Flexibility of Light Meromyosin and Other Coiled-Coil α-Helical Proteins

Søren Hvidt and John D. Ferry*

Department of Chemistry, University of Wisconsin, Madison, Wisconsin 53706

Daniel L. Roelke and Marion L. Greaser

Muscle Biology Laboratory, University of Wisconsin, Madison, Wisconsin 53706.
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ABSTRACT: The storage and loss shear moduli, G' and G'', have been measured for solutions of rabbit light meromyosin in a solvent containing 50.6% glycerol at 0.4, 5.0, and 10.0 °C by use of the Birnboim–Schrag multiple lumped resonator. The intrinsic viscosities of light meromyosin and tropomyosin were measured by capillary viscometry at 10.0 °C. The intrinsic reduced moduli showed very good agreement with an empirical hybrid model for semiflexible rods. The rotational and longest bending relaxation times, reduced to water at 20 °C, were 6.7 μs and 350 ns for light meromyosin. The results and previous viscoelastic and hydrodynamic data for other coiled-coil α-helical proteins show that these proteins are well modeled as semiflexible rods with a persistence length of 1300 ± 400 Å at low temperatures.

Introduction

The flexibilities of both natural and synthetic rodlike macromolecules have been studied extensively by use of many different techniques. The degree of flexibility of an elongated molecule is most commonly given in terms of its persistence length[1] or bending force constant.[1,2] Only for contour lengths, L, much smaller than the persistence length, $L \ll q$, will the physical properties approach those of a rigid rod.[1] The properties of flexible coils are approached when the contour length is much larger ($L \gg q$) than the persistence length. Semiflexible rods are expected to show properties in between those of a rigid rod and a flexible coil.

Viscoelastic methods have been used in studies of a large variety of macromolecules.[3] The frequency dependence of the complex shear modulus extrapolated to infinite dilution has given information about the dynamics of macromolecules in solution,[3,4] including flexible coils,[5] rigid rods,[6] and several semiflexible rods.[7-9] The principal quantities that can be obtained from viscoelastic measurements of semiflexible rods are the intrinsic viscosity, $[\eta]$, the rotational relaxation time, τ_0, and the relaxation time for the slowest bending motion, τ_1.

Following recent theoretical work, the flexibility and the persistence length can be obtained from viscoelastic measurements in three independent ways: from $[\eta]/[\eta]_R$ (Yamakawa and Yoshizaki[10]), τ_0/τ_{0R} (Hagerman and Zimm[11]), or τ_0/τ_1, (Rosser et al.[9]), where $[\eta]_R$ and τ_{0R} are the intrinsic viscosity and the rotational relaxation time for a rigid rod with the same contour length. We have exploited the last method several times, but we have not until now made detailed comparisons of the three methods.

Tropomyosin, paramyosin, and myosin rod are all coiled-coil α-helical proteins,[12,13] with a diameter[12] of 20 Å and a 1.49-Å translation along the helical axes per residue.[14,15] Tropomyosin has been completely sequenced,[16] and part of the myosin rod has also been sequenced by Elzinga and co-workers[17] as reported by Parry.[18] The sequence results show a heptapeptide residue repeat of which positions 2 and 6, where the two helices face each other, are occupied by nonpolar residues and the other five positions are primarily occupied by polar or charged amino acids.[17,18] Hydrophobic interactions between neighboring nonpolar residues of the two chains are believed[19] to be largely responsible for the rigidity of these systems.

Hydrodynamic and light scattering data of coiled-coil α-helical proteins have usually been analyzed in terms of rigid rod or rigid ellipsoid models,[12,20-22] but viscoelastic measurements on paramyosin[9] and myosin rod[23] showed that these proteins could be better modelled as semiflexible rods at low temperatures. We could not, however, rule out the possibility that a part of the rod, light meromyosin (LMM), was nearly rigid from the viscoelastic measurements on myosin rod. The remainder of the rod is myosin subfragment 2 (S-2).

Here we report viscoelastic measurements on LMM and capillary viscosity measurements on LMM and tropomyosin (TM). Our results will be compared with the findings for myosin rod and paramyosin (PM), and it will be shown that the coiled-coil α-helical proteins are well described as semiflexible rods with a persistence length of 1300 ± 400 Å.

