Infinite-Dilution Viscoelastic Properties of Myosin

Robin W. Rosser,[1a] F. Henry M. Nestler,[1a] John L. Schrag,[1a] John D. Ferry,*[1a] and Marion Greaser[1b]

Departments of Chemistry and Meat and Animal Science, University of Wisconsin, Madison, Wisconsin 53706. *Received August 14, 1978*

ABSTRACT: The storage and loss shear moduli, G' and G'', of dilute myosin solutions have been measured and the results have been extrapolated to infinite dilution. The Birnboim–Schrag multiple-lumped resonator technique was used in the frequency range from 150–8000 Hz. Measurements were performed in the concentration range 0.72–7.4 × 10^{-3} g/mL in water and glycerol–water mixtures containing potassium chloride, phosphate buffer, EDTA, and in some cases glycine. The temperatures were 0.0 and 6.0 °C. Data were matched reasonably well by the hybrid theory for slighly flexible rodlike molecules; the rotational relaxation time agrees extremely well with theoretical predictions of Wada. The Young's modulus of an individual molecule, as deduced from the ratio of the two longest relaxation times, appears to be similar to, but slightly lower than, that reported for paramyosin. In the presence of glycerol without glycine, the molecular configuration appears to be altered.

Measurements of the viscoelastic properties of dilute polymer solutions have been performed in recent years for a wide variety of molecules.[2] The results, extrapolated to infinite dilution, have proved valuable in testing molecular models and in devising new theories for describing macromolecular dynamics. In particular, for several linearly extended molecules, data have been fit very well by the so-called hybrid model, which has a relaxation spectrum consisting of one long relaxation time corresponding to end-over-end rotation of the entire molecule, together with a series of shorter ones attributed to internal modes of motion such as flexure. For one rodlike protein, paramyosin, viscoelastic measurements have provided an estimation of the Young's modulus of an individual molecule, from the ratio of the longest two relaxation times.[2b]

In the present experiments, myosin, a molecule with a more complicated structure, has been examined. This has the form of a double-headed mace; it consists of a long rodlike portion which is a coiled coil of two α helices, connected at one end to two globular portions, or heads.[3] The molecular weight of the entire molecule is 470 000.[4] The behavior of myosin is further complicated by a tendency to aggregate. Keeping the phosphate concentration high reduces, but does not eliminate, this tendency. According to Harrington, the dimerization constant in 0.5 M KCl, 0.5 M phosphate, and 0.01 M EDTA at pH 7.3 is 130 mL/g.[5]

Theory

For comparison of experimental viscoelasticity results and theoretical predictions it is convenient to use the reduced intrinsic storage and loss shear moduli defined respectively as

$$[G']_R \equiv (M/RT) \lim_{c \to 0} (G'/c) \qquad (1)$$

$$[G'']_R \equiv (M/RT) \lim_{c \to 0} \{(G'' - \omega\eta_s)/c\} \qquad (2)$$

where M is the molecular weight, R the gas constant, T the absolute temperature, c the polymer concentration in g/mL, G' and G'' the storage and loss shear moduli, ω the radian frequency of deformation, and η_s the solvent viscosity. The intrinsic viscosity, $[\eta]$, is related by the expression

$$[\eta] = (RT/M) \lim_{\omega \to 0} [G'']_R/\omega\eta_s \qquad (3)$$

For the hybrid theory,[2b]

$$[G']_R = m_1\omega^2\tau_0^2(1 + \omega^2\tau_0^2)^{-1} + zZ'(\omega\tau_1) \qquad (4)$$

$$[G'']_R = \omega\tau_0[m_1(1 + \omega^2\tau_0^2)^{-1} + m_2] + zZ''(\omega\tau_1) \qquad (5)$$

