Viscoelastic Properties of Very Dilute Paramyosin Solutions

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ABSTRACT: The storage and loss shear moduli, G' and G'', have been measured for dilute solutions of paramyosin from the clam *Mercenaria mercenaria* in water and glycerol–water mixtures containing potassium chloride and phosphate buffer. The Birnboim–Schrag multiple-lumped resonator was used in the frequency range from 150 to 8100 Hz; the concentration range was 0.7 to 2×10^{-3} g/mL and the temperature range was 0.0 to 6.0 °C. The intrinsic moduli were obtained by extrapolation to infinite dilution. When compared with predictions of Yamakawa for a rigid cylindrical molecule, they agreed at low frequencies but diverged at high frequencies. Excellent agreement was obtained with calculations for a hybrid model whose relaxation times are attributed to rigid-body end-over-end rotation together with some internal modes of motion, probably flexural. The rotational relaxation time agreed rather well with that determined by DeLaney and Krause from electrical birefringence measurements. From the ratio of the rotational to the longest flexural relaxation time, the flexural rigidity and Young's modulus of the paramyosin molecule were estimated by relations derived by Wada and collaborators; the modulus was 1.2×10^{10} dyn/cm^2.

Recent measurements of dynamic (oscillatory) viscoelastic properties of dilute solutions of macromolecules with a more or less extended rodlike configuration have provided information about the internal stiffness of such molecules. The magnitudes and frequency dependences of the storage and loss shear moduli, G' and G'', after extrapolation of the data to infinite dilution to eliminate effects of molecular interaction, can be compared with predictions of molecular theories. It was concluded that tobacco mosaic virus[2] and fibrin oligomer[3] are stiff rods whose motions can be described by a single rotatory relaxation time. However, the behavior of the helical molecules poly(γ-benzyl L-glutamate)[4] and poly(n-hexyl isocyanate)[5] can be described by a hybrid relaxation spectrum in which the longest relaxation time is attributed to rigid-body rotation and the others to flexural modes of motion. Wada and collaborators[6] have shown how the flexural rigidity of the molecule can be calculated from such information.

Paramyosin is a rodlike macromolecule with two α helices in a coiled coil structure. In an earlier study of viscoelastic properties in dilute solution,[7,8] it was concluded that the low-frequency behavior was consistent with rigid body end-over-end rotation, but at higher frequencies the behavior diverged from that predicted for a rigid rod or ellipsoid. However, the experimental method then available necessitated concentrations ($c \geq 0.004$ g/mL) at which significant intermolecular interactions might be expected, and the low frequencies (0.04 to 400 Hz) necessitated use of a solvent containing a very high proportion of glycerol to elevate the viscosity. Under these conditions, denaturation occurred unless glycine was also present. The multiple-lumped resonator[9] now permits measurements at higher frequencies and lower concentrations. The viscoelastic properties of this protein have therefore been reexamined, in solutions with lower protein concentrations and a smaller proportion of glycerol, and interpreted in terms of more recent theory.

Theory

For comparison of experimental data with theory, it is convenient to express the viscoelastic properties in terms of the reduced intrinsic storage and loss shear moduli, $[G']_R$ and $[G'']_R$, defined as follows:

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

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

where c is the concentration (g/mL), M is the molecular weight, R is the gas constant, T is the absolute temperature, η_s is the solvent viscosity, and ω is the radian frequency of oscillation. For each molecular model, there is a characteristic frequency dependence of $[G']_R$ and $[G'']_R$.

If paramyosin is represented as a rigid rod, the best model is probably the cylinder treated by Yamakawa,[10] for which the reduced moduli can be expressed as

$$[G']_R = m_1\omega^2\tau_0^2(1 + \omega^2\tau_0^2)^{-1} \quad (3)$$

$$[G'']_R = \omega\tau_0[m_1(1 + \omega^2\tau_0^2)^{-1} + m_2] \quad (4)$$

$$\tau_0 = m[\eta]\eta_s M/RT \quad (5)$$

with $m_1 = 3/5$, $m_2 = 4/(5\gamma) - 3/5$, and $m = 5\gamma/4$; γ is a parameter related to the axial ratio of the cylinder, which for the dimensions of paramyosin can be taken as 0.90; $[\eta]$ is the intrinsic viscosity. The relaxation time τ_0 corresponds to end-over-end rotation.

