Translational and Rotational Dynamics of Collagen in Dilute Solution

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The translational and rotational dynamics, including the translational−rotational coupling, of collagen in an aqueous 0.25 mg/mL solution of 0.1 N HCl were studied by polarized dynamic light scattering. An autocorrelator with variably spaced delayed times allowed computation of dynamic light-scattering intensity autocorrelation functions over a wide dynamic range. Methods of data analysis included the method of cumulants and the inverse Laplace transform−regularization method as embodied in Provencher's CONTIN program. The value determined for the translational diffusion coefficient using the method of cumulants is 8.4×10^{-12} m^2/s. This value is smaller than the value of 9.5×10^{-12} m^2/s found using CONTIN. The method of cumulants appears to result in an erroneous value of D due to the presence of dust or aggregates. The D value found from CONTIN analysis corresponds to a hydrodynamic length of 2390 Å using Broersma's relations and assuming the diameter $d = 13.6$ Å. The rotational diffusion coefficient was determined to be 1110 s^{-1}. The Hagerman−Zimm and Yoshizaki−Yamakawa theories for the ratio of the value of Θ expected for a rigid rod to that for a semirigid rod of the same contour length were used to determine values of 1600 and 1650 Å for the persistence length of collagen.

Introduction

One of the fundamental goals of polymer physics is to gain an understanding of the dynamics of macromolecules in solution. The molecular dynamics is related to macroscopic properties of importance in both industrial and biological applications. Rodlike macromolecules provide relatively simple model systems that can be used to test basic theoretical approaches to molecular solution dynamics as well as to test and help develop experimental methods for studying them. There is much interest in gaining a detailed understanding of the translational and rotational dynamics of such materials in a range of concentration regimes as well as in testing methods for extracting the required information from the experimental data. In addition, many macromolecules of biological and materials importance are rodlike. In this work dynamic light scattering (DLS) is used to study the translational and rotational dynamics of the protein collagen in dilute solution. One of the novel features of the present work as opposed to previous work on collagen is the use of an autocorrelator with variably spaced delay times. The variable spacing allows data to be accumulated without the need for splicing autocorrelation functions obtained in different time ranges. The current work, among other things, tests the consistency of various methods of analyzing data on long rigid rod polymers. It also provides dilute solution data on readily available standard preparations of collagen that can be used as a reference for investigating the interesting effects that occur in more concentrated solutions in which intermolecular interactions between the rodlike polymers become important.

Collagen is an abundant protein in the animal world. It provides the body with a strong yet flexible framework to contain the tissues and cells. Boedtker and Doty[1] concluded that the monomer was a rod 3000 Å long and 13.6 Å in diameter. The monomer is composed of three polypeptide chains each wrapped in an extended left-handed helix. These three chains are then wrapped together in a right-handed super helix, forming a coiled-coil structure. This helical structure imparts stiffness to the rod. Considering both its structure and function, the collagen monomer is expected to be fairly stiff. Dilute solution viscoelastic and sedimentation experiments have yielded persistence lengths P between 1600 and 1800 Å.[2−4] The persistence length of collagen is thus thought to be larger than half its contour length. It might be expected that, as a first approximation, theories developed for rigid rods could be used to treat collagen dynamics. Polarized and depolarized dynamic light scattering[5−11] and electric birefringence decay[12−14] are among the experimental techniques that have been used to examine the dynamics of various forms of collagen. Much of this previous work is contradictory, and questions about sample preparation, correlator sampling times (DLS), methods of data analysis (cumulants, inverse Laplace transforms), fitting formulas, etc. are still not yet settled.

For long, rodlike molecules in dilute solution the polarized DLS intensity time autocorrelation function contains contributions from the macromolecular translational diffusion and rotational diffusion.[15] The relative contributions of translational and rotational motion depend on the product of the scattering vector length, q, and the polymer length, L. For small qL the translational motion dominates the time autocorrelation function. As qL increases, the contributions of terms also containing rotational and/or internal motion become significant, eventually giving a multiexponential form to the correlation function. In this work, DLS is used to characterize the dilute solution dynamics of collagen by studying the polarized DLS time correlation function over a range of q values. A variety of techniques are available for analysis of DLS time correlation data. The method of cumulants is convenient for studying translational diffusion[16] but may be affected by the presence of dust particles or aggregates. The FORTRAN program CONTIN[17] written by Provencher allows for a straightforward simultaneous analysis of both translational and rotational dynamics. The program also accounts and corrects for the presence of small amounts of dust or aggregates. Both cumulants and CONTIN are used in this work.

Theory

In dynamic light scattering, the scattered light intensity autocorrelation function is measured:

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$$G_2(t) = \langle I(0)I(t)\rangle \quad (1)$$

This may be normalized by the base line to give the second-order correlation function

$$g_2(t) = G_2(t)/\langle I\rangle^2 \quad (2)$$

If the fluctuations in the scattered field have a Gaussian distribution, the second-order correlation function may be related to the first-order correlation function,

$$g_2(t) = 1 + \beta|g_1|(t)^2 \quad (3)$$

with β an adjustable parameter independent of t.[18] The first-order correlation function may then be written for various systems. For collections of molecules, $g_1(t)$ may be expressed by a continuous distribution of decays:

$$g_1(t) = \int_0^\infty \mathrm{d}\Gamma\, G(\Gamma)\exp(-\Gamma t) \quad (4)$$

where $g_1(t)$ is the Laplace transform of the decay rate distribution function $G(\Gamma)$. $G(\Gamma)$ gives the relative intensity of light scattered with decay constant Γ and is a function of the number and size of the scatterers.