Here, τ_0 is the rotational relaxation time, and $Z'(\omega\tau_1)$ and $Z''(\omega\tau_1)$ are the reduced moduli for the Zimm theory for random coils,[6] for which τ_1 is the longest relaxation time. The parameters m_1, m_2, and z and the ratio τ_0/τ_1 are adjustable; with $m_1 = 0.6$ and $m_2 = 0.2$ the first term in eq 4 or 5 is that for the Kirkwood–Auer theory for rigid rods;[7] if $z = 1$, the second term in both equations is simply that of the unmodified Zimm theory. The relaxation time τ_0 may be calculated from experimental observables and theoretical parameters as

$$\tau_0 = (m_1 + m_2 + 2.37z\tau_1/\tau_0)^{-1}[\eta]\eta_s M/RT \qquad (6)$$

For a rodlike macromolecule, τ_1 probably reflects a flexural, rather than a longitudinal, mode of motion,[8,9] and the equivalent Young's modulus of an individual molecule has been shown to be[2b]

$$E = 2kT(L/d^4)(\tau_0/\tau_1) \qquad (7)$$

where k is Boltzmann's constant and L and d are the molecular length and diameter. For myosin, of course, this result is not directly applicable because of the more complicated molecular structure.

Experimental Section

Materials. Myosin was prepared from rabbit skeletal muscle by the procedure of Nauss et al.[10] and purified on a DEAE–Sephadex A50 column.[11] Four separate preparations, labeled A–D, were prepared, and each was characterized by polyacrylamide gel electrophoresis under reducing conditions in the presence of SDS. The gels showed a heavy chain band at about M = 210 000 as well as the lower molecular weight bands (light chains) characteristic of myosin.[12] In addition, varying amounts of somewhat degraded myosin heavy chains were observed, roughly two-thirds of which was of only slightly lower molecular weight, namely, 150 000 to 200 000. Based on absorption scans of the gels, the weight percents of partially degraded heavy-chain material in the preparations were as follows: A, 15%; B, 8%; C, 50%; D, 15%.

Three different solvents were used for the viscoelastic measurements. All contained 0.5 M KCl, 0.5 M phosphate buffer, and 0.1 M EDTA. An aqueous solvent containing only the above ingredients, with a pH of 7.3, was used with preparation A. Preparations B and C were measured in a solvent which also contained 0.6 M glycine and of which 50% of the total volume was glycerol. Preparation D was measured in a solvent containing no glycine, and in which the water to glycerol ratio was 40:60 by weight. Glycerol was purchased from Aldrich Chemical Co.

Each preparation was first dialyzed into the aqueous solvent described above in connection with preparation A. In those cases where glycerol was used, solutions were prepared by adding concentrated myosin in the aqueous buffer to a mixture containing enough additional water, KCl, phosphate, EDTA, glycerol, and glycine such that the final concentrations of all components were approximately correct. Exact proportions were then obtained by dialysis against solvent with the exact required composition. Since glycine and glycerol will turn yellow on standing[13] at room

0024-9297/78/2211-1239$01.00/0

Table I
Summary of Solution Properties

solvent[a]	T, °C	η_s, Poise	ρ_s, g/mL	no. of solutions	myosin concn range, g/mL × 10^3	myosin prep[b]
aqueous	0	0.0200	1.093			
	6	0.0173	1.092	7	1.01-4.96	A (15%)
0.6 M glycine, 50% glycerol by vol	0	0.53	1.247	10	0.717-4.48	B (8%)
0.6 M glycine, 50% glycerol by vol	0	0.53	1.247	6	0.568-3.17	C (50%)
40/60 water-glycerol by wt	0	0.41	1.226	6	1.01-7.40	D (15%)

[a] All solvents contain 0.5 M KCl, 0.5 M phosphate, and 0.01 M EDTA in addition to the components listed.
[b] Numbers in parentheses indicate percent of materials showing as partially degraded in gel electrophoresis.

temperature, and since glycerol can slowly cause cross-linking of concentrated protein solutions,[14] all solvents and solutions were stored in a refrigerated room and experiments were performed within 1 week.

