Notes

Spectral Distribution of Light Quasielastically Scattered from Straight and Curved Rods

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Measurements of spectral distribution of light quasielastically scattered from solutions of macromolecules can provide information on dynamics of these molecules.[2] For a rodlike molecule, spectral distribution, $S(K, f)$, in the homodyne case has the form[3]

$$S(K,f) = \sum_{n,m:\text{even}}\sum P_n(KL)P_m(KL)\,[(\Gamma_{nm}/\pi)/(f^2 + \Gamma_{nm}{}^2)]$$

$$2\pi\Gamma_{nm} = 2DK^2 + \{n(n+1) + m(m+1)\}\Theta \qquad (1)$$

$$K = (4\pi/\lambda)\sin(\phi/2)$$

where D and Θ are, respectively, translational and rotational diffusion coefficients, f is the frequency in hertz, λ is the wavelength of the incident light in a medium, ϕ is the scattering angle, and L is the length of a rod. Scattering form factors $P_n(KL)$ have been calculated.[4] For $KL \leq 10$, $n\ (m) = 0$ and 2 are important. The spectrum of light quasielastically scattered from tobacco mosaic virus has been successfully analyzed by use of eq 1.[3,5] In this note, we wish to present the spectral analysis of solutions of straight and curved rods.

As to the scatterers, we prepared short fragments of *Salmonella* flagella (Figure 1) according to the previous method.[2b] The spectrometer used in this study was the same as the previous one.[5] The bandwidth of the wave analyzer was 3 Hz. A light source was a 15-mW He–Ne laser ($\lambda_0 = 632.8$ nm). Results for rods having mean lengths of about 0.3 and 0.5 μm are shown in Figure 2, where any appreciable difference was not observed between Γ's of straight and curved rods. The values of D were estimated to be 0.38×10^{-7} and 0.22×10^{-7} cm^2/sec for 0.3- and 0.5-μm flagella, respectively. These values are about two-thirds of the corresponding Perrin values. This probably came partly from sample polydispersity.[6] At concentrations lower than 0.5 mg/ml of flagella, however, Γ ($=DK^2/\pi$) of straight rods increased appreciably (*e.g.*, at 0.1 mg/ml of flagella, D was about 0.47×10^{-7} cm^2/sec for $L = 0.3$ μm), while that of curved rods stayed constant. This probably suggests the difference in an intermolecular interaction between both types of flagella, as was observed in the case of tobacco mosaic virus.[5]

For solutions of flagella with a mean length of about 0.7 μm, the results were very different between straight and curved rods.

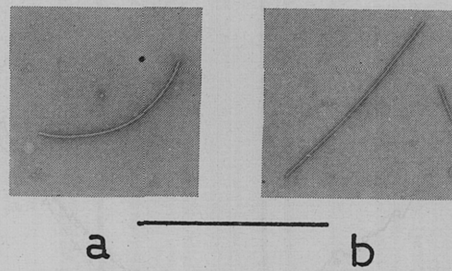

Figure 1. Electron micrographs of short fragments of bacterial flagella of *Salmonella:* (a) normal type (curved rod), (b) straight type; diameter of flagellum about 20 nm, scale 1 μm.

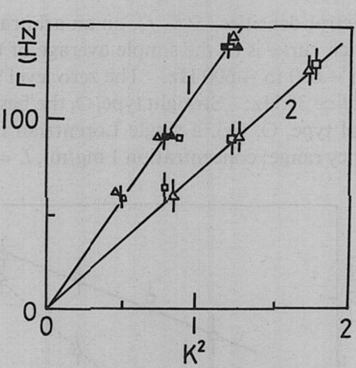

Figure 2. Γ *vs.* K^2. The abscissas in this and Figures 4 and 5 are scaled in units of $10^{-10}K^2$ (cm^{-2}). Concentration 1 mg/ml; (□, ■) normal type, (Δ, ▲) straight type; (1) $L = 0.3$ μm (two different preparations of sample are distinguished by open and filled symbols), (2) $L = 0.5$ μm.