For a dilute solution of spherical, monodisperse particles undergoing Brownian diffusion $G(\Gamma)$ may be represented with the delta function $G(\Gamma) = \delta(\Gamma - q^2D)$ giving

$$g_1(t) = \exp(-q^2Dt) \quad (5)$$

where D is the translational diffusion coefficient and q is the magnitude of the scattering vector

$$q = (4\pi n/\lambda)\sin\theta/2 \quad (6)$$

defined in terms of the solution refractive index, n, the wavelength in vacuum of the light used, λ, and the scattering angle, θ.

For polydisperse particles or particles with modes other than translation present, $g_1(t)$ is no longer a single-exponential function and may be modeled as a weighted sum of exponential decays. In this study two methods of analysis were used to determine the distribution of decay times $G(\Gamma)$ leading to the correlation function decay. These methods were the method of cumulants and the FORTRAN program CONTIN.[17]

A cumulants expansion may be used to determine the average decay rate, $\bar{\Gamma}$,

$$g_1(t) = \exp(-\bar{\Gamma}t)[1 + (\mu_2/(2!))t^2 - (\mu_3/(3!))t^3 + ...] \quad (7)$$

where μ_i is the ith moment around $\bar{\Gamma}$ of the decay rate distribution. This method gives the weighted average diffusion coefficient $\bar{\Gamma}/q^2$. The polydispersity or broadness of the distribution may be determined from the second moment μ_2 normalized by $\bar{\Gamma}^2$. Maeda[16] gives an expression for the apparent diffusion coefficient (first cumulant) for a dilute solution of rigid rods

$$\bar{\Gamma}/q^2 = [D + (L^2/12)\Theta f_1(k) - (D_{\|} - D_{\perp})\{1/3 - f_2(k)\}] \quad (8)$$

where $k = qL/2$, $D_{\|}$ and $D_{\perp}$ are the coefficients for translation parallel and perpendicular to the rod's axis, Θ is the rotational diffusion coefficient, and D is the overall translational diffusion coefficient of the rod. The functions $f_1(k)$ and $\{1/3 - f_2(k)\}$ are weighting factors depending only on k. The term $f_1(k)$ accounts for the contribution of rotation to $\bar{\Gamma}/q^2$. The term $\{1/3 - f_2(k)\}$ accounts for the contribution of translational anisotropy, or difference between parallel and perpendicular diffusion, defined as $D_{\|} - D_{\perp}$. Explicit forms of these weighting factors may be found in ref 16.

In the limit of $q \to 0$, $f_1(k) \to 0$ and $f_2(k) \to 1/3$. Therefore, the extrapolated value of $\bar{\Gamma}/q^2$ to $q^2 = 0$ gives the translational diffusion coefficient. Unfortunately, the complicated q dependence of $\bar{\Gamma}/q^2$ makes determination of Θ using higher q data difficult.

The program CONTIN is useful for analysis of more complicated data, since it may be used to calculate the entire distribution of decay times rather than merely the average decay and width of the distribution as found in cumulants. The program determines $G(\Gamma)$ by performing an inverse Laplace transform on eq 4. The computer searches for the smoothest nonnegative solution for $G(\Gamma)$ that is consistent with the data. The correct amount of smoothing is chosen using a Fisher F-distribution and confidence region. The individual decay times and relative contributions of these decays to the distribution function may then be used in order to study both translational and rotational dynamics.

For a dilute solution of long, optically isotropic rodlike molecules of length L, the polarized dynamic light-scattering time correlation function may be written as a series of exponentials,

$$I_{vv}(q,t) = N\alpha^2 \sum_{l=0,\mathrm{even}} S_l(qL)\exp(-t/\tau_l) \quad (9)$$

where N is the number density of molecules and α is the molecular polarizability. The τ_l are the relaxation times. The $S_l(qL)$ are the amplitudes for each relaxation mode and are often referred to as the dynamic form factors. Forms for the τ_l and $S_l(qL)$ depend on the dynamical theory used. Pecora[15] treated the case where the rod undergoes independent translational and rotational diffusion with respective translational and rotational diffusion coefficients D and Θ and found that

$$1/\tau_l = q^2D + (l)(l+1)\Theta \quad (10)$$

with the first two terms

$$1/\tau_0 = q^2D \quad (11)$$

and

$$1/\tau_2 = q^2D + 6\Theta \quad (12)$$

giving the expression

$$I_{vv}(q,t) = N\alpha^2[S_0(qL)\exp(-q^2D)t + S_2(qL) \times \exp[-(q^2D + 6\Theta)t] + ...] \quad (13)$$

where S_0 and S_2 are the first two dynamic form factors

$$S_0(qL) = [(2/(qL))\int_0^{qL/2}(\sin z/z)\,\mathrm{d}z]^2 \quad (14)$$

$$S_2(qL) = (5/(qL)^2)[-3\{(2/(qL))^2\sin(qL/2) - (2/(qL))\cos(qL/2)\} + \int_0^{qL/2}(\sin z/z)\,\mathrm{d}z]^2 \quad (15)$$

As $qL \to 0$, $S_0(qL) \to 1$ and all higher $S_l(qL) \to 0$. At low angles the first term whose decay depends only on D dominates the time correlation function, and as qL increases, the term with $l = 2$ containing D and Θ becomes important and the time correlation function becomes effectively a two-exponential form.

