Coil–Globule Transition of Poly(*N*-isopropylacrylamide): A Study of Segment Mobility by Fluorescence Depolarization

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ABSTRACT: Time-resolved measurements of fluorescence polarization anisotropy were used to monitor the "freezing" of local motions due to the temperature-induced conformational transition of poly(*N*-isopropylacrylamide) in aqueous solutions. The label dye dansyl was covalently bound to polymers containing aminostyrene which were synthesized by radical polymerization. The reorientational relaxation of the labels is anisotropic and can be resolved into two components. These two relaxation modes are interpreted in terms of a simple model as (i) rotations of the label around a free axis (this mode is sensitive to microviscosity) and (ii) polymer-coupled reorientations reflecting the chain flexibility. Both relaxation times diverge above a transition temperature of 31 °C. Our findings confirm the picture of a conformational transition from extended coils to a compact globular state of the polymer.

1. Introduction

The properties of macromolecular solutions may exhibit a remarkably strong dependence on temperature. An example well-known from everyday experience is the denaturation of protein solutions upon heating, where the temperature-induced conformational changes result in an abrupt loss of biological activity. To study the fundamental microphysical backgrounds of such phenomena simple model systems are needed and helpful. A model system of increasing popularity is poly(*N*-isopropylacrylamide) [poly(NIPAM)] in water: Aqueous solutions of poly(NIPAM) show a lower critical solution temperature; i.e., when heated above a critical temperature T_c (values between 31 and 35 °C are reported), they turn opaque.[1-3] Apparently the solvent becomes poor at higher temperatures and aggregation of the polymer molecules takes place, producing large particles and strong scattering. Another manifestation of this temperature-induced transition of poly(NIPAM) can be observed when the polymer is cross-linked into a network.[4,5] Below T_c the resulting gels are swollen but they deswell reversibly, expelling most of the solvent when the temperature is raised. Apparently the increase of temperature induces substantial changes of the conformation of the polymer. Such conformational changes have been demonstrated in fluorescence studies on fluorescently labeled poly(NIPAM): The transition is accompanied by a strong decrease of pyrene excimer fluorescence,[6] and the shrinking of the coils is indicated by a strong enhancement of the nonradiative energy transfer between the donor naphthalene and the acceptor pyrene bound on doubly labeled polymers.[7] Previous light scattering studies[8,9] have yielded evidence that in dilute solutions the conformational change may be a coil–globule transition, but the quantitative interpretation of such measurements has been difficult because of the aggregation of the polymer. In a recent work we have been able to prevent the aggregation by the addition of a very small amount of a surfactant and study thus the conformational changes of isolated poly(NIPAM) molecules by both static and dynamic light scattering:[10,11] Below T_c the polymer molecules are dissolved as extended coils (radius of gyration R_g = 135 nm for a molecular weight $M = 7 \times 10^6$) and above T_c they collapse into small compact globules (R_g = 16 nm). The aim of the present work is to investigate the "details" of the transition, i.e., the temperature-induced changes of local mobility on the length scale of a few polymer segments: We have prepared fluorescently labeled derivatives of poly(NIPAM) and measured the Brownian (spontaneous) reorientational dynamics of the fluorescent label groups by means of time-resolved fluorescence depolarization.

2. Synthesis and Characterization

To prepare fluorescent poly(NIPAM) derivatives we have first copolymerized *N*-isopropylacrylamide (NIPAM) with *p*-aminostyrene (AS). The NH_2 group of AS is an active site for the binding of fluorescing dyes containing isothiocyanate or sulfonyl chloride groups. The radical polymerization was carried out in methanol using α,α'-azobisisobutyronitrile (AIBN) as initiator (the alcoholic reaction medium was necessary to dissolve AS, but it appears to limit the obtainable molecular weight of the polymer). The concentration of NIPAM was 100 mg/L, the NIPAM/AS molar ratio amounted to 500:1, and the NIPAM/AIBN ratio was 25:1. The reaction solution was held for ca. 10 h under argon at 40 °C and stirred. The polymerization was stopped by precipitation with diethyl ether. The product was prepurified by repeated dissolution in methanol and precipitation with diethyl ether and finally dried in vacuo. In a second step, the copolymer was covalently labeled with the dye dansyl (in the form of dansyl-Cl, i.e., 1-(dimethylamino)naphthalene-5-sulfonyl chloride). Methanolic solutions of 15 g/L of copolymer and 0.45 g/L of dansyl-Cl (corresponding to 5 dansyl per AS) were held at room temperature for 6 days. Again the labeled copolymer was precipitated by adding diethyl ether and dried, thereby separating free dansyl.