Theory

Viscoelastic measurements provide a determination of the frequency dependence of G' and G'', the storage and loss shear moduli, respectively. It is convenient to use the reduced storage and loss shear moduli defined[4] respectively as

$$G'_R = (M/cRT)G' \tag{1}$$

$$G''_R = (M/cRT)(G'' - \omega\eta_s) \tag{2}$$

where M is the molecular weight, c the concentration in g/mL, R the gas constant, T the absolute temperature, ω the radian frequency of oscillation, and η_s the solvent viscosity in poise.

Molecular theories are most often given in terms of the reduced intrinsic storage and loss shear moduli $[G']_R$ and $[G'']_R$, which can be compared with measured values of G'_R and G''_R extrapolated to vanishing concentration. The intrinsic viscosity $[\eta]$ can be obtained from capillary viscosity measurements but is also related to the reduced intrinsic loss shear modulus at low frequencies by the general relation

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

The theory for rigid cylinders[24] shows that one relaxation time, τ_0, due to end-over-end rotation, contributes to G' and G''

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

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

$$\tau_0 = m[\eta]\eta_s M/RT \tag{6}$$

with $m_1 = 3/5$ and $m = (m_1 + m_2)^{-1}$. The parameter m_2

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depends slightly on the axial ratio and is 0.2 for long cylinders.[24]

The empirical hybrid model has been used to describe the properties of semiflexible rods.[7-9,20] The shear modulus is given as a sum of contributions from a rigid cylinder and a flexible coil

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

$$[G'']_R = \omega\tau_0[m_1(1 + \omega^2\tau_0^2)^{-1} + m_2] + Z''(\omega\tau_1) \quad (8)$$

$$m = (m_1 + m_2 + 2.37\tau_1/\tau_0)^{-1} \quad (9)$$

where Z' and Z'' are the reduced moduli specified by the Zimm theory for dominant hydrodynamic interaction[25] and τ_1 is the longest Zimm-like relaxation time attributed to the slowest flexural mode of weakly bending rods.[9] For this model τ_0 is given by eq 6 with the value of m from eq 9.

The intrinsic viscosity of a helical wormlike chain has recently been calculated by Yamakawa and Yoshizaki.[10] The model used is an elastic wire of total contour length L with spherical caps at the ends. The intrinsic viscosity of a chain with a persistence length q and a diameter d is

$$[\eta] = [\eta]_R f(L/q) \quad (10)$$

$$[\eta]_R = (\pi N_A L^3/24M)F(L/d) \quad (11)$$

where N_A is Avogadro's number and expressions for $f(L/q)$ and $F(L/d)$ are given in their paper.[10] The equations are only valid for $L < 4.5q$ and $d < 0.2q$.

Hagerman and Zimm[11] have calculated the rotational relaxation time for semiflexible rods. Their model employed a chain of touching beads, and the range of validity is $0.1 < L/q < 5$ and $L/d > 20$. Their results can be written in a form similar to eq 10 and 11:

$$\tau_0 = \tau_{OR}R_c(L/q) \quad (12)$$

$$\tau_{OR} = \pi\eta_s L^3(18kT[\ln(2L/d) - 1.57 + 7[1/\ln(2L/d) - 0.28]^2])^{-1} \quad (13)$$

where the function $R_c(L/q)$ is given in their paper.[11] The functions f in eq 10 and R_c in eq 12 depend only on the ratio L/q and are monotonically decreasing functions of L/q. Their values are 1 in the rigid rod limit ($L \ll q$).

The fundamental bending relaxation time for an elastic rod was calculated by Ookubo, Komatsubara, Nakajima, and Wada,[26] and their results can be written as

$$\tau_F = 5.53 \times 10^{-3}\pi\eta_s L^4/(qkT \ln(L/d)) \quad (14)$$

The persistence length can then be obtained from this equation and the fit of eq 7 and 8 to viscoelastic data with the assumption[9] that τ_1 equals τ_F. Two other estimates of q can be obtained from $[\eta]$ and τ_0 by use of eq 10 and 12, respectively.

Experimental Section

Protein Preparation. Proteins were prepared from rabbit skeletal muscle (leg and back) by following published procedures. Myosin was prepared as described by Nauss et al.[20] LMM was obtained from myosin by digestion with TPCK-trypsin (Worthington) by following the procedure of Lowey and Cohen.[21] Tropomyosin was obtained as described by Greaser and Gergely.[27]

Column Purification. LMM and tropomyosin were purified on a hydroxyapatite column and eluted with the same linear gradient that was used for myosin rod preparations.[23] The purest fractions, as determined by gel electrophoresis (see below), were pooled and the proteins were precipitated by lowering the pH to 4.6 for tropomyosin and 4.8 for LMM by dropwise addition of 2 N HCl with continuous stirring. The precipitates were collected by centrifugation at 10000g for 20 min, resuspended in small volumes of buffer A (see below), and dialyzed against that solvent for at least 16 h.