D and Θ may be related to the rod length using the Broersma[19−21] or Tirado and Garcia de la Torre[22,23] relations. These theories calculate diffusion coefficients in term of the ratio of rod length to the cross-sectional diameter, L/d, and the solvent viscosity, η. Both theories utilize hydrodynamic methods assuming nonslip boundary conditions.

Broersma estimates that his relations apply to rods with $L/d > 5$. The Broersma relation for the rod translational diffusion coefficient may be written as

$$D = (k_b T/(3\pi\eta L))[\delta - (1/2)(\gamma_{||} + \gamma_{\perp})] \tag{16}$$

where

$$\delta = \ln(2L/d)$$

$$\gamma_{||} = 0.807 + 0.15/\delta + 13.5/\delta^2 - 37/\delta^3 + 22/\delta^4$$

$$\gamma_{\perp} = -0.193 + 0.15/\delta + 8.1/\delta^2 - 18/\delta^3 + 9/\delta^4$$

$$L = \text{rod length}$$

$$d = \text{rod diameter}$$

The rotational diffusion coefficient is given by

$$\Theta = (3k_b T/(\pi\eta L^3))(\delta - \xi) \tag{17}$$

where

$$\xi = 1.14 + 0.2/\delta + 16/\delta^2 - 63/\delta^3 + 62/\delta^4$$

The Tirado and Garcia de la Torre relations, estimated to be valid for $2 \le p \le 30$ may be written as

$$D = (k_b T/(3\pi\eta L))(\ln p + \nu) \tag{18}$$

where

$$p = L/d$$

$$\nu = 0.312 + 0.565p^{-1} - 0.100p^{-2}$$

and the rotational diffusion coefficient is given b

$$\Theta = (3k_b T/(\pi\eta L^3))(\ln p + \delta) \tag{19}$$

where

$$\delta = -0.662 + 0.917p^{-1} - 0.050p^{-2}$$

Since the collagen molecule's dimensions L and d are so different, the rod will be expected to translate in a direction parallel to its axis at a different rate from that perpendicular to it. The translational and rotational motions are coupled and are no longer independent.

The coefficients for translation in each direction for the Broersma relations are given by

$$D_{||} = (k_b T/(2\pi\eta L))(\delta - \gamma_{||}) \tag{20}$$

$$D_{\perp} = (k_b T/(4\pi\eta L))(\delta - \gamma_{\perp})$$

The difference in these rates is the diffusive anisotropy:

$$\Delta D = D_{||} - D_{\perp} \tag{21}$$

Many authors have considered the case where the translational and rotational motions become coupled because of the anisotropy in the translational diffusion coefficient for long rigid rods.[16,24−31] The approximate expressions given by Rallison and Leal[28] as corrected by Kubota et al.[30] and Maeda and Fujime[16] are convenient for application to the collagen molecule:

$$1/\tau_0 = q^2 D - (2q^4(\Delta D)^2)/(135\Theta) + ... \tag{22}$$

$$1/\tau_2 = q^2 D + 4q^2\Delta D/21 + 6\Theta + ... \tag{23}$$

giving the expression

$$I_{vv}(q,t) = N\alpha^2[a_0(qL)\exp[-\{q^2 D - 2q^4\Delta D^2/(135\Theta) + ...\}t] + a_2(qL)\exp[-\{q^2 D + 4q^2\Delta D/21 + 6\Theta + ...\}t + ...]] \tag{24}$$

with dynamic form factors a_0 and a_2 given by

$$a_0 = S_0(qL) + (2q^4\Delta D/(45\Theta))(5S_0S_2)^{1/2} - (q^4\Delta D^2/(405\Theta^2))(S_0) + ... \tag{25}$$

$$a_2 = S_1(qL) - (2q^2\Delta D/(45\Theta))(5S_0S_2)^{1/2} + (q^4\Delta D^2/(405\Theta^2))(S_0) - ... \tag{26}$$

In the limit of $\Delta D \rightarrow 0$ eqs 22−26 reduce to eqs 10−15 above given by Pecora.

The q dependence of the decay times and dynamic form factors described above may be used to determine dynamic quantities of interest, and the Broersma[19−21] or Tirado and Garcia de la Torre[22,23] relations may be used to relate the quantities D, Θ, L, and ΔD.

The measured value of Θ may be used to determine the quantity P, the persistence length of the molecule. The persistence length is a measure of the molecular flexibility. P is the distance at which the projection of a tangent vector along the chain to that at another point along the contour length falls to 1/e of its initial, zero-distance value. The rotational diffusion coefficient is very sensitive to rod stiffness and is highly dependent on the chain contour length L (see eqs 17 and 19). If a molecule behaves hyrdrodynamically as if it were shorter than the given contour length, Θ will be affected.

An expression relating the persistence length and the measured rotational diffusion coefficient has been given by Hagerman and Zimm.[32] The theory relates Θ predicted by the original Broersma relation[19] for a rigid rod of length L to the experimentally measured Θ. Their expression is given as

$$\Theta_{\text{Broersma}}/\Theta_{\text{measured}} = R_a(1 - Y) \tag{27}$$

with

$$R_a = 1.0120 - 0.24813X + 0.033703X^2 - 0.0019177X^3$$

$$Y = 0.06469X - 0.01153X^2 + 0.0009893X^3$$

$$X = L/P$$

An alternative theory has been presented by Yoshizaki and Yamakawa.[33] This theory relates the ratio of the rotational diffusion coefficient predicted by Yoshizaki and Yamakawa for a rigid rod to that for a semistiff polymer made up of N_k Kuhn segments , where $N_k = L/(2P)$:

$$\Theta_{\text{rigid rod}}/\Theta_{\text{measured}} = [N_k + 0.5(\exp(-2N_k) - 1)]^{3/2}N_k^{-3} \times [1 + 0.539526\ln(1 + N_k)] \tag{28}$$

Both these expressions may be used to determine P from the experimentally determined rotational diffusion coefficient.