The final purification was carried out by HPLC with controlled pore glass (CPG-10 Polyol from Electro-Nucleonics, pore size 100 nm) as matrix and methanol as the mobile phase. A fraction corresponding to the main (first) peak of the fluorescence as well as the refractometer signal was taken as the desired product.

Because of the fluorescence of the sample, the average molecular weight of this fraction was not measured by static light scattering but estimated by viscosimetry, using the established Mark–Houwink relation[12] for poly(NIPAM) in methanol. It amounts to $M = 3.5 \times 10^5$, corresponding to approximately 3100 monomers. Quasielastic light scattering was used to measure the hydrodynamic radius R_h = 17.5 nm in aqueous solutions at 25 °C. This is almost the same as the value we found for the unlabeled copolymer (17.8 nm), indicating that the binding of dye did not induce important structural changes of the chains.

The samples for fluorescence investigations were prepared by redissolving the polymer to a concentration of 1 g/L in water containing 0.1 M $NaHCO_3$ and 0.012 M NaN_3. The $NaHCO_3$ content ensures complete deprotonation of the nitrogen site of dansyl, NaN_3 prevents bacterial growth, and the high ionic strength screens any coulombic interactions of possible residual

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charged groups. Since we are here interested in the local aspects of the transition which are presumably not affected by aggregation, the solutions do not contain surfactant. Prior to the measurements the samples were filtered through a 0.22-μm filter.

The degree of labeling was estimated at 2.6×10^{-4} dansyl labels per monomer (i.e., 1 dye/polymer) by comparing the fluorescence intensity of the labeled polymer with the intensity of a reference solution of the free dye (the water-soluble acid dansyl-OH). We have assumed that the quantum yield of the dye is not affected by the labeling as there are no significant differences between the fluorescence spectra of the free dye and of the bound labels, and their fluorescence lifetimes differ only slightly. We have chosen to use fluorescence because the degree of labeling is low and the UV absorption band of dansyl is masked by the absorption of the coupler aminostyrene and also by the extinction due to the absorption and scattering of the polymer. The upper limit for the degree of labeling is given by the aminostyrene content of the polymer to 0.0026. In any case we can safely neglect the influence of dansyl–dansyl nonradiative energy transfer on the fluorescence polarization: The Förster radius estimated from spectral overlap is rather large (0.8 nm), and there would be only 0.008 dansyl molecules within one Förster sphere even at the upper limit of labeling in bulk polymer.

As a final check of the success of the preparation procedure, we have measured the light scattering of the solutions (at an angle of 90°) versus temperature. The intensity showed a sharp increase between 30.8 and 31.4 °C. The labeling thus does not essentially affect the transition; there is only a shift of transition temperature with respect to the homopolymer.[10,11]

3. Method

The method of time-resolved fluorescence depolarization measurements is described in detail by O'Connor and Phillips.[13] The polarized and the depolarized components $I_P(t)$ and $I_D(t)$ of the time course of the fluorescence following the excitation of the labels by a narrow pulse of light (polarized in the z direction) are recorded with single photon counting. From these two components one gets the sum $S(t) = I_P(t) + 2I_D(t)$, which corresponds to the unpolarized ("natural") fluorescence, and the (frequently rather small) difference $D(t) = I_P(t) - I_D(t)$, which is related to the product of the unpolarized fluorescence and the time-dependent anisotropy $r(t)$. Both $S(t)$ and $D(t)$ are convolutions with the response function of the given apparatus.

The anisotropy $r(t)$ can be shown to be a reorientational correlation function of the absorption and emission transition moments of the labels