After the first solution of each preparation had been measured, subsequent measurements were performed on solutions diluted sequentially with stock solvent. For solutions containing glycerol, dialysis was repeated after several dilutions to insure that η_s was always the same for the solution as for the solvent used in instrument calibration.

Methods. The storage and loss shear moduli, G' and G'', of all solutions were measured by use of the Birnboim-Schrag multiple-lumped resonator[15] (MLR) with a computerized data acquisition and processing system.[16] The resonator housing was machined from pure titanium, and the resonator, Mark VIII-B, from a single piece of a heat-treated titanium alloy.[17] It exhibits five working frequencies in the range from 150-8000 Hz.

The apparatus was filled by a 50-mL syringe barrel with a 15-gauge needle. Solutions were allowed to flow in by gravity. The apparatus was usually wet with solvent, so after filling with solution, it was drained and then refilled to insure homogeneity of the solution in the chamber. Any bubbles adhering to the resonator were removed before the measurements by use of Teflon-tipped tool.[8] After measurement, the chamber was cleaned with a trypsin solution followed by a 40% urea solution, and then rinsed several times with water and finally solvent. Solvent measurements, used for calibration, were performed either immediately before or immediately after all solution measurements.

Solvent densities (ρ_s) and viscosities at the operating temperatures of 0.0 and 6.0 °C were measured by pycnometry and capillary viscometry.

Myosin concentrations were determined after each MLR measurement by UV absorption. Equal volumes of solution and solvent were diluted with aqueous solvent and the relative absorbances were used to calculate c from the equation[18] $c(\text{g/mL}) = (A_{280} - A_{320})/558$, where the A's are the absorbances at the respective wavelengths. Error caused by the presence of glycerol was shown to be negligible by also performing measurements in which glycerol solvent was used as the diluent, instead of the aqueous buffer. A modified Beckman DU spectrometer with digital readout was used for all measurements.[19]

A summary of solvent and solution properties is given in Table I.

As a check for mechanical degradation of myosin with repeated handling, a fresh solution of preparation B with intermediate concentration was prepared, measured, diluted, and remeasured. Both results agreed with data for solutions of similar concentration prepared by repeated dilution of the original starting solution.

Results

The reduced intrinsic storage and loss shear moduli, $[G']_R$ and $[G'']_R$, were obtained by extrapolating $(G'M/cRT)^{1/2}$ and $(G'' - \omega\eta_s)M/cRT$ at each frequency to zero concentration.[17] Representative plots are shown in Figures 1 and 2, for preparations A at 6.0 °C and B at 0.0 °C, respectively. Here, M was taken[4] as 470 000. The results for preparations A and B are plotted logarithmically versus the conventional reduced frequency, $\omega\eta_s[\eta]M/RT$, in Figure 3. (The wide range is due to the large variation in solvent viscosity, η_s.) The intrinsic viscosity, as determined by use of eq 3, was 235 mL/g. The data are compared to the predictions of the hybrid model with m_1, m_2, and z having values of 0.6, 0.2, and 1.0, respectively; these are the values of the unmodified Kirkwood-Auer[7] and Zimm theories.[6] The ratio of τ_0/τ_1 is 6.0. The fit is excellent for $[G'']_R$ over the entire frequency range. The observed $[G']_R$ data, however, are larger than theory at both high and low frequencies. The first effect, not observed previously for rodlike molecules, may reflect motions of the heads such as wagging. The deviation at low frequencies is symptomatic of a small amount of aggregation.[8] This is reasonable since for the most dilute aqueous solution, roughly 10% of the material by weight (or 5% by mol) should be dimer, according to the equi-