Although it is less dependent on rod stiffness than the rotational diffusion coefficient, the translational diffusion coefficient may also be used to determine the rod's persistence length. Yamakawa and Fujii[34] have given expressions relating the translational diffusion coefficient to the reduced rod length ($L/(2P)$) and the reduced rod diameter ($d/(2P)$). The Yamakawa−Fujii relation provides an additional method for the determination of the persistence length.

Experimental Section

Materials and Preparations. Vitrogen 100 purified collagen was purchased from Celtrix Corporation, Santa Clara, CA. Vitrogen 100 is a sterile solution of purified, pepsin-solubilized bovine dermal collagen dissolved in 0.012 N HCl. It is 99.9% pure collagen and 95−98% type I collagen with the remainder comprised of type III collagen. The stock solution had a concentration of 3 mg/mL. Samples of 0.25 mg/mL solutions were prepared by diluting the stock solution with 0.012 N HCl prepared with doubly distilled water. Higher and lower concentration collagen solutions were also prepared and studied by DLS. The 0.25 mg/mL solution was the lowest concentration at which good signal-to-noise was obtained in the DLS time autocorrelation functions. Doubly distilled water was prepared by redistilling house-deionized water with a Corning Mega-Pure still.

The cells used for DLS studies were 10 mm path length square cells. An initial cleaning of the cells was done by rinsing each cell for a minimum of 24 h with house-deionized water. To clean and remove dust from the cells, each cell was rinsed for a minimum of 24 h with doubly distilled water cycled through a filtering setup. The water was cycled using a Manostat cassette pump through Gelman Sciences Acrodisc PF filters containing a 0.8 μm prefilter and a 0.2 μm Supor filter. The cells were inspected for dust by observing a focused 200 mW 488 nm wavelength laser beam in the cell under 5× magnification. If dust was seen, the cell was replaced in the filtering setup.

Once a cell was adequately cleaned, it was dried upside down in an oven. The collagen solution was then filtered directly into the clean, dry cell. Prior to filtration of the collagen solution, filters were rinsed with 200 mL of doubly distilled water and 100 mL of 0.012 N HCl prepared with doubly distilled water. Two different filtration methods were used. In the initial method the collagen solution was filtered through a tandem setup of three Gelman Sciences Acrodisc PF filters containing a 0.8 μm prefilter and a 0.2 μm Supor filter. To ensure that the 0.2 μm Supor filter did not cleave or damage the collagen monomer, an additional sample was prepared using two Gelman Sciences Supor Acrodisc 25 0.45 μm filters in tandem. Data from both samples were compared to ensure that the monomer size was equal with both filtration methods. Samples were stored refrigerated at 4 °C and were centrifuged overnight in a DuPont Instruments Sorvall RC-5B refrigerated superspeed centrifuge at 4000 rpm prior to DLS experiments. During DLS experiments, the sample temperature was maintained constant at 20.00 ± .05 °C using a VWR Scientific temperature control bath.

Data Collection and Analysis. The optics of the light-scattering apparatus were of standard design and have been described previously.[35] Experimental photon correlation functions were measured using a Brookhaven Model BI-9000AT digital correlator. Delay channels were ratio spaced from 2−40 000 μs for the lowest angle to 2−10 000 μs for the highest angles. The samples were studied at angles 33.00, 57.00, 62.59, 68.13, 73.62, 79.10, 84.55, 90.00, 95.45, 100.90, 106.38, and 123.00°. This angular range spans a decade in q^2. Only data with a base line percentage difference less than 0.10 were accepted. Samples were studied from 4 to 8 days with no appreciable increase in the base line percentage difference. If an increase in base line percentage difference was seen, the sample was recentrifuged overnight at 4000 rpm.

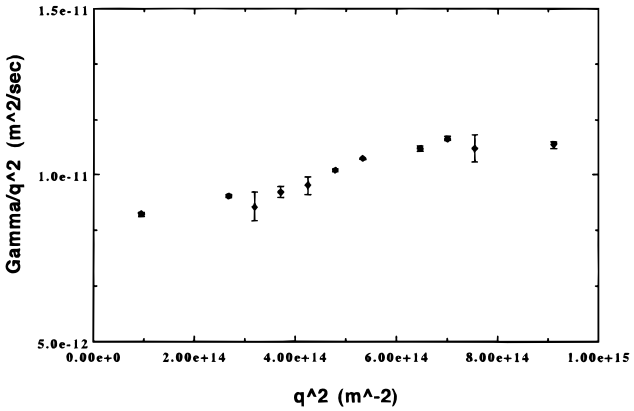

Figure 1. $\bar{\Gamma}/q^2$ (m^2/s) from second-order cumulants fit as a function of q^2 (m^{-2}).

