taken at a scattering angle of 36.1 deg. Spectrum (c) was taken from the same point on the cell as spectrum (b), immediately after addition of para-chloromercuribenzoate, a streaming inhibitor. Each of these spectra was collected in about 30 sec. The points are the output of the spectrum analyzer, and the dark lines have been drawn merely to make the data more perspicuous and to emphasize the reproducible features of the data.

power to less than 0.2 mW. All measurements were made at 23°C.

Representative light-scattering spectra obtained from Nitella internodal cells are presented in Fig. 1. The horizontal axis is in frequency, but it may be helpful to think of the horizontal axis as a velocity axis. The velocity conversion factors can be calculated from the experimental geometry and the Doppler equation. For spectrum (a) in Fig. 1 a shift of 10 Hz corresponds to a velocity of 14.1 μm/sec, and for spectra (b) and (c) in Fig. 1 a shift of 10 Hz corresponds to a velocity of 8.0 μm/sec. The experimental geometry was such that the velocity component being observed is along the long dimension of the internodal cell. Spectra (a) and (b) are taken at different scattering angles and at different points on the same cell. The centers of the shifted peaks correspond to streaming velocities along the length of the cell of about 50 μm/sec for (a) and about 60 μm/sec for (b), which represent differences in velocities at different points in the cell. Spectrum (c) was obtained immediately after spectrum (b), except that para-chloromercuribenzoate, a known streaming inhibitor,[8] was added just a few seconds before the spectrum was taken. Clearly all evidence of streaming has disappeared. Observation of a change in light-scattering spectra upon quenching of streaming has been previously reported.[9]

The width of the spectrum about zero frequency in Fig. 1(c) is a measure of the diffusion of the various components in the protoplasm. This diffusion width is almost exactly equal to the width in spectrum (b), suggesting that the width in spectrum (b) is due to diffusion rather than to the variation in streaming velocities. Even if only part of the widths of the shifted spectra are due to diffusion, we can conclude that the velocity distribution of the particles represented in these shifted peaks is quite narrow. It was observed that the quenching of the streaming by para-chloromercuribenzoate was reversible simply by washing the cells with their normal nutrient solution, and it was often the case that cells reversed in this way showed a 10–20% increase over their initial streaming velocity, suggesting either that low levels of para-chloromercuribenzoate act as a stimulant to streaming or that the cells streamed faster as a result of their dormant period.

In addition to the main peaks in spectra such as (a) and (b) in Fig. 1, there is considerable intensity at higher and lower frequencies, corresponding to higher and lower velocities. Movement at lower velocities is easy to interpret as hindered motion of larger particles, although this interpretation may not be valid because the velocities corresponding to the main peaks correlate well with the velocities of very large particles seen in microscopes. The intensity at higher frequencies is even more intriguing. Although the high-frequency intensity may be due to experimental artifacts (one of which will be discussed later), it would be very interesting if this part of the spectrum indicates that some particles in the cell are moving much faster than the bulk of the protoplasm and hence faster than previous microscopic observations have reported. This high-veloci-

ty motion could be coming from macromolecules which are too small to be observed in a microscope, or may be related to some form of local motion which is responsible for the streaming of the entire protoplasm. Further investigations are needed to resolve these interesting possibilities.

The flow pattern in the Nitella internodal cell is helically wound in a pattern similar to a peppermint stick with alternating bands flowing in opposite directions. The spacing of the bands is approximately 4 mm. The diameter of the focused laser beam used in these experiments is approximately 0.1 mm, so that we were able to probe the local velocities anywhere on the cell with high resolution. These velocities are normally uniform[8]; but we observed that one set of cells (an old specimen which had become infested with parasites) often showed a constant and reproducible difference in flow velocity between the bands which flow in opposite directions. Spectra (a) and (b) in Fig. 1 are examples of this. The difference in velocities observed was usually 10–20% and was always the same for any given cell. This velocity difference was seen on both edges of the cell and throughout its entire length. This kind of anomaly, of which we are aware of no previous report, was never seen on fresh and healthy cells.

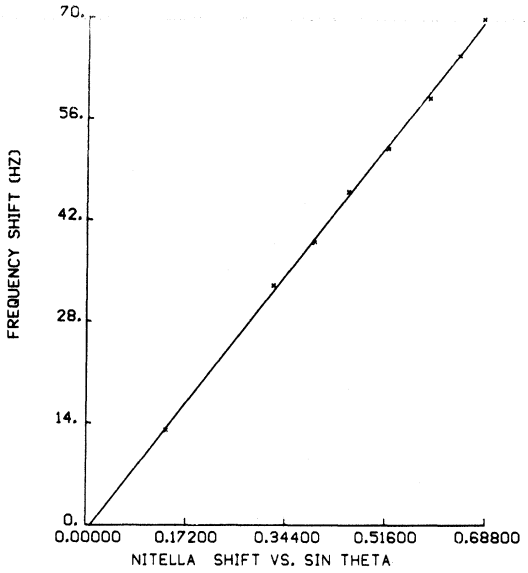

FIG. 2. A plot of the magnitude of the Doppler shift from a fixed point on a Nitella cell as a function of the sine of the scattering angle θ, the angle between the propagation vectors of the incident and scattered light. The line is the best least-squares fit to the points. The predicted linear dependence is verified, and the deviations from the line provide an estimate of the experimental precision.