The hybrid spectrum which has been used to describe the behavior of helical molecules (of not too high molecular weight) provides a frequency dependence which can be formulated as follows:[4]

$$[G']_R = m_1\omega^2\tau_0^2(1 + \omega^2\tau_0^2)^{-1} + zZ'(\omega\tau_1) \quad (6)$$

$$[G'']_R = \omega\tau_0[m_1(1 + \omega^2\tau_0^2)^{-1} + m_2] + zZ''(\omega\tau_1) \quad (7)$$

where m_2 was originally set equal to zero and z to unity. Here Z' and Z'' are the reduced moduli specified by the Zimm theory[11] for flexible random coils with the number of submolecules N indefinitely large and the hydrodynamic interaction factor h^* equal to 0.25;[12] z is a measure of the relative strength of the Zimm spectrum. In fitting previous experimental data, m_1 and τ_0/τ_1 were taken as adjustable parameters. The relaxation time τ_0 was associated with end-over-end rotation, and the Zimm-like relaxation times, of which the longest is τ_1, were attributed not to the configurational changes of the original Zimm theory but to flexural modes of motion of the weakly bending rod. For this model, eq 5 holds with

$$m = (m_1 + m_2 + zS_{1z}\tau_1/\tau_0)^{-1} \quad (8)$$

where $S_{1z} = \Sigma\tau_p/\tau_1$, the τ_p being the individual relaxation times of the Zimm spectrum; $S_{1z} = 2.37$.

The predictions of both these models will be compared with data for paramyosin. In the case of the hybrid, the parameter m_2 has been assigned a finite value and z has been set equal to unity.

Experimental Section

Materials. The paramyosin was the same preparation, from *Mercenaria mercenaria* adductor muscle, as used by Allis.[7,8] It had

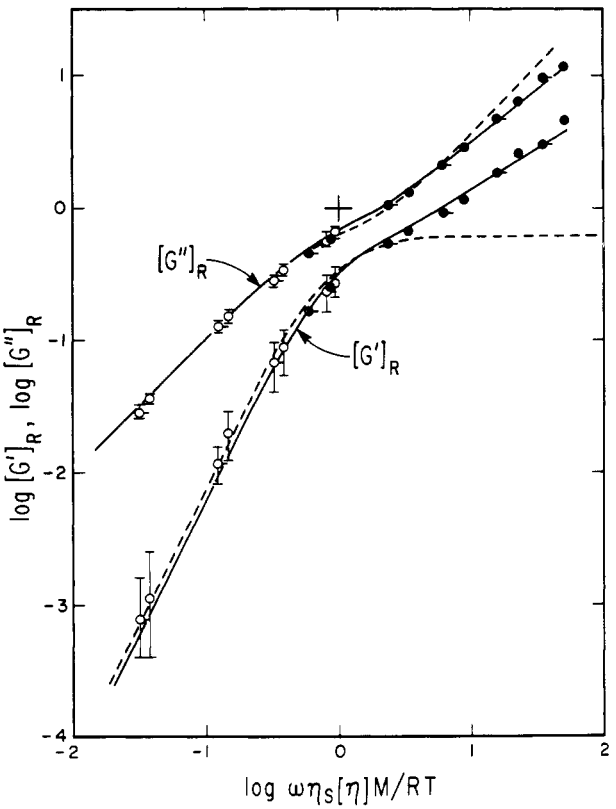

Figure 1. Logarithmic plots of $[G']_R$ and $[G'']_R$ against $\omega\eta_s[\eta]M/RT$. Open circles, in aqueous 0.6 M potassium chloride and 0.05 M phosphate buffer, pH 7; no pip 1.0 °C, with pip 6.0 °C. Filled circles, in solvent with 62% glycerol; no pip 0.0 °C, with pip 6.0 °C. Solid curves, hybrid model with $m_1 = 0.6$, $m_2 = 0.1$, $\tau_0/\tau_1 = 8.43$, $z = 1$; dashed curves, Yamakawa theory for cylinder with $\gamma = 0.9$.

been freeze dried from a solution in 0.6 M potassium chloride and 0.05 M phosphate buffer, pH 7.0, and stored at 3 °C in a desiccator for 12 years. It was completely soluble except for a trace of undissolved material. Sedimentation in the ultracentrifuge (Spinco Model E) in the same solvent as the original potassium chloride–phosphate buffer at a protein concentration of 2×10^{-3} g/mL showed a single peak with a sedimentation coefficient of 3.1 ± 0.1 S at 20 °C, compared with 2.95 ± 0.05 interpolated from the data of Riddiford and Scheraga.[13] The intrinsic viscosity as derived from viscoelastic measurements was between 260 and 280 mL/g, compared with 221 originally measured by capillary viscometry.[8] Polyacrylamide gel electrophoresis after reduction and solubilization by dithiothreitol and sodium dodecyl sulfate[14] showed one band corresponding roughly to a subunit molecular weight 100 000 and a second, amounting to less than 5% of the total material, with molecular weight twice that value; there were no fragments of smaller molecular weight. It may be concluded that the two native polypeptide chains, each of molecular weight approximately 100 000, were intact. Gel filtration experiments with agarose Biogel A-50M, 100–200 mesh,[15] indicated the possibility of a small amount of material of higher molecular weight than the principal component. This may be responsible for the somewhat higher intrinsic viscosity, but should not affect the conclusions drawn from the viscoelastic measurements. The ratio of absorbances at 280 and 260 nm was 2.1, indicating freedom from any contamination with nucleotides.[8,16]

From the method of preparation[13] it may be inferred that the paramyosin was the β form, with a molecular weight of 200 000 and a length of 1180 Å.[16] On the basis of a circular cylindrical model, with density[8] 1.37, this would correspond to a diameter of 16 Å.