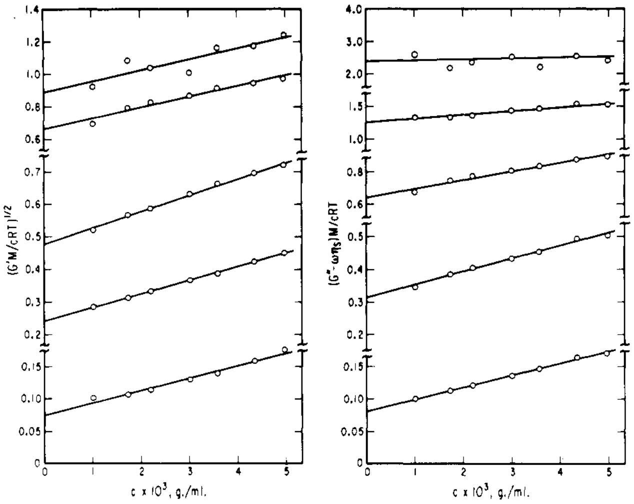

Figure 1. Plots of $(G'M/cRT)^{1/2}$ and $(G'' - \omega\eta_s)M/cRT$ against concentration for preparation A in aqueous solvent at 6.0 °C. The frequencies of measurement are, from bottom to top, 145, 576, 1499, 3758, and 8057 Hz.

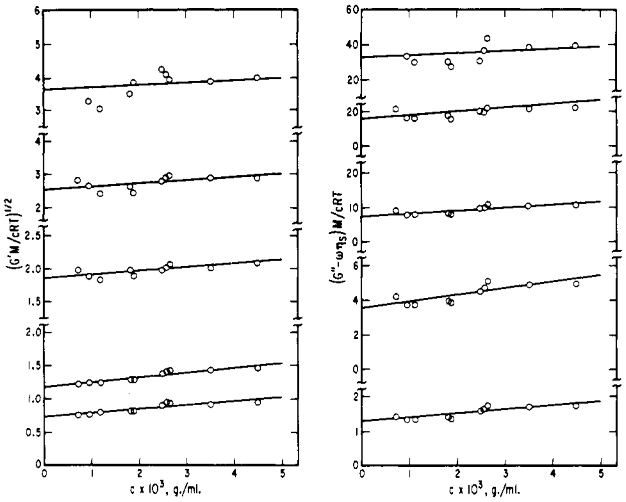

Figure 2. Plots of $(G'M/cRT)^{1/2}$ and $(G'' - \omega\eta_s)M/cRT$ against concentration for preparation B in solvent containing glycerol at 0.0 °C. The frequencies of measurement are, from bottom to top, 144, 573, 1496, 3756, and 8058 Hz.

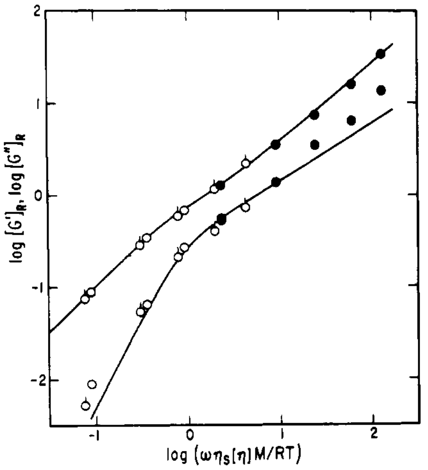

Figure 3. Reduced intrinsic moduli, $[G'']_R$ (upper points) and $[G']_R$ (lower points), plotted logarithmically vs. reduced frequency, $\omega\eta_s[\eta]M/RT$. Open circles are for preparation A (pip up, 6.0 °C; no pip, 0.0 °C) and filled circles are for preparation B at 0.0 °C. Curves drawn for hybrid theory with $m_1 = 0.6$, $m_2 = 0.2$, $z = 1.0$, $\tau_0/\tau_1 = 6.0$.