$$r(t) = 3\langle A_z^2(0)\,(B_z^2(t) - B_y^2(t))\rangle \qquad (1)$$

where **A** and **B** are unit vectors in the direction of these moments. The interpretation of measurements of the time-resolved anisotropy depends on the model chosen for the reorientational motions of the labels. The most simple behavior, often observed for the rotational diffusion of free dye molecules like dansyl, consists in isotropic reorientations; i.e., the molecules behave like spheres and the anisotropy takes the form of a single exponential, $r(t) = r_0 \exp(-6D_{\text{rot}}t)$ containing only one dynamical parameter, the rotational diffusion constant D_{rot}. The radius of the equivalent sphere may be obtained from the rotational Einstein relation $D_{\text{rot}} = kT/8\pi\eta a^3$. For nonspherical freely rotating rigid bodies there are in general three principal diffusion constants and one has to expect up to five exponentials[14] in $r(t)$, whereas the anisotropy of flexible polymers may exhibit a complex spectrum of relaxation times.[15] The usefulness of the fluorescence depolarization technique for polymer conformational transitions has been demonstrated in a number of publications (e.g., refs 16 and 17 and references therein) concerned mainly with polyelectrolytes such as poly(methacrylic acid) and more recently also by careful time-resolved measurements.[18,19] However, these studies reveal also the complexity of the problem, resorting throughout to semiempirical interpretations of the measured relaxation times. We shall return to the question of a quantitative interpretation in section 6.

4. Experimental Method

The apparatus for polarized time-correlated photon counting used in this work is described elsewhere.[20] The excitation light source was a free-running spark gap operated in air at atmospheric pressure at a frequency of ca. 10 kHz and illuminating the sample through an interference filter selecting the excitation wavelength of 337 nm. To eliminate the effects of drifts and instabilities, the accumulation of data is organized in cycles of typically 10 s, each including three measurements of I_P, I_D, and I_R, respectively, where I_R is the fluorescence intensity produced by a reference sample adequate to use relative deconvolution[21] in the subsequent evaluation (we used fluorescein as reference in this investigation). The direction of the polarizer for the exciting light is changed to switch between I_P and I_D, and an auxiliary photon counter is used to normalize I_P and I_D.

The samples scatter strongly above T_c and care must be taken to minimize the contribution of scattering to the detected light: In addition to the use of double blocking filters (Schott KV470 and KV480), the fluorescence profiles were corrected by subtraction of blanks obtained from measurements of unlabeled copolymer. The detection efficiencies were typically 2%, and a complete measurement at one temperature took about 6 h to obtain sufficient counting statistics. The evaluation of the parameters was done by standard nonlinear least-squares procedures, working with properly normalized functions $S(t)$ and $D(t)$ and using model functions comprising the sum of two exponentials for both.

5. Results

Before entering the discussion of labeled poly(NIPAM), we briefly summarize some results obtained with the free dye (which are needed to verify if the polarization anisotropy of the label indeed reflects the local dynamics of the polymer chain). The fluorescence decay of free dansyl is a single exponential (at the given pH); the decay time does not depend on temperature (25–35 °C) and amounts to $\tau_f = 13.0 \pm 0.1$ ns. The rotation of the free dye in aqueous solutions is too fast to be measurable with the present setup. Therefore we have measured the anisotropy relaxation of the free dye in water–glycerol mixtures. The relaxation was found to be sphere-like; i.e., the anisotropy is an exponential $r(t) = r_0 \exp(-k_r t) = r_0 \exp(-6D_{\text{rot}}t)$. The reduced relaxation rate

$$\mu = k_r(\eta/kT) = 1/V_{\text{sph}} \qquad (2)$$

(where T is the absolute temperature, k is the Boltzmann constant, and η is the viscosity of the solvent) does not depend on temperature. Recalling the rotational Stokes–Einstein relation, we can interpret $1/\mu$ as the volume of the equivalent sphere V_{sph}. The radius a of the equivalent sphere amounts to 0.42 nm for dansyl-OH, whereas the derivative dansylaminostyrene yields a slightly larger value (0.46 nm).

The most important results of the time-dependent depolarization measurements on the labeled copolymer are compiled in Figures 1–3. Figure 1 is an example of the raw data and their least-squares fits as obtained at 27 °C. The sum $S(t)$ (Figure 1a) is almost a single-exponential

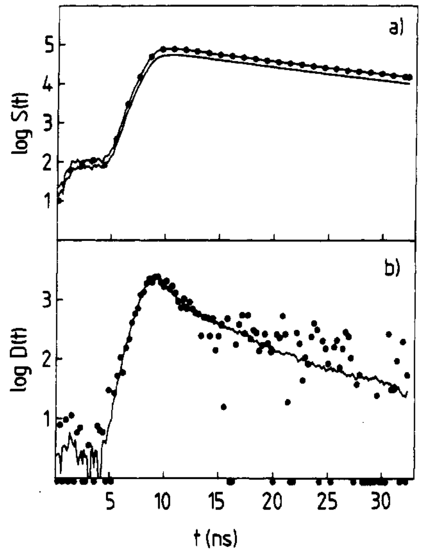

Figure 1. Sum and difference of time-dependent, polarized components of fluorescence at 27 °C (semilogarithmic): (a) (●) the measured unpolarized fluorescence decay $S(t)$, (—) fit with two exponentials and (shifted) fit with a single exponential; (b) (●) the measured difference $D(t) = I_P - I_D$, (—) fit with two exponentials. Note that $D(t)$ is a difference of two photocount distributions and therefore contains negative values; these are indicated by points below the picture frame.