Examples of spectral densities are shown in Figure 3. Spectral densities of straight rods were approximated fairly well with a *single* Lorentzian (Figure 3).[7] On the other hand, spectral densities of curved rods could not be approximated with a single Lorentzian, but at least with two Lorentzians (Figure 3). For straight rods, the results (Δ in Figure 4) could roughly be approximated by $\Gamma = DK^2/\pi$, with $D = 0.21 \times 10^{-7}$ cm^2/sec. However, since the lowest rotational mode might contribute at $K \geq 1 \times 10^5$ cm^{-1} for $L = 0.7$ μm, this interpretation is not reasonable. The dotted curve in Figure 4 is the result of a single-Lorentzian fit of $S(K, f)$ in eq 1, computed by assuming $D = 0.16 \times 10^{-7}$ cm^2/sec and $\Theta = 25$ sec^{-1}, and the dashed line indicates $\Gamma = DK^2/\pi$, with $D = 0.16 \times 10^{-7}$ cm^2/sec. For curved rods, the tail of the spectrum was at first fitted by a wide Lorentzian (□) and the residue at the low frequency range by a narrow Lorentzian (■).[8] (No physical basis exists about the present two-Lorentzian fit. It is only tentative.) If we regarded that the half-width of the wide Lorentzian was Γ_{20} (Γ_{22}), the Θ value was estimated to be 100 sec^{-1} (50 sec^{-1}). This is much larger than the Perrin value.

(1) (a) Department of Physics; (b) Institute of Molecular Biology.

(2) (a) S. Fujime, "Advances in Biophysics," Vol. 3, University of Tokyo Press, Tokyo, 1972, p 1; (b) S. Fujime, M. Maruyama, and S. Asakura, *J. Mol. Biol.*, **68**, 347 (1972).

(3) H. Z. Cummins, F. D. Carlson, T. J. Herbert, and G. Woods, *Biophys. J.*, **9**, 518 (1969).

(4) R. Pecora, *J. Chem. Phys.*, **48**, 4126 (1968).

(5) S. Fujime, *J. Phys. Soc. Jap.*, **29**, 416 (1970).

(6) R. Pecora and Y. Tagami, *J. Chem. Phys.*, **51**, 3298 (1969).

(7) The term "a single-Lorentzian fit" means that the experimental spectrum can be fitted fairly well with "a single Lorentzian having a proper width."

(8) Because of the rapid decrease of the amplifier gain below 20 Hz, Γ's of the narrow component are not accurate.

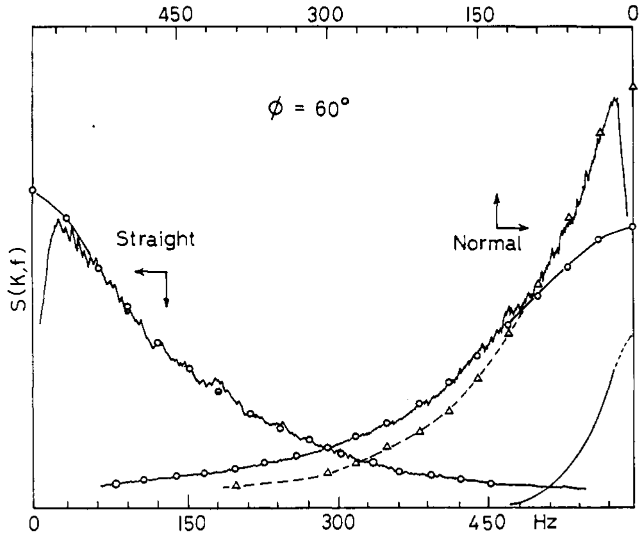

Figure 3. Spectral densities, $S(K, f)$, on an arbitrary scale against frequency. Each curve is of the simple average of three successive scannings from −600 to +600 Hz. The zero level was determined from $S(Kf,)$ at $f \simeq 3$ kHz: Straight type, O, the best single Lorentzian fit; normal type, O, (Δ), a single Lorentzian fit at the higher (lower) frequency range; concentration 1 mg/ml, L = 0.7 μm.

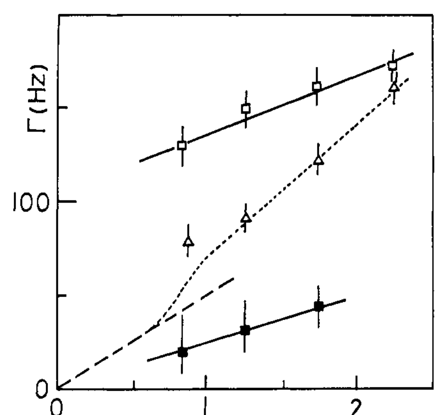

Figure 4. Γ *vs.* K^2; concentration 1 mg/ml, L = 0.7 μm; (□, ■) normal type, (Δ) straight type. For details, see text.