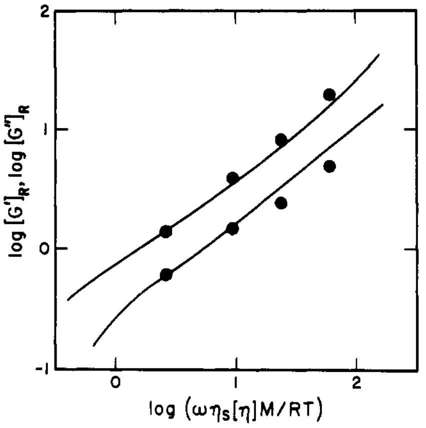

Figure 4. Reduced intrinsic moduli, $[G'']_R$ and $[G']_R$, plotted as in Figure 3. Points are for preparation C at 0.0 °C; curves correspond to data points of Figure 3.

librium constant discussed above.[5]

It should be noted that the excellent superposition of the two sets of data in Figure 3 is strong evidence that the presence of glycerol with glycine does not modify the molecular structure.

For preparation C, which contained considerably more partially degraded material than any of the others, final data are shown in Figure 4, and compared to the data of Figure 3 (the curves correspond to the *data points* in Figure 3). The only appreciable difference is that $[G']_R$ is slightly lower for preparation C, and thus in closer agreement with the hybrid theory predictions. This is reasonable since myosin is most subject to enzymatic attack toward the head end of the molecule.[3] Hence the degraded material might be expected to behave more like other flexible rodlike molecules than would the intact myosin.

For preparation D in the solvent containing glycerol, but not glycine, final data are shown in Figure 5 and again compared to those of Figure 3. Both $[G']_R$ and $[G'']_R$ are considerably reduced, indicating some modification of the molecular configuration. This is in agreement with similar results for paramyosin.[13]

Discussion

The longest relaxation time, τ_0, for myosin may be calculated using the hybrid theory fitting parameters of

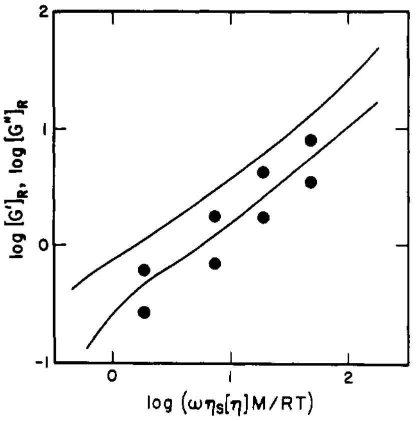

Figure 5. Reduced intrinsic moduli, $[G'']_R$ and $[G']_R$, plotted as in Figure 3. Points are for preparation D at 0.0 °C; curves correspond to data points of Figure 3.

Figure 3. When the results are reduced to water at 20 °C, eq 6 gives $\tau_0 = 38$ μs. This agrees extremely well with a recent theoretical result of Nakajima and Wada based on modelling the molecule with a rigid assembly of Stokes spheres,[20] which yields $\tau_0 = 37$ μs. As noted earlier, however, it is more difficult to calculate the effective Young's modulus of myosin using the ratio (τ_0/τ_1), because of the complicating presence of the head portion of the molecule. A rough approximation may still be obtained, however, if the seemingly plausible approximation is made that flexure of the rodlike portion is essentially unaffected by the heads. Hence the experimental τ_1 may be used for the rod alone. A value of τ_0 for the rod alone may be calculated using Wada's results; it is approximately two-thirds of the value for the entire molecule. Hence, for the rod alone $\tau_0/\tau_1 \simeq 4$, and from eq 7, $E \simeq 0.9 \times 10^{10}$ dyn/cm^2, calculated using a rod length of 1350 Å and a diameter of 15 Å.[20] This is in good agreement with the value of 1.2×10^{10} dyn/cm^2 obtained previously for paramyosin,[2b] another double α-helical, coiled coil molecule. The fact that the present result is somewhat lower may be consistent with other evidence that the rodlike portion of myosin contains a short segment which is more flexible than the rest.[3] Recent measurements of fluorescence depolarization by Harvey and Cheung[21] are also consistent with a rodlike structure which is nearly rigid but capable of slight bending with a restoring force.