Table I
Properties of Buffer G

property	temperature, °C 0.4	5.0	10.0
viscosity, cP	51.7	38.9	29.3
density, g/cm^3	1.245	1.243	1.240

The proteins were finally purified by gel chromatography on a Sephacryl 300 Superfine (Pharmacia) column (height 90 cm; diameter 2.5 cm; flow rate 20 mL/h) in order to remove any lower molecular weight polypeptide material. About 80 mg of protein with a concentration of 10 mg/mL was applied with each column run. The gel chromatography step was not used for the LMM preparation for viscoelastic measurements because large amounts were required for these measurements.

Solvents. Two solvents were used for the viscoelastic and capillary viscosity measurements. Buffer A contained 0.5 M KCl, 0.5 M potassium phosphate, and 10 mM EDTA (pH 7.3). Buffer G contained the same salts and in addition 0.6 M glycine and 50.6% glycerol by weight. The same solvents were used previously for the experiments on myosin rod.[23] Solutions of LMM in buffer G were prepared as described for myosin rod,[23] dialyzed against large volumes of solvents for at least 36 h, and clarified by centrifugation at 100000g for 1 h before any measurements were made.

Viscoelastic Measurements. The storage and loss shear moduli, G' and G'', of the LMM solutions were measured by use of the Birnboim–Schrag multiple-lumped resonator apparatus[28] with a computerized data acquisition and processing system.[29,23] A titanium resonator with five working frequencies from 150 to 8000 Hz was used in a titanium resonator housing.[6] The handling of solutions and the cleaning of the apparatus have been described elsewhere;[23] the only difference was that the apparatus was always cleaned with ethanol and methanol and dried before solvents and solutions were filled in the apparatus. Consecutive solutions were made gravimetrically by dilution with dialysate. Solvent calibration runs were performed daily. Measurements were made at 10, 5, and 0.4 °C with concentrations from 1.37 to 4.9 mg/mL.

Capillary Viscosity Measurements. Cannon-Fenske 50 and 150 viscometers were used for solvent and intrinsic viscosity measurements in buffers A and G, respectively. The solvent flow times for 4-mL solutions were 360 and 240 s at 10.0 ± 0.05 °C in a thermostated bath. The flow time was measured three times on each solution and was accepted if it was constant to within 0.1 s. Solutions were made up gravimetrically from clarified stock solution and solvent. Solvent densities were determined by use of a 25-mL pycnometer that had been calibrated with water. The properties of buffer G are summarized in Table I.

Concentration Determinations. The concentration of protein was determined by UV absorption at 280 nm for LMM and at 278 nm for tropomyosin. The extinction coefficients used were 3.0 for LMM[30] and 3.14 for tropomyosin.[12] The ratios of absorption at 280 nm to those at 260 nm were from 2.0 to 2.3 for both LMM and tropomyosin preparations. The refractive index increments at 546 nm were also measured for five LMM solutions with concentrations less than 2.5 mg/mL by use of a Brice-Phoenix type apparatus with a 100-cm-long optical path length. The temperature was 25.0 °C. A differential refractive index increment of 0.179 mL/g was obtained from a linear least-squares fit. This value is in good agreement with a reported value[31] of 40 fringes/1%, which corresponds to 0.182 mL/g.

Gel Electrophoresis. A Tris/glycine tube stacking gel system[32] was used with some minor modifications.[23] Apparent SDS molecular weights were obtained by comigrating samples with molecular weight standards from 40000 to 200000 (Bio-Rad). Gels were scanned by use of a Gilford spectrophotometer equipped with a linear transport system.