Two solvents were used for viscoelastic measurements. The first was the original potassium chloride–phosphate buffer at pH 7.0 cited above. The second contained the same concentrations of salt and buffer plus 0.6 M glycine (found by Allis[8] to eliminate the tendency to denaturation at glycerol concentrations higher than used here) in a glycerol–water mixture in the ratio 62:38 by weight. This solvent was not allowed to stand for long periods since interaction of glycerol with glycine produces a yellow discoloration.

In calculating $G'' - \omega\eta_s$ in eq 2, the difference is relatively very small in dilute solution and it is essential that the composition of the solution exclusive of protein be identical with that of the solvent. The most concentrated solution used for viscoelastic measurements was dialyzed against the solvent and the dialyzate was subsequently used both for dilution to lower concentrations and for the solvent measurements used in calibrating the instrument. Protein concentrations were determined by ultraviolet absorption at 277 nm.[13]

Method. The storage and loss moduli G' and G'' of the solutions were measured with the Birnboim–Schrag multiple-lumped resonator with computerized data acquisition and processing system.[17] One titanium alloy resonator,[2] with five working resonance frequencies from 150 to 8100 Hz, was used. The resonator housing was constructed of pure titanium. Some minor modifications in procedure and calculations are described elsewhere.[18] In particular, solvent and solution runs were always made on the same day, to avoid effects of small drifts in calibration on the differences calculated.

Measurements in the aqueous solvent were made at 1.0 and 6.0 °C, over the concentration range from 0.74 to 2.02×10^{-3} g/mL, and in the glycerol–water solvent at 0.0 and 6.0 °C, over the concentration range from 0.82 to 1.18×10^{-3} g/mL.

Results

The data in the aqueous solvent were extrapolated to zero c by plotting $(G'/c)^{1/2}$ and $(G'' - \omega\eta_s)/c$ against c as previously described.[2-5] Because of the low concentrations, there was considerable uncertainty, especially in obtaining $[G']_R$. In the glycerol–water solvent, there was no significant change in G'/c or $(G'' - \omega\eta_s)/c$ with concentration and the values measured at 0.82×10^{-3} g/mL were used in eq 1 and 2 without extrapolation. This behavior is not surprising since the solvent viscosity is about 20 times that of the aqueous salt–buffer and the reduced frequency $\omega\eta_s[\eta]M/RT$ (see below) is in a range where the concentration dependence has been observed to be very small for both flexible coils[19] and weakly bending helices.[4]

The dimensionless quantities $[G']_R$ and $[G'']_R$ were calculated from eq 1 and 2, taking $M = 200\,000$ and $[\eta] = 278$ mL/g, and are plotted logarithmically against $\omega\eta_s[\eta]M/RT$ in Figure 1. (As shown by eq 5, this dimensionless reduced frequency differs from $\omega\tau_0$ only by the factor m, close to unity, which depends on the model chosen.) The data are compared first with the Yamakawa rigid cylinder prediction (dashed curves). They agree fairly well at low frequencies but not at all at high frequencies, indicating that the response observed at low frequencies reflects rigid body rotation but at higher frequencies internal motions are seen. By applying the hybrid spectrum, calculations from eq 6 and 7 give excellent agreement with $m_1 = 0.6$, $m_2 = 0.1$, $\tau_0/\tau_1 = 8.43$, and $z = 1$; $m = 1.02$. (Other choices of parameters can also give fairly good fits, for example, by increasing both τ_0/τ_1 and z.) The fact that m_2 must be taken as finite, rather than zero as in poly(γ-benzyl L-glutamate) and poly(n-hexyl isocyanate), may be associated with the structure of two parallel helices as contrasted with a single helix; for all *rigid* elongated structures, m_2 is finite (e.g., 0.29 for the Yamakawa cylinder with $\gamma = 0.9$).