Another example is shown in Figure 5. The length distribution of flagella used was examined by electron microscopy (Figure 6). The mean lengths and the degree of polydispersity of both straight and curved flagella were nearly equal to each other. On the other hand, the half-widths at half-height of a single Lorentzian fit were greatly different between straight and curved rods. The results for straight rods (O) could roughly be approximated by $\Gamma = DK^2/\pi$, with $D = 0.10 \times 10^{-7}$ cm²/sec. This value of D is close to the Perrin value of D, but this interpretation is not as reasonable as before because of the complete neglect of the contribution of rotational modes. Although an accurate treatment was not possible because of sample polydispersity, computer simulation using eq 1 (taking n (m) up to 8) suggested that the results on straight rods were compatible with the existing theory,[4] whereas the results on curved rods could not reasonably be interpreted.

A definite explanation for these results is difficult at present. One possible origin of the above difference may be in a difference of the degree of coupling between translational and rotational modes of motion.[5,9,10] However, the above-mentioned large difference may not be explained only by

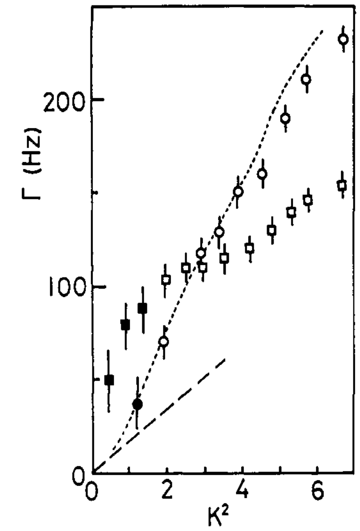

Figure 5. Γ *vs.* K²; concentration 1 mg/ml; (□) normal type, (O) straight type. Filled symbols indicate that there were large deviations from a single Lorentzian fit. L = 0.9 μm (see Figure 6). The dotted line is the half-widths of a single Lorentzian fit of $S(K,f)$ in eq 1, assuming Θ = 13 sec⁻¹ and $D = 0.05 \times 10^{-7}$ cm²/sec (about one-half of the Perrin value). Noting that KL = 23 at the highest scattering angle, the contribution of rotational modes up to n = 8 must be taken into account [see S. Fujime, *J. Phys. Soc. Jap.*, **31**, 1805 (1971)]. The dashed line indicates $\Gamma = DK^2/\pi$, with $D = 0.05 \times 10^{-7}$ cm²/sec.

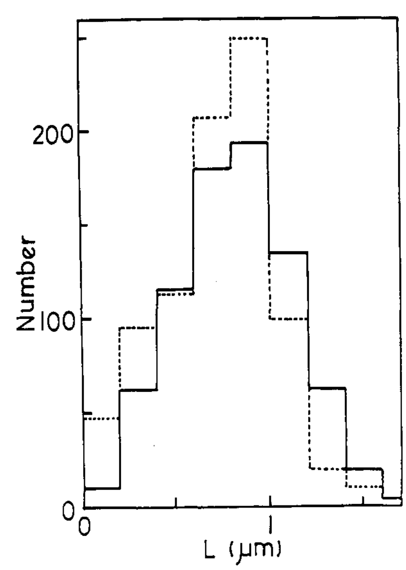

Figure 6. Distribution of flagella in length: (——) normal type, (. . . .) straight type.

this reason. Qualitatively speaking, on the other hand, the difference presumably comes from the geometrical shape of curved rods. For a straight rod the rotational fluctuation about its long axis does not contribute to the line broadening, whereas for a curved rod the corresponding fluctuation will produce a time-dependent local fluctuation in the dielectric constant of the solution, resulting in the line broadening.[11] Thus the spectral density will also be a function of the radius of curvature of a rod. To sum up, it is necessary to develop a theory of light scattering which takes into account the rotational fluctuation of a curved rod.

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(11) S. Fujime, *J. Phys. Soc. Jap.*, **31**, 1805 (1971).