Results

Characterization of Proteins. Gel electrophoresis of the LMM preparations showed one major band on gels with the appropriate[30,33] apparent molecular weight of 75000. The main contaminant at high loads was a fine

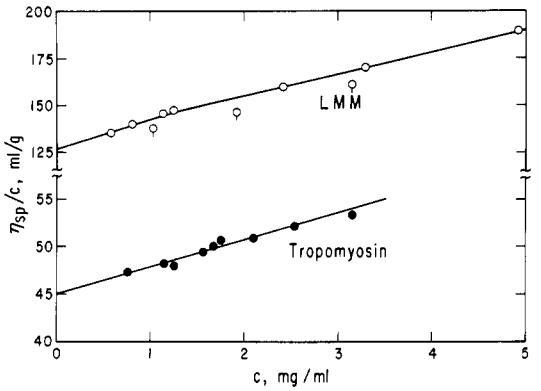

Figure 1. Plots of η_{sp}/c against c for LMM and tropomyosin at 10.0 °C. Circles without pips, in aqueous buffer A; with pips, in buffer G (50.6% glycerol).

doublet with an apparent molecular weight of about 92000. The doublet content was about 3–5% (three preparations) as judged from densitometric scans of gels. The same doublet was found to be the main contaminant in rod preparations.[23]

The preparations of tropomyosin showed the characteristic pattern of a strong α-TM and a weaker β-TM band on gels. No other bands could be detected even at loads of 60 μg per gel.

Intrinsic Viscosities. The intrinsic viscosities of LMM and TM at 10 °C in buffer A were obtained from plots of η_{sp}/c against concentration, as shown in Figure 1. The data for LMM in buffer A showed some curvature, as has been reported by others;[31] an intrinsic viscosity of 127 mL/g was obtained from a smooth extrapolation to vanishing concentrations. The Huggins constant k in the equation $\eta_{sp}/c = [\eta] + k[\eta]^2c + \ldots$ was 0.8. A few measurements on LMM in buffer G showed the same intrinsic viscosity but a lower Huggins constant (0.6). A linear least-squares fit for TM gave an intercept for 45 mL/g with an estimated uncertainty of ±1 mL/g. The Huggins constant was found to be 1.6.

Viscoelastic Results. The quantities $G'_R{}^{1/2}$ and G''_R were calculated from the measured G' and G'' for LMM solutions by use of eq 1 and 2. The molecular weight was taken[30] to be 130000. The corresponding intrinsic moduli $[G']_R$ and $[G'']_R$ were obtained as the intercepts of plots against c, in most cases from a linear least-squares fit. This extrapolation is illustrated in Figure 2 for the LMM data at 10 °C. The extrapolations for the data at 0.4 and 5 °C were very similar. The uncertainties in the intercepts are about 10% at the highest frequency and are considerably smaller at lower frequencies.

The reduced intrinsic moduli at the three temperatures are plotted logarithmically against the conventional reduced angular frequency, $\omega\eta_s[\eta]M/RT$, in Figure 3. The logarithmic uncertainty corresponding to a 10% uncertainty in $[G']_R$ or $[G'']_R$ is shown in the upper left corner. The data are first compared with theoretical curves (dashed) for a rigid rod as calculated from eq 4 and 5. The value of m_2 was calculated[24] to be 0.29 from the axial ratio of LMM (see below). The good agreement for $[G'']_R$ at low frequencies shows that the intrinsic viscosity determined from viscoelastic measurements in buffer G agrees remarkably well with the value determined from capillary viscosity measurements in buffer A and G; actually the $[\eta]$ value obtained from $[G'']_R$ at the lowest frequency by use of eq 3 is 123 mL/g, close to the expected value of 127 mL/g at vanishing frequencies.

The rigid rod model gives a fairly good fit to data at low frequencies, but diverges widely at higher frequencies.

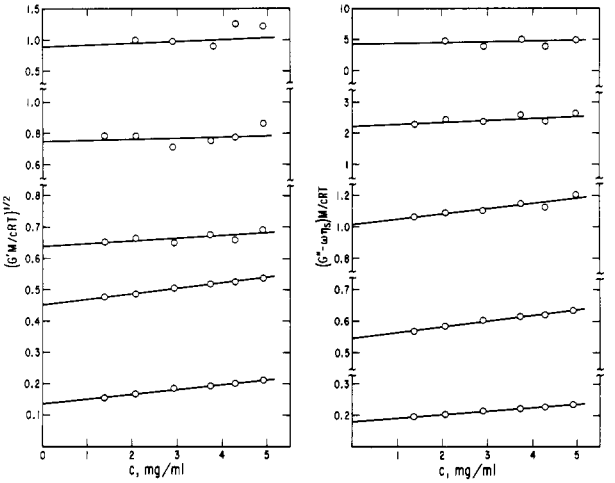

Figure 2. Plots of $(G'M/cRT)^{1/2}$ and $(G''-\omega\eta_s)M/cRT$ against c for LMM in buffer G at 10.0 °C. The frequencies of measurements are, from bottom to top, 144, 573, 1494, 3749, and 8037 Hz.