Since the uncertainties at low frequencies in Figure 1 are rather serious, a plot was also made for the data in the aqueous solvent at the highest protein concentration of 2.02×10^{-3} g/mL, which are more reliable. Here, in Figure 2, the coordinates are G'/RT and $(G'' - \omega\eta_s)/RT$ vs. $\omega\eta_s/RT$. These data are fitted also to the hybrid calculation and match the shapes very well although arbitrary shifts of the origin of Figure 1 are necessary, as previously employed for fibrinogen at finite concentration.[3] The position of the origin on the ordinate scale determines an apparent value of M/c as 1.4×10^8, whereas the true value is 1.0×10^8. The position on the abscissa scale determines an apparent relaxation time as given below. In any case, Figure 2 reinforces the conclusion that the hybrid model applies over the whole frequency range.

From the infinite-dilution data, the rigid rotational relaxation time τ_0 can be calculated from eq 5 with the appropriate

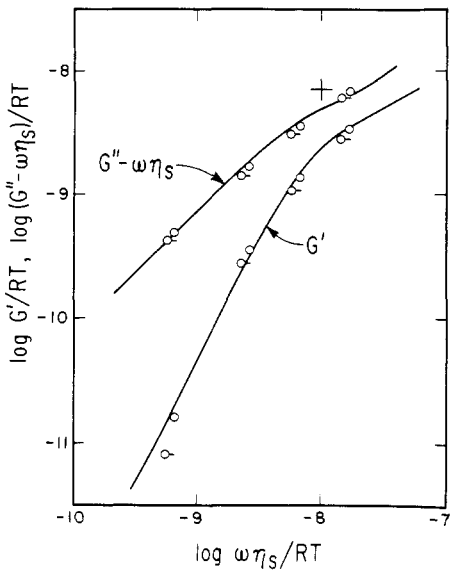

Figure 2. Logarithmic plots of G'/RT and $(G'' - \omega\eta_s)/RT$ against $\omega\eta_s/RT$, at paramyosin concentration of 2.02×10^{-3} g/mL, in aqueous solvent; key to temperatures same as in Figure 1. Curves drawn for hybrid model with same parameters as in Figure 1 and arbitrary shifts of coordinates with the cross corresponding to the origin of Figure 1.

value of m once the fit has been established. From Figure 2, τ_0 can be calculated from the position of the cross, which corresponds to $\log \omega\tau_0/m = 0$. In making these calculations, the viscosity of water at 20 °C has been used (0.0100 P) to provide values which can be compared with conventional protein data. These are given in Table I. At infinite dilution, τ_0 is practically the same for either rigid or weakly bending model. The value, 23 to 25 μs, compares favorably with recent very careful determinations from electrical birefringence by DeLaney and Krause[16] (19 μs for the β form, 23 μs for the α form at infinite dilution). The slight difference may be due to a small amount of aggregated material in our sample.

Discussion

The significance of the longest relaxation time τ_0 appears to be well established. The shorter relaxation times have been interpreted as due to flexural modes of motion,[4] although the possibility of additional internal modes such as twisting or elongation cannot be excluded at present. An analysis of flexure was made by Wada and collaborators,[6] although they focussed attention on a single time τ_F associated with the fundamental flexural mode which can be approximately identified with τ_1 in our analysis. The ratio τ_0/τ_1 is then related to the flexural rigidity B as follows:[6]

$$B \simeq 0.10LkT\tau_0/\tau_1 \quad (9)$$

where L is the rod length. Since $\tau_0/\tau_1 = 8.43$, we obtain for B, a measure of the resistance of the paramyosin molecule to bending, a value of 4.0×10^{-19} dyn-cm^2 at 20 °C. This may be compared with 0.75×10^{-19} for poly(L-alanine) and 5×10^{-19} for poly(γ-benzyl L-glutamate) in which the side groups contribute substantially to the stiffness; the figures for these latter single helices were estimated by Wada from persistence lengths.[6]

Table I
Relaxation Times τ_0 Reduced to Water at 20 °C (s)

Model	Log τ_0, Infinite dilution	Log τ_0, $c = 2 \times 10^{-3}$
Yamakawa cylinder	−4.60	−4.54
Hybrid spectrum	−4.64	−4.38

The flexural rigidity of a rod depends on both the Young's modulus and the cross-section area and internal structure. If the bending rod is a cylinder of uniform structure, B is in turn related to Young's modulus E by the equation

$$E = 64B/\pi d^4 \quad (10)$$

where d is the rod diameter. For paramyosin we obtain $E = 1.2 \times 10^{10}$ dyn/cm^2. Alternatively, if one considers B to be the sum of two contributions from the individual helices whose cross-section areas are half that of the single-cylinder model, we obtain $E = 2.4 \times 10^{10}$ dyn/cm^2. These are quite high values, similar to that of a polymer in the glassy state. The fact that the molecule is flexible, in spite of the high calculated modulus, reflects its small diameter and small d/L ratio.

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