Cumulants analysis was performed using software directly on the Brookhaven correlator. Data were fit up to the second cumulant according to eq 7. For CONTIN analysis, data were transferred for calculation on a VAXStation 3200 using VMS Kermit-32 Version 3.0.051. The program was run with output of scattered intensity as a function of hydrodynamic radius, R_h. R_h is defined in terms of decay time τ ($\tau = 1/\Gamma$), scattering vector length q, solvent viscosity η, temperature T, and Boltzmann's constant k_b as

$$R_h = q^2 \tau k_b T/(6\pi\eta) \tag{29}$$

and is the size of a sphere predicted by the Stokes−Einstein relation to translate with a diffusion coefficient corresponding to the relaxation time of the peak. R_h may be easily converted to find this "apparent" diffusion coefficient, D'

$$D' = k_b T/(6\pi\eta R_h) \tag{30}$$

Results

Cumulants analysis was performed in order to determine the translational diffusion coefficient, D. D may be estimated from a plot of $\bar{\Gamma}/q^2$ vs q^2 using eq 8. A plot of $\bar{\Gamma}/q^2$ vs q^2 is found in Figure 1. The value of D obtained from this plot using low-angle data is 8.4×10^{-12} m^2/s. This value was used to determine a hydrodynamic length for the collagen molecule using eqs 16 and 18. Broersma's relation gave a length of 2820 Å, and the Tirado and Garcia de la Torre relation gave a length of 2910 Å. In this work all calculations involving hydrodynamic length assumed the diameter of the molecule $d = 13.6$ Å. Table 2 shows the corresponding Θ and ΔD values found using eqs 17 and 21 (Broersma) for the hydrodynamic length 2820 Å. The Broersma relations were used to relate these quantities because the ratio L/d for the collagen molecule is ≅220, and the Tirado and Garcia de la Torre relations are predicted to be valid for L/d up to ≅30.

The program CONTIN was used to determine both the apparent R_h and the relative amplitude of peaks found in the frequency spectrum. Typical CONTIN output are shown in Figure 2 for two different scattering angles, 57° and 90°. CONTIN consistently showed a peak at the large R_h end of the window with a relative spectral amplitude of less than 10%.

TABLE 1: Peak Positions (nm) and Relative Amplitudes for the $l = 0$ and $l = 2$ Peaks[a]

scattering angle (deg)	$q \times 10^{-7}$ (m^{-1})	$l = 0$ R_h (nm)	relative amplitude	$l = 2$ R_h (nm)	relative amplitude
33.00	0.98	23.0 ± .8			
57.00	1.64	23.7 ± 1.4	0.93 ± 0.02	5.9 ± 1.1	0.07 ± 0.02
62.59	1.78	22.8 ± 1.3	0.90 ± 0.06	6.0 ± 1.2	0.10 ± 0.06
68.13	1.92	22.1 ± 1.7	0.83 ± 0.09	6.9 ± 1.1	0.17 ± 0.09
73.62	2.06	22.5 ± 2.3	0.87 ± 0.06	7.1 ± 1.3	0.13 ± 0.06
79.10	2.19	21.8 ± 1.9	0.84 ± 0.06	7.4 ± 1.3	0.16 ± 0.06
84.55	2.31	22.8 ± 2.6	0.84 ± 0.06	7.6 ± 1.2	0.16 ± 0.06
90.00	2.43	22.9 ± 3.2	0.77 ± 0.06	8.6 ± 1.1	0.23 ± 0.06
95.45	2.54	22.3 ± 2.7	0.79 ± 0.07	7.8 ± 1.2	0.21 ± 0.07
100.90	2.65	22.1 ± 2.4	0.79 ± 0.08	8.0 ± 1.6	0.21 ± 0.08
106.38	2.75	22.8 ± 3.2	0.76 ± 0.09	8.8 ± 1.4	0.24 ± 0.09
123.00	3.01	22.1 ± 1.9	0.71 ± 0.10	8.5 ± 1.2	0.29 ± 0.10
average all angles		22.6 ± 0.5			

[a] Error bars are the standard deviations from the average quantity determined at each angle.

TABLE 2: Results of Fits to the Dynamic Light-Scattering Data[a]

	D (m^2/s)	Θ (s^{-1})	L (Å)	ΔD (m^2/s)
D (cumulants)	$\mathbf{8.36 \times 10^{-12}}$	800	2820	4.5×10^{-12}
D (CONTIN)	$\mathbf{9.58 \times 10^{-12}}$	1270	2390	5.0×10^{-12}
	Fits to Decay Times			
fit to τ_o				
optically isotropic rod, no coupling (Broersma restricted, Pecora, 1968)	9.51×10^{-12}	1240	**2410**	5.0×10^{-12}
optically isotropic rod with coupling (Broersma restricted, Rallison and Leal, 1981)	9.64×10^{-12}	1300	**2370**	5.0×10^{-12}
fit to τ_2				
Pecora	1.03×10^{-11}	1630	**2180**	5.4 × 10−12
Rallsion and Leal	1.01×10^{-12}	1530	**2230**	5.3×10^{-12}
fit to both τ_o and τ_2	9.84×10^{-12}	1390	**2310**	5.1×10^{-12}
	Fit to D′ vs $1/q^2$			
τ_o (D) and τ_2 (Θ, L)				
D not included in fit	8.43×10^{-12}	**820**	2800	4.5×10^{-12}
D included	9.20×10^{-12}	**1110**	2510	4.8×10^{-12}
D constrained	1.02×10^{-11}	**1580**	2210	5.3×10^{-12}
	Fit to Amplitudes			
Pecora	9.48×10^{-12}	1220	**2420**	5.0×10^{-12}
Rallison and Leal	7.92×10^{-12}	660	**3020**	4.2×10^{-12}
	Predicted Values from Broersma's Relations			
$L = 3000$ Å, $d = 13.6$ Å	7.97×10^{-12}	670	3000	4.3×10^{-12}

[a] For each method, the quantity determined directly is in bold face. Other quantities were calculated using Broersma's relations.