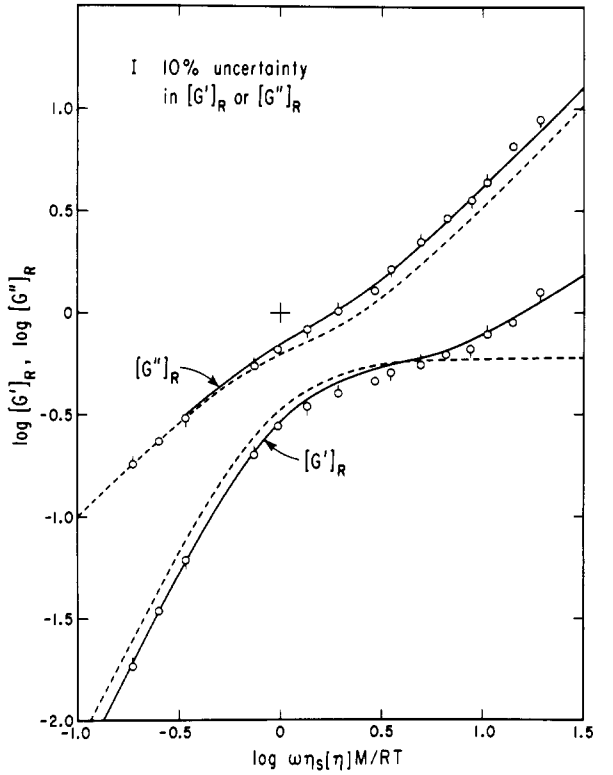

Figure 3. Reduced intrinsic moduli, $[G']_R$ and $[G'']_R$, of LMM plotted logarithmically against reduced frequency. Temperatures of measurements: pip down, 0.4 °C; no pip, 5.0 °C; pip up, 10.0 °C. Dashed curve, Yamakawa theory for rigid rod, eq 4 and 5 with $m_2 = 0.29$; solid curve, hybrid theory with $m_2 = 0.29$, $\tau_0/\tau_1 = 19$.

Even at low frequencies, the measured $[G']_R$ is lower than expected for a rigid rod. The theoretical bump in $[G'']_R$ at intermediate frequencies is hardly seen; $[G'']_R$ at high frequencies is above the rigid rod curve; $[G']_R$ does not level off at high frequencies. These differences are all indicative of a semiflexible rod.[7-9,23]

The data were therefore fitted to the hybrid model eq 7 and 8 with τ_0/τ_1 as an adjustable parameter. The values of m_1 and m_2 were kept at the values for the rigid rod. The best fit was obtained with $\tau_0/\tau_1 = 19 \pm 4$ and is shown with the full curves in Figure 3. The agreement is excellent for $[G'']_R$ over the entire frequency range and very good for $[G']_R$ except at intermediate frequencies. This discrepancy

Table II
Hydrodynamic Data for Coiled-Coil α-Helical Proteins[a]

protein	M_r	L, Å	$[\eta]$, mL/g	$[\eta]_R$,[b] mL/g	$\tau_{0,w}$,[20] μs	τ_{0R},[20 b] μs	τ_F, μs	τ_0/τ_F
myosin rod	220 000[c]	1440[d]	293[e] 270-290[f] 265[h]	348	25.3[e] 24.1[g]	37.5	3.2[e]	8[e]
paramyosin	208000[i]	1345[j]	260[k] 254[l]	307	22.7[k] 19-23[m]	31.2	2.7[k]	8.43[k]
LMM	130 000[c]	833[j]	127[n] 123[h] 120[o]	139	6.7[n] 7.6[g]	8.69	0.35[n]	19[n]
S-2	101 000[d]	650[d]	76[d]	94	3.5[g]	4.54		
tropomyosin	65 500[p]	423[q]	45[n] 45-47[s]	48.9	1.30[r]	1.50		

[a] Results from viscoelastic measurements and other types of hydrodynamic measurements. [b] Calculated by use of eq 11 and 13. [c] Reference 30. [d] Reference 33. [e] Reference 23. [f] Reference 54. [g] Reference 39. [h] Reference 31. [i] Reference 52. [j] Calculated as described in text. [k] Reference 9. [l] Reference 37. [m] Reference 22. [n] This study. [o] Reference 38. [p] Reference 16. [q] Reference 19. [r] Calculated by use of eq 13 from a measured apparent length of 400 Å, ref 53. [s] Reference 12.

could indicate a lower m_1 value, but such an optimization was not attempted.