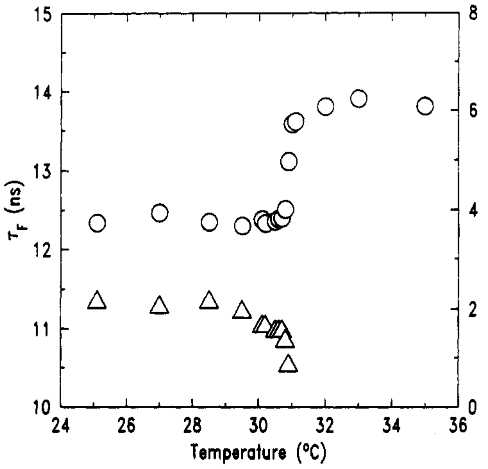

Figure 2. Temperature-dependent parameters of unpolarized fluorescence. (Left, ○) The decay time τ_f of the slow component increases sharply at the transition. (Right, △) The fraction α of the fast component which has an almost constant decay time of 1.1 ns drops from 2% to zero at the transition.

function; i.e., the unpolarized fluorescence exhibits a major component with a decay time of 12.4 ns, and it contains only approximately 2% of a fast decay (1.3 ns). As can be seen in Figure 1a the difference between the single- and double-exponential fits is hardly perceivable. Such slight nonexponentialities are frequently observed with labeled polymers.[22] The percentage of the fast component diminishes to 1% between 30.0 and 30.9 °C and then completely disappears, whereas the decay time of the slow component increases to 13.8 ns at temperatures higher than 30.9 °C (see Figure 2).

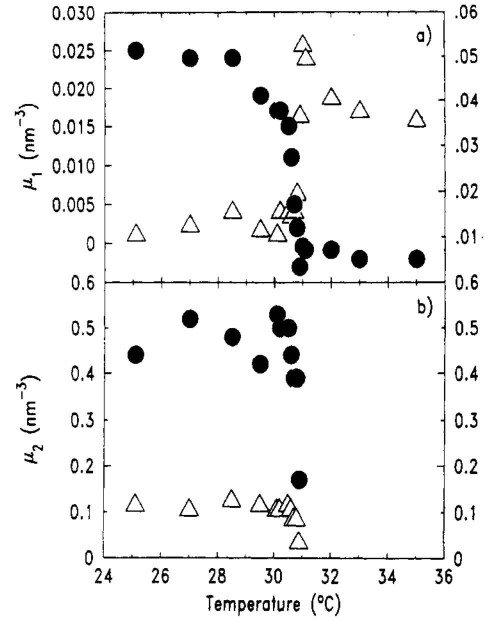

Figure 3. Reduced reorientational relaxation rates μ_i and their amplitudes β_i versus temperature: (a) (left, ●) μ_1, (right, △) β_1 (the reorientational mode reflecting backbone motions); (b) (left, ●) μ_2, (right, △) β_2 (the reorientational mode reflecting mainly label motions).

Table I

T, °C	k_{r1}, ns^{-1}	k_{r2}, ns^{-1}	β_1	β_2	a, nm	p, nm
25.1	0.11 (1)	2.0 (1)	0.011 (3)	0.12 (1)	0.72	3.28
27.0	0.11 (1)	2.5 (1)	0.013 (4)	0.11 (1)	0.68	3.49
28.5	0.11 (1)	2.2 (1)	0.016 (6)	0.13 (1)	0.70	3.42
29.5	0.091 (8)	2.1 (1)	0.012 (4)	0.12 (1)	0.73	3.86
30.1	0.083 (7)	2.8 (1)	0.011 (4)	0.11 (1)	0.67	4.36
30.2	0.083 (7)	2.6 (2)	0.016 (5)	0.11 (1)	0.69	4.28
30.5	0.08 (1)	2.6 (1)	0.016 (5)	0.12 (1)	0.69	4.62
30.6	0.06 (1)	2.2 (1)	0.015 (4)	0.11 (1)	0.71	5.45
30.7	0.03 (2)	2.0 (4)	0.016 (3)	0.09 (1)	0.74	8.35
30.8	0.008 (7)	2.0 (4)	0.020 (2)	0.09 (1)	0.74	13.74
30.9	−0.017 (4)	0.9 (2)	0.037 (2)	0.04 (1)	0.98	∞
31.0	−0.002 (2)		0.053 (1)			
31.1	−0.004 (2)		0.050 (2)			
32.0	−0.005 (4)		0.041 (1)			
33.0	−0.009 (5)		0.038 (2)			
35.0	−0.009 (5)		0.036 (1)			