This "slow mode" may be attributable to dust in the sample or to higher-order aggregates of monomers. An additional peak with a small amplitude was found at the small R_h end of the window and was considered to be an artifact. Similar small R_h artifacts have been found by other researchers with both experimental and simulated data.[36] This peak could be due to photomultiplier tube afterpulsing, internal motions, or an artifact of the data analysis.

For all but the lowest scattering angle two peaks were consistently found in the CONTIN analysis of the data. The position of the main peak found at all angles was independent of q. This peak may be attributed to translational diffusion of the molecule and the term corresponding to $l = 0$ in eq 9. For all but the lowest scattering angle a second peak dependent on q was found located at a smaller R_h than the dominant peak of the spectrum. This peak's R_h position and relative contribution to total intensity increased as q increased (see Figure 2), as would be expected for a mode corresponding to rotational and/or internal motions of the molecule. A significant contribution from this mode is not expected and was not seen at the lowest scattering angle. This peak may be attributed to the term corresponding to $l = 2$ in eq 9.

The results from the two samples studied were compared and found to be identical within the error of the method. The translational peak averaged over all angles was found to be 22.39 ± 0.70 nm for the sample filtered through 0.2 μm filters and 22.69 ± 1.00 nm for the sample filtered through 0.45 μm filters. The data from the two samples were averaged. The resulting decay times (in the form of R_h) and relative amplitude of each peak are found in Table 1. The translational diffusion coefficient determined from these data is 9.48×10^{-12} m^2/s. This corresponds to a hydrodynamic length of 2420 (Broersma) and 2590 Å (Tirado and Garcia de la Torre) using eqs 16 and 18.

Fits to the decay times τ_0 and τ_2 predicted by the Pecora and Rallison and Leal theories were performed using length as the adjustable parameter. Fits were first performed for τ_0 and τ_2 individually, and then both were weighted equally. The best fit to τ_0 was found for the Pecora theory for a length of 2410 Å. For the Rallison and Leal theory, error was minimized for a length of 2370 Å. Fits to τ_2 gave a length of 2180 Å for the Pecora theory and 2230 Å for the Rallison and Leal theory. The L dependencies of D, ΔD, and Θ were calculated using Broersma's relations and are shown in Table 2. For both theories, a length of 2310 Å gave the best fit to the data for both τ_0 and τ_2 . Figure 3 shows the q dependence of experimentally determined R_h values and for R_h values calculated for both theories using this length. Table 2 contains the corresponding values for D, ΔD, and Θ.

An alternative, direct method of obtaining the rotational diffusion coefficient involves the apparent diffusion coefficient

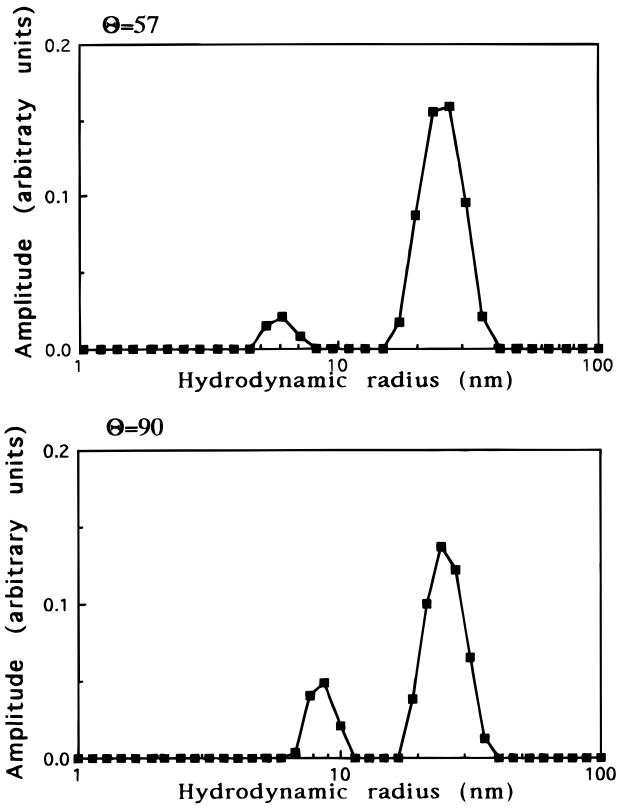

Figure 2. Typical CONTIN output shown for angles 57° and 90°. Amplitudes are displayed in arbitrary units as a function of hydrodynamic radius (nm).

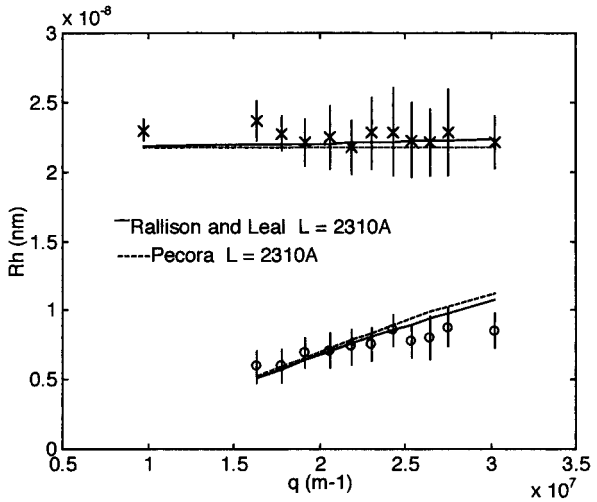

Figure 3. Experimental R_h values (nm) ($\times$ = slow decay, $\circ$ = fast decay) and best fit to theories (solid line = Rallison and Leal, L = 2310 Å; dotted line = Pecora, L = 2310 Å) as a function of q (m^{-1}).