The rotational relaxation time and the longest bending time reduced to water at 20 °C were then obtained from the fit by use of eq 6 and 9 and were found to be 6.7 μs and 350 ns, respectively.

Discussion

Flexibility and conformational changes within the coiled-coil α-helical proteins at physiological temperatures are believed to play important roles during muscle contraction.[34] A large body of evidence[33-35] shows that a part of the S-2 fragment near the LMM–S-2 junction can undergo a conformational change with a transition temperature of 42–46 °C. Fluorescence and circular dichroism measurements on tropomyosin indicated[36] a localized unfolding transition between 25 and 40 °C that involves the region of the molecule near Cys-190, whereas they were completely folded below 25 °C. Optical rotary dispersion measurements on paramyosin[37] showed that this protein is fully α-helical below 23 °C but exhibited a transition around 44 °C. This transition, however, does not appear to affect the rotational relaxation time up to 50 °C as determined from electric birefringence measurements.[22] Our viscoelastic and intrinsic viscosity measurements on these proteins have been made at temperatures from 0 to 10 °C, well below these transitions, since we are primarily interested in the intrinsic rigidity of the coiled-coil structure.

The intrinsic viscosity of tropomyosin has been measured at 20 °C by Holtzer and co-workers[12] and was found to vary between 35 and 47 mL/g, depending on the amount of salt in the solvent. In a solvent with 0.5 M KCl and 0.1 M potassium phosphate (pH 7.2) the value was 45 mL/g with a Huggins constant of 1.7, in very good agreement with our findings. Our value for the intrinsic viscosity of LMM, 127 mL/g, determined from capillary measurements, is slightly higher than literature values[35,38] of 120–123 mL/g. The small difference is most likely due to the small amounts of the contaminating doublet in our sample or to possible removal of components of lower molecular weight; we believe that the intrinsic viscosity of LMM without the doublet is at most 127 mL/g.

Our rotational relaxation of 6.7 μs for LMM is somewhat smaller than the relaxation time of 7.6 μs obtained from electric birefringence measurements[39] in 2 mM pyrophosphate (pH 9.3) at 3 °C. The source of this difference is not clear to us but may be due to either different solvent conditions or differences in samples.

The viscoelastic data for LMM in Figure 3 shows that a good fit was obtained when LMM was modelled as a semiflexible rod. Similar conclusions were reached for paramyosin[9] and for myosin rod[23] where the measurements extended to higher reduced frequencies and the effects due to the spectrum of bending relaxation times could be more clearly observed. Tropomyosin and S-2 are shorter than LMM, and viscoelastic measurements with our apparatus would not give very conclusive information about their flexibility. Some information can however be obtained from values of the rotational relaxation times and intrinsic viscosities by use of eq 10 and 12, provided reasonably accurate values of the contour length and molecular weights are known.

We have chosen equilibrium sedimentation and sequence results as reliable sources for molecular weights. The values are summarized in Table II. The values for S-2 and LMM are somewhat uncertain since enzyme digestion can result in fragments of varying length.[21,33,38,40] The value of 130 000 for LMM was determined[30] for preparations following the same procedure that we have used.[21] The length of the proteins can be obtained from the helical structure, the molecular weight, and mean residue molecular weight, since each residue gives rise[14,15] to a 1.49-Å translation along the direction of the helix. The values for myosin rod,[33] S-2,[33] and tropomyosin[19] are summarized in Table II. The lengths of paramyosin and LMM are calculated from the molecular weights and the mean residue molecular weights[37,38] of 115.2 and 116.3, respectively. The calculated value for paramyosin is in good agreement with the findings from electron microscopy[41] and X-ray diffraction,[42] which suggest a length of at least 1275 Å. The electron microscopy value[43,44] of 1440 Å for myosin rod is also in excellent agreement with the calculated helical length.