Acknowledgment. This work was supported in part by the National Institutes of Health under Grants GM21652 and HL18612, and by the College of Agricultural and Life Sciences of the University of Wisconsin—Madison. We are indebted to Mr. David J. Deubler for help with calculations and Mr. Richard Bain for help with the myosin preparation.

References and Notes

(1) (a) Department of Chemistry; (b) Department of Meat and Animal Science.
(2) (a) J. D. Ferry, *Acc. Chem. Res.*, **6**, 60 (1973); (b) R. W. Rosser, J. L. Schrag, J. D. Ferry, and M. Greaser, *Macromolecules*, **10**, 978 (1977).
(3) S. Highsmith, K. M. Kretzschmar, C. T. O'Konski, and M. F. Morales, *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 4986 (1977).
(4) J. Godfrey and W. Harrington, *Biochemistry*, **9**, 894 (1970).
(5) W. Harrington, *Biochemistry*, **11**, 1448 (1972).
(6) B. H. Zimm, *J. Chem. Phys.*, **24**, 269 (1956).
(7) J. G. Kirkwood and P. L. Auer, *J. Chem. Phys.*, **19**, 281 (1951).
(8) R. W. Rosser, Ph.D. thesis, University of Wisconsin—Madison, 1977.
(9) R. W. Rosser, *Macromolecules*, **11**, 1060 (1978).
(10) K. W. Nauss, S. Kitagawa, and J. Gergely, *J. Biol. Chem.*, **244**, 755 (1969).
(11) G. Offer, C. Moos, and R. Starr, *J. Mol. Biol.*, **74**, 653 (1973).

(12) S. Sarkar and P. H. Cooke, *Biochem. Biophys. Res. Commun.*, **41**, 918 (1970).
(13) J. W. Allis, Ph.D. Thesis, University of Wisconsin—Madison, 1965.
(14) J. Bello and H. R. Bello, *Arch. Biochem. Biophys.*, **172**, 608 (1976).
(15) J. L. Schrag and R. M. Johnson, *Rev. Sci. Instrum.*, **42**, 224 (1971).
(16) D. J. Massa and J. L. Schrag, *J. Polym. Sci., Part A-2*, **10**, 71 (1972).
(17) N. Nemoto, J. L. Schrag, J. D. Ferry, and R. W. Fulton, *Biopolymers*, **14**, 409 (1975).
(18) J. Godfrey and W. Harrington, *Biochemistry*, **9**, 886 (1970).
(19) Update Instrument, Inc., Madison, Wis. 53705.
(20) H. Nakajima and Y. Wada, *Biopolymers*, **16**, 875 (1977).
(21) S. C. Harvey and H. C. Cheung, *Biochemistry*, **16**, 5181 (1977).

Rayleigh–Brillouin Spectroscopy and Molecular Motion in Toluene Solutions of Polystyrene

G. D. Patterson,* C. P. Lindsey, and G. R. Alms[+]

Bell Laboratories, Murray Hill, New Jersey 07974. *Received April 25, 1978*

ABSTRACT: The Rayleigh–Brillouin spectra of toluene and solutions of 110 000 molecular weight polystyrene are studied. The reorientation relaxation time of the toluene is relatively unaffected by the presence of the polystyrene even up to concentrations of 150 mg/mL of polymer. Also, the hypersonic loss determined by Brillouin scattering is unaffected within experimental error. Thus, the local viscosity as probed by Rayleigh–Brillouin spectroscopy remains similar to the solvent viscosity even though the macroscopic viscosity increased by almost two orders of magnitude.

The presence of a small fraction of high molecular weight polymer in a solvent can have dramatic effects on the macroscopic properties of the solution.[1] However, the effects of polymer on the microscopic dynamics of the solvent may be quite different. In order to study such effects we have measured the Rayleigh–Brillouin spectra of toluene and its solutions with 110 000 molecular weight polystyrene.