The theory of this technique predicts that the magnitude of the Doppler shift should be proportional to the sine of the scattering angle θ (the angle between the vectors of propagation of the incident and scattered light).[6] To verify this, we have measured a series of spectra as a function of the scattering angle. Throughout this series the laser beam was impinging on the same spot on the same Nitella cell. The plot of the Doppler shift as a function of scattering angle is shown in Fig. 2. The predicted linear dependence is observed.

It is occasionally observed that the spectrum will have a second small peak at a frequency equal to twice the frequency of the main peak [see spectrum (a), Fig. 1]. This arises from the fact that the detection in laser-light–scattering experiments is by optical beating. In these experiments the detection is of the heterodyne type, in which the scattered light is mixed at a photomultiplier tube with an unshifted component of the incident light to produce the low-frequency electronic beats which we observe. However, if the light being scattered from one of the directions of flow is beat against light scattered from the other direction of flow, then, because their relative velocities are approximately twice the absolute velocity, the beat will be of approximately twice the fundamental frequency. We have seen a definite correlation between the presence of these peaks and the position of the laser beam on the cell, with the double-frequency beat appearing when the beam is traversing both directions of flow. We have also observed a relative increase in this double-frequency beat when the intensity of the unshifted beating component (the "local oscillator") is reduced. These double-frequency beats from broad distributions of velocities could account for some of the higher-frequency intensity seen in our spectra.

We have measured the various components of the velocity in the Nitella internodal cell by rotating the cell with respect to the scattering vector. The maximum velocity is seen when the velocity is along the scattering vector, and the magnitude of this velocity is 70–75 μm/sec. We have also observed the response of the cell to excess light. If an intense laser beam is focused on the cell, the cell will stop streaming in that area, but it will continue to stream elsewhere,

even a few tenths of a millimeter from the intense beam. This is consistent with a very local mechanism for the streaming. Similar responses to other types of perturbations have been observed microscopically.[8]

We have also observed protoplasmic streaming in the whorled leaf cells of Nitella. In these cells the flow pattern seems to follow the leaflike contour of the cells, and the maximum velocity was determined to be 55–60 μm/sec. This flow is also reversibly quenched by para-chloromercuribenzoate.

We believe that the technique of laser-light-scattering spectroscopy is ideally suited to the measurement of flow patterns within cells. Some of the advantages which we have found this technique to have in comparison with microscopy are as follows: (1) It is objective; i.e., all motion within the scattering region is viewed, rather than just the motion of selected particles. (2) The information is directly interpretable in terms of velocities. (3) It is exceedingly fast. Complete velocity distributions (spectra) are obtained in a few seconds. (4) Any desired component of the velocity can be measured just by changing the orientation of the cell with respect to the scattering vector. (5) The motion of submicroscopic particles can readily be observed. This technique may have a great role to play in the elucidation of the mechanism of protoplasmic streaming and in the study of other phenomena in which protoplasmic movement is an important factor.

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Nuclear Spin-Lattice Relaxation in Liquid Crystals by Fluctuations in the Nematic Director

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We show that a finite cutoff of the hydrodynamic modes (order fluctuations) in the nematic liquid-crystalline phase explains the measured spin-lattice relaxation in this phase. A calculation of T_1 based upon such a cutoff and a fit to the data of MBBA (4-*n*-methoxybenzylidene-4′-*n*-butylaniline) show this cutoff to be 1 molecular length. At $T_{1\rho}$ frequencies, far from the cutoff, order fluctuations are shown to be the dominant relaxation mechanism. This is done by comparing the predicted angular dependence of $T_{1\rho}$ for these modes with that measured in MBBA.

The published measurements[1-13] of spin-lattice relaxation in nematic liquid crystals have not lent themselves to a single interpretation. For example, one compound, PAA (*p*-azoxyanisole) has been shown to exhibit a $\nu^{1/2}$ frequency dependence characteristic of thermal fluctuations of the director, whereas the compound MBBA (4-*n*-methoxybenzylidene-4′-*n*-butylaniline) has not shown this dependence over the same frequency range.[11] It has not been clear why one compound should obey one mechanism while another compound in the same phase appears to obey another.

In this paper we shall show that this behavior can be explained by a cutoff of the collective or-