D' for the second decay time, τ_2 . By examination of the form for τ_2 in eqs 12 and 23, it is seen that a plot of D' versus $1/q^2$ for both the Pecora and Rallison and Leal theories should give a straight line with a slope of 6Θ. A plot of these values is shown in Figure 4. A fit to the data gave a rotational diffusion coefficient Θ of 820 s^{-1}, which using Broersma's relations corresponds to L = 2800 Å as shown with D, ΔD, and Θ for this length in Table 2. According to the Pecora theory, the intercept of this line with the y axis should occur at the translational diffusion coefficient, D ($y = D + 6\Theta/q^2$). The above fit gave a value of 1.91×10^{-11} m^2/s for the intercept, a value nearly twice that found from averaging D obtained from τ_0 at each angle. The Rallison and Leal theory predicts a difference between the y intercept and D due to diffusive

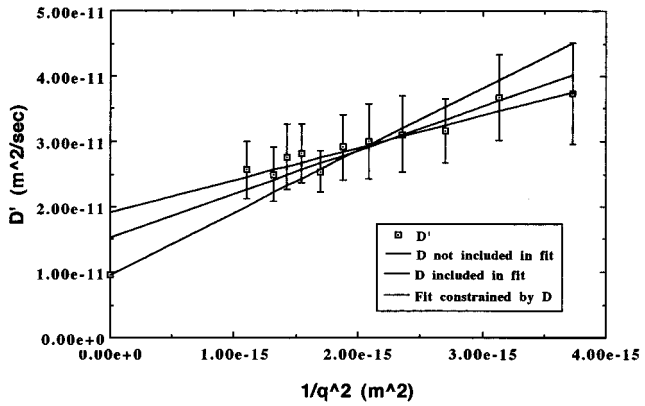

Figure 4. Fit to fast decay for D' (m^2/s) vs $1/q^2$ (m^2) (solid line = D not included in fit, larger dotted line = D included in fit, alternating dotted line = fit constrained by D).

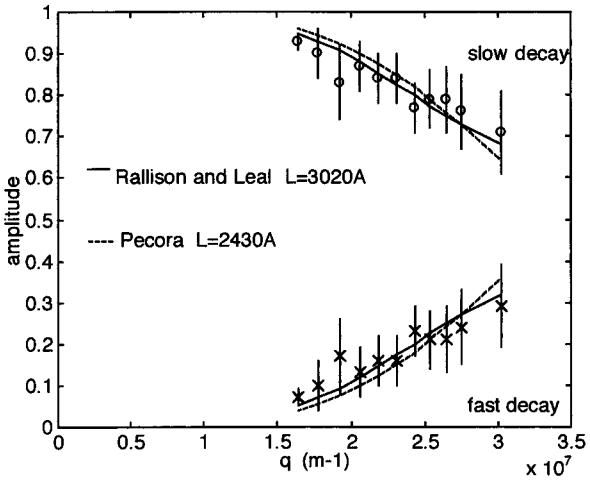

Figure 5. Experimental amplitudes ($\circ$ = slow decay, $\times$ = fast decay) and best fit to theories (solid line = Rallison and Leal, L = 3020 Å; dotted line = Pecora, L = 2430 Å) as a function of q (m^{-1}).

anisotropy ($y = D + 4\Delta D/21 + 6\Theta/q^2$). However, this difference is an order of magnitude larger than that predicted by their theory. If the value for D found using τ_0 is included as a point at $1/q^2 = 0$, the slope of the line from the resulting fit gave Θ = 1110 s^{-1}. Constraining the fit such that the line passes through the translational diffusion coefficient D yields Θ = 1580 s^{-1}. These fits are shown in Figure 4, and corresponding D, ΔD, and Θ for these fits are found in Table 2.

The relative amplitude of the peaks varied with q as would be expected from eqs 14, 15, 23, and 24. The relative amplitude of the $l = 0$ peak decreased as q increased, while the amplitude of the $l = 2$ peak increased with increasing q. The amplitude values are found in Table 1 and are shown graphically in Figure 5. A fit to these amplitudes was performed again using L as the adjustable parameter with D, ΔD, and Θ related to L using Broersma's relations. For the Pecora theory, error was minimized with L = 2420 Å, the same hydrodynamic length found using the average diffusion coefficient from CONTIN analysis. This corresponds to Θ = 1220 s^{-1} and $D = 9.48 \times 10^{-12}$ m^2/s as shown in Table 2. For the Rallison and Leal theory, error was minimized for L = 3020 Å, giving Θ = 660s^{-1} and $D = 7.92 \times 10^{-12}$ m^2/s.