Theory

The depolarized (I_{VH}) Rayleigh spectra of liquids such as toluene have been extensively studied.[2-6] The spectrum consists of a Lorentzian line centered at the incident frequency with width $\Gamma_{VH} = (2\pi\tau_{ls})^{-1}$ where τ_{ls} is the collective reorientation relaxation time for the liquid. It has been observed that [2,6]

$$\tau_{ls} = \frac{g_2}{J_2}\left[\frac{C'\eta_s}{T} + \tau_0\right] \qquad (1)$$

where g_2 is a static pair orientation correlation parameter, J_2 is a dynamic pair orientation correlation parameter, C' depends only on the size and shape of the molecule, η_s is the shear viscosity, T is the temperature, and τ_0 is the inertial limit for the single molecule reorientation correlation time. If polystyrene is added to toluene, the macroscopic shear viscosity will be greatly increased. The present study examines the effect of added polystyrene on the molecular reorientation time determined for the toluene. The polymer itself will give rise to a very narrow depolarized Rayleigh peak[7] compared to the toluene and the spectrum can be fit to a sum of two peaks.

The Brillouin spectrum of liquids consists of two shifted peaks with splitting given by

$$\pm\frac{\Delta\omega_l}{\omega_0} = \frac{2nV_l}{C}\sin\frac{\theta}{2} \qquad (2)$$

where ω_0 is the incident frequency, V_l is the longitudinal hypersonic velocity, C is the speed of light in a vacuum, n is the refractive index, and θ is the scattering angle in the scattering plane. The line width is

$$\Gamma_l = \frac{q^2}{2\rho}\left[\eta_v + \frac{4}{3}\eta_s\right] + \frac{\kappa q^2}{2\rho C_p}(\gamma-1) \qquad (3)$$

where $q = (4\pi n/\lambda)\sin\theta/2$ is the magnitude of the scattering vector and λ is the wavelength of the incident light in a vacuum, ρ is the density, η_v is the volume viscosity, κ is the thermal conductivity, C_p is the specific heat at constant pressure, and γ is the ratio of specific heats C_p/C_v. For most organic liquids κ is very small and the contribution to Γ_l due to thermal conductivity can be ignored. The volume and shear viscosities that are appropriate in eq 3 must be evaluated at the frequency of the hypersonic phonons and hence may differ appreciably from the zero frequency macroscopic viscosity. The phonon velocity is given by

$$V_l = \left(\gamma\frac{M'}{\rho}\right)^{1/2} \qquad (4)$$

where M' is the real part of the longitudinal modulus $M = K + {}^4/_3G$ where K is the modulus of compression and G is the shear modulus. Again, M' must be evaluated at the frequency of the hypersonic waves.

The collective reorientation relaxation time τ_{ls} given in eq 1 is proportional to the shear viscosity η_s. However, the reorientational motions of small molecules are sensitive only to the local environment composed of the first and at most second nearest neighbor shells of molecules. The shear strain created by the rotation is thus very short ranged and the appropriate viscosity would be a more general q-dependent shear viscosity $\eta_s(q)$, where the value of q is determined by the size and shape of the reorienting unit. The shear strain associated with the longitudinal acoustic waves probed by Brillouin scattering is much longer ranged ($q \approx 0.002$ Å^{-1}), but it has a definite frequency given by $\Delta\omega_l$. Thus, the appropriate shear viscosity to use in eq 3 is

$$\eta_s(q,\omega) = \sum_j \frac{G_j(q)\tau_i}{1 + (\omega\tau_j)^2} \qquad (5)$$

where the $\{G_j(q)\}$ are q-dependent relaxation strengths and the $\{\tau_j\}$ are the corresponding relaxation times for the modes of motion which determine η_s. The value of $\eta_s(q,\omega)$

[+] Elastomers departments, E.I. du Pont de Nemours and Company, Wilmington, Delaware 19898.

0024-9297/78/2211-1242$01.00/0