The intrinsic viscosity and rotational relaxation time of the proteins modeled as rigid rods were calculated by use of eq 11 and 13 from the lengths and molecular weights and are summarized in Table II. The values of $[\eta]$, τ_0, and τ_F from viscoelastic measurements are listed together with literature values of $[\eta]$ and τ_0 (from electric birefringence). The data from Table II are compared with theoretical predictions for semiflexible rods in Figure 4. The circles in the bottom figure show the measured intrinsic viscosity divided by the intrinsic viscosity of a rigid rod against contour length. The three curves are calculated by use of eq 10 for three different values of the persistence length. The middle figure shows the measured τ_0 divided by τ_{0R} against contour length. The three curves are calculated

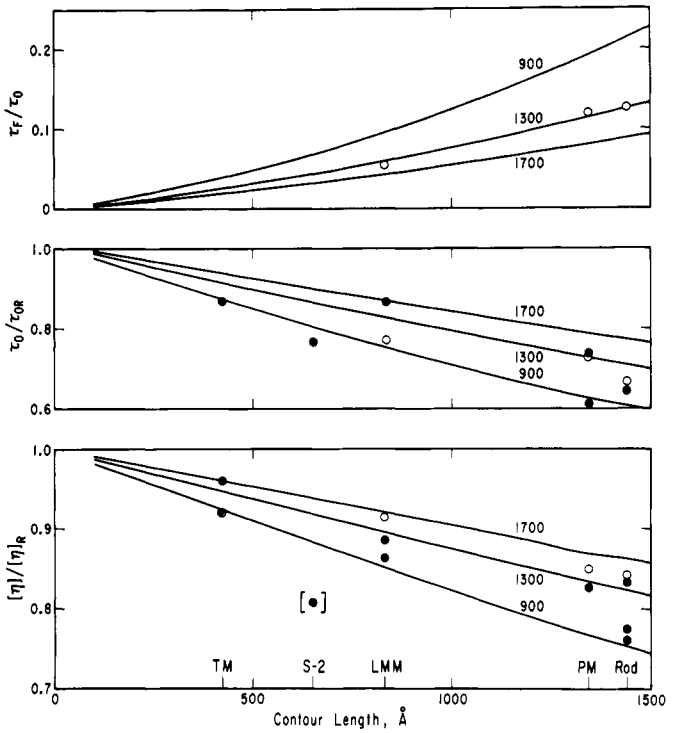

Figure 4. Plots of τ_F/τ_0, τ_0/τ_{0R}, and $[\eta]/[\eta]_R$ against contour length L. Solid curves drawn for three values of persistence length by use of eq 10–16; open circles, data from this laboratory; black circles, data from the literature (Table II).

from eq 12 for the same three values of the persistence length. The top figure shows a plot of the measured τ_1/τ_0 values from the hybrid fit against length. The theoretical dependence of τ_F/τ_0 as a function of length is shown with the full curves for the three values of the persistence length. The values of τ_F and τ_0 were calculated for flexible rods by use of eq 12 and 14.

It is seen from Figure 4 that all the measured values of $[\eta]$ and τ_0 fall below the rigid rod case, which corresponds to a value of 1 on these plots. Taken together with the observed bending motions from the frequency dependence of the shear modulus for the three systems, this gives evidence that the coiled-coil α-helical proteins are flexible rather than rigid.

A comparison between the measured values and the theoretical curves for semiflexible rods allows for up to three estimates of the persistence length for each system. There is clearly considerable scatter in the measured values of $[\eta]$ and τ_0, but most of the data points fall between the theoretical curves corresponding to 900 and 1700 Å with only the intrinsic viscosity of S-2 falling significantly outside. The lower value could be due to contamination with shorter S-2. The value of 76 mL/g is for an 80–90% clean S-2 preparation[33] at 10 °C, with some more degraded S-2, which was shown[33] to have an intrinsic viscosity of only 40 mL/g. We did not manage to prepare sufficient amounts of S-2 with satisfactory purity to give reliable intrinsic viscosity results. The experimental uncertainties in τ_0, $[\eta]$, and length prevent very precise estimates of the persistence lengths, but all three methods indicate that a persistence length of 1300 ± 400 Å is characteristic for this group of proteins with the possibility that S-2 is slightly more flexible.

The estimates of q from τ_1/τ_0 show relatively little scatter. This is presumably due to a weaker dependence on length as seen from eq 13 and 14. The ratio is approximately proportional to L/q, so a small uncertainty in L will only result in a small uncertainty in q. The estimates of q from $[\eta]$ and τ_0 on the other hand are obtained from the differences between measured values and rigid rod values, which depend quite strongly on L as seen from eq 11 and 13. Viscoelastic measurements are especially useful for determinations of whether an elongated molecule is rigid or not in cases where only approximate information about lengths are available, since the frequency dependences of the reduced shear modulus are qualitatively different for rigid rods and semiflexible rods.