A persistence length was determined using eqs 27 and 28. The value used for $\Theta_{measured}$ was that obtained from the technique resulting in the direct determination of Θ, the fit to D' vs $1/q^2$, with D included in the fit. This gave a value for the persistence length of 1600 Å using the Hagerman and Zimm

TABLE 3: Comparison of Experimental Values of the Translational Diffusion Coefficient *D*

	D (m^2/s)
reference	
Maeda et al.[11]	7.6×10^{-12}
Kubota et al.[10]	7.80×10^{-12}
Hwang and Cummins[8]	8.64×10^{-12}
Fletcher[6]	8.6×10^{-12}
present work	
contin analysis	9.5×10^{-12}
cumulants analysis	8.4×10^{-12}

TABLE 4: Comparison of Experimental Values of the Rotational Diffusion Coefficient Θ

	technique[a]	Θ (s^{-1})
reference		
Hwang and Cummins[8]	DLS (pol)	890
Thomas and Fletcher[7]	DLS (depol)	1080
Bernengo et al.[14]	EB	1040
Ananthanarayanan and Veis[13]	EB	810
Yoshioka and O'Konski[12]	EB	970
present work[b]		
D not included in fit		820
D included in fit		1110

[a] DLS (pol) = polarized dynamic light scattering. DLS (depol) = depolarized dynamic light scattering. EB = electric birefringence. [b] Results from fit to D' vs $1/q^2$ (see text).

model and a value of 1650 Å using the Yoshizaki and Yamakawa theory. These values for the persistence length were used along with a diameter $d = 13.6$ Å and the translational diffusion coefficient found from the CONTIN analysis (9.48×10^{-12} m^2 s^{-1}) in the Yamakawa−Fujii[34] relation for the translational diffusion coefficient of a wormlike chain to determine the rod contour length. The value for the contour length obtained by this procedure is ∼2600 Å.

Discussion

Table 3 shows experimentally determined values of the translational diffusion coefficient D from both previous and present work. In this work, the value determined for D using the program CONTIN is larger than that found using the method of cumulants and larger than those found in the previous studies as shown. The method of cumulants may result in an erroneous value for D due to the inability of the method to account for dust or higher-order aggregates of collagen. A "slow mode" was consistently found in the CONTIN analysis. The average decay, $\bar{\Gamma}$, would incorrectly include contributions from this slow mode, resulting in an experimentally determined D smaller than the true value. The value of D found in this work from cumulants analysis is within experimental error (∼10%) of the values determined by previous researchers. Prior work may also show a contribution of dust or aggregates in the determined D value. It should also be noted that two of the studies cited[10,11] involved systems of lathyritic rat skin collagen rather than enzyme-treated collagen. The alternative method of preparation may result in a system having slightly different properties, as may different pH conditions, although the monomer length should essentially be identical. In particular, aggregation may be more likely in the pepsin-treated system.

Table 4 contains a comparison of values of the rotational diffusion coefficient, Θ, experimentally determined using a variety of techniques as shown. Values shown from this work were determined from the fit to D' versus $1/q^2$, since this provided a direct measurement of the value of Θ. These values are in agreement with previous studies as shown. Table 2 contains values of Θ related from other hydrodynamic values using the Broersma relations assuming rigid rod behavior. These values are in excellent agreement, given the large experimental uncertainty in the determination of Θ.

The methods of data analysis used in this work determined a number of different hydrodynamic properties of the system. Table 2 displays the relationship between these quantities using the Broersma relations assuming rigid rod behavior. The agreement of the values obtained from these different methods of data analysis indicates that the theories for rigid rods seem to be applicable to this system. The Pecora and Rallison and Leal theories are in very good agreement when predicting a length from decay times. When the amplitude data are fitted, the Rallison and Leal theory finds a value for the length larger than that found with the Pecora theory. The length found with the Pecora theory is identical with that found using the average diffusion coefficient from CONTIN analysis.

Broersma's theory predicts that a rod with length 3000 Å and diameter 13.6 Å will have $\Theta = 670$ s^{-1} and $D = 7.97 \times 10^{-12}$ m^2/s as shown in Table 2. Experimental results show that collagen behaves dynamically as a rod shorter than 3000 Å. This is expected, since collagen is not a perfectly rigid rod, and the flexibility of the rod will cause it to appear shorter hydrodynamically.

For both the Pecora and Rallison and Leal theories, fits to the second decay time τ_2 yielded a slightly shorter contour length than other fitting methods. Bending and flexing motions in the rod may contribute hydrodynamically as an increase in the rotational diffusion coefficient. This would result in the rod appearing shorter when applying theories of rigid rod rotation to this system. Since τ_2 has a greater dependence on the motions seen as Θ than τ_0, a shorter contour length is found with methods dependent on τ_2 than found using τ_0. This effect of flexibility on rotational diffusion was used to determine the persistence length P, using both the Hagerman and Zimm and the Yoshizaki and Yamakawa models. These theories gave values for the persistence length P of 1600 and 1650 Å, respectively. Both values are in excellent agreement with those found by previous researchers[2−4] and with values determined using the Yamakawa−Fujii[34] relation for the translational diffusion coefficient of a wormlike chain.

Conclusion

Dynamic light scattering has been used to study the translational and rotational diffusion and the translational−rotational coupling of collagen in dilute solution. The agreement between the hydrodynamic quantities determined using a variety of data analysis techniques indicates that DLS is an excellent tool for such studies. CONTIN appears to be especially well suited for data analysis in systems of this type. The cumulants method may give erroneous values for the diffusion coefficients, since it also averages in dust or aggregates. The assumption of rigid rodlike behavior provides a self-consistent framework for relating the quantities L, D, and Θ using Broersma's relations. These methods should be applicable to other rodlike macromolecules of comparable stiffness in dilute solution. They may also serve as a reference for DLS experiments on collagen in more concentrated solutions where intermolecular interactions become very important.[37] The solution studied here corresponds to a concentration of approximately 12 rods in a cubical volume L^3. The relation between the DLS time correlation function measured in more concentrated solutions and the self diffusion coefficient (D), the translational anisotropy (ΔD), and the single molecule rotational diffusion coefficient (Θ) deserves further study.[37−39] DLS studies of collagen at near neutral pH values

where dimers and higher aggregates form would also be valuable for elucidating the initial steps in collagen self-assembly into fibers.

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