The fact that myosin rod does not seem to have a higher flexibility than its two fragments, LMM and S-2, is taken as additional[23] evidence against a hinge between these two fragments at low temperatures. Results from electric birefringence[39] and depolarized light scattering[45] have been taken in support of a model[45] in which the rod is composed of two rigid fragments, LMM and S-2, connected by a hinge that allows free diffusion up to a maximum angle of 128° with a correlation time of 3.2 μs. Our results indicate that the fragments are flexible and their correlation time is equal to the longest bending time for the rod as seen in Table II. The spectrum of quasielastically scattered light from a semiflexible rod with L = 1440 Å and q = 1300 Å should have a component due to the slowest bending motion with τ_0/τ_1 about 8–10, as seen from Figure 1 of ref 46.

The difference in hydrodynamic properties between a rigid rod and a semiflexible rod becomes more important the longer the contour length, as illustrated in Figure 4. Tropomyosin is the shortest one and is therefore expected to be most nearly rigid. Two independent groups have concluded that tropomyosin is flexible on the basis of X-ray diffraction studies[47] and fluorescence depolarization measurements.[48] The fluorescence experiments on labeled monomeric tropomyosin showed a complicated decay of anisotropy with time; correlation times of 3 and 69 ns at 3 °C were obtained from a two-exponential fit to the decay. The longest bending time for tropomyosin is expected to be 60 ns at 3 °C in water, as calculated by use of eq 14 with L = 423 Å and q = 1300 Å, quite close to the slowest correlation time observed from the fluorescence decay.

The fact that tropomyosin is wrapped around the groove of the thin filament[19] with a half pitch of 385 Å and a diameter of 20–22 Å is hard to reconcile with a rigid rod model. The energy of a bent rod[1] is $^1/_2qkTL/R_c^2$, where R_c is the radius of curvature. The groove pitch and a diameter of 22 Å give $R_c = (22^2 + (385/\pi)^2)/22 = 705$ Å and an energy of $0.55kT$ for L = 423 Å and q = 1300 Å. Only energies close to thermal energies ($^1/_2kT$) are therefore needed to bend tropomyosin around the actin filament. It has been proposed[19] that tropomyosin regulates myosin–actin binding by rolling on the thin filament. Tropomyosin appears to be flexible enough that such a motion would necessitate only small energies.

The persistence length of about 1300 Å shows that the coiled-coil α-helices are remarkably stiff. The persistence length of DNA, another double-stranded macromolecule, is only[11] 400–600 Å. The persistence length of 1700 Å for the triple-stranded collagen[49,50] is only slightly higher than the value for the coiled-coil α-helical proteins. A theoretical persistence length of 400–650 Å for single α-helices of polyglycine andd poly(L-alanine) has been calculated[51] from the fluctuations of bond angles in the helices. A double-stranded coiled-coil α-helix would presumably have at least twice the persistence length and rigidity, very similar to the 1300 Å determined from hydrodynamic and viscoelastic measurements.

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Infrared Study of Helix Reversal in "Nafion"[1] Perfluorinated Membranes and Precursors†

William Y. Hsu

Central Research and Development Department, E. I. du Pont de Nemours and Company, Experimental Station, Wilmington, Delaware 19898. *Received June 26, 1982*

ABSTRACT: Backbone disorders, known as helix reversals in poly(tetrafluoroethylene) (PTFE), are observed in "Nafion" perfluorinated membranes and precursors. The formation energy is 1 kcal/mol and is independent of the nature and number of vinyl ether side groups. The soliton- or domain-like nature of these defects is discussed.

Introduction

"Nafion" perfluorinated membranes have been studied extensively because of their extraordinary electrochemical properties and commercial application in chloralkali cells.[2,3] The sulfonate ion-exchange polymer is obtained by hydrolysis from a precursor copolymer (XR) of tetrafluoroethylene and the sulfonyl vinyl ether CF_2=$CFOCF_2C$-$F(CF_3)OCF_2CF_2SO_2F$.[2,3] The precursor can also react with ethylenediamine (EDA) to form a sulfonamide polymer.[4]

The unique transport properties[5,6] observed in these perfluorinated ionomers when submerged in an electrolyte have been correlated to the spontaneous segregation of the aqueous phase into conductive ion-containing domains randomly distributed in the insulating fluorocarbon matrix.[6,7] According to a recent elastic model,[8] the average size of these domains is controlled by the tensile property of the polymer and several other factors such that, qualitatively, a flexible and deformable polymer can support larger clusters than a stiff and rigid one. It is then important to know whether a rigid or flexible rod model would be more appropriate for "Nafion" perfluorinated membranes.

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