The anisotropy of fluorescence was found to be rather low, especially at temperatures below T_c. For the example in Figure 1b the time average $\bar{r}$ of $r(t)$ amounts to only 0.01, thus leading to a corresponding scatter of data points. The low value of $\bar{r}$ is due to the rather low initial anisotropy $r(0)$. Nevertheless the quality of the data is sufficient to resolve two exponentials in the difference $D(t)$; in fact, unlike the evaluation of $S(t)$, two exponentials are necessary to obtain a satisfactory fit. The parameters of the fits with

$$r(t) = \beta_1 \exp(-k_{r1}t) + \beta_2 \exp(-k_{r2}t) \tag{3}$$

are shown in Table I. In their evaluation we have neglected the slight nonexponentiality of $S(t)$. The reduced values $\mu_i = (\eta/kT)k_{ri}$ of the reorientational relaxation rates k_{ri} are plotted as a function of temperature in Figure 3. There is a sharp decrease of the reorientational relaxation rates at the transition; i.e., we observe "freezing" of the label motion. The overall rotation of the collapsed (and aggregated) polymers is too slow to affect the anisotropy. This finding clearly supports our view[10] of the transition as a collapse from the coil state of the polymer to a densely packed state no longer allowing individual movements of the labels. The negative values of the reorientational relaxation rate k_{r1} above 31 °C (evaluated with a single exponential) may indicate the limiting systematic error appearing when movements are virtually absent, but we would not exclude a repolarization effect as an alternative explanation.

6. Interpretation

Our description of time-dependent polarization anisotropy in terms of two exponentials is so far purely empirical and it is desirable to explore its physical grounds. The complexity of theoretical expressions proposed for time-dependent fluorescence depolarization of flexible polymers indicates the complexity of the problem (see for example Yoshizaki[15] and references therein). Most of the theories assume that the label is incorporated into the chain; i.e., the label is treated as one of the polymer segments. In our case, however, the label is a side group. An inspection of a space-filling model (Figure 4) shows that the fluorescent group is capable of reorientational motions independent of the motions of the chain backbone, and this fact complicates the analysis even more. A formalism appropriate for this case is outlined by Viovy et al.,[23] but no simple closed expression is available.

To simplify the analysis we adopt the following model already proven useful[24,25] for the interpretation of polymer label systems: In the coil state (below the transition), due to the flexibility of the chain, the label does not "feel" more than a few of the polymer segments. Therefore, one can forget about the polymer being a coil but rather represent it by a large, almost immobile sphere. The coupling of the label to this "blob" is mediated by a chain segment consisting of a few monomers. The length of such a dynamic equivalent segment reflects the flexibility of the polymer. In ref 24 and 25 we have shown experimentally that it correlates with the static persistence length of the polymer.

To parametrize this picture we simplify it further: The label is represented by a sphere. Its radius a should roughly correspond to the rotational hydrodynamic radius of the free dye (see beginning of section 5 for experimental results). The sphere is attached to the polymer molecule by means of a lever of length $d + p$, where d corresponds to the distance between the center of the photophysical active part of the label (i.e., the naphthalene structure of dansyl) and the joint of the label to the polymer chain and p is the dynamic persistence length. The reorientational diffusion of the label in such a structure is characterized by two diffusion constants, namely, D_3 for rotational motions around the lever axis

$$D_3 = kT/8\pi\eta a^3 \quad (4)$$

and $D_1 = D_2$ (for motions perpendicular to the lever axis) which can be related to the parameters $p + d$ and a by use of the lever rule and the translational Stokes–Einstein relation for spheres:

$$D_1 = D_2 = \frac{kT}{(p + d)^2(6\pi\eta a)} \quad (5)$$

Note that the Einstein type of behavior, $D \sim kT/\eta$, is assumed: Since the label is rather bulky and p is comprised of many monomers, we disregard the internal friction (i.e., the isomerization barriers and their activation energies[26,27]) of the polymer backbone. For a stiff polymer such as poly(NIPAM) we expect $p + d > a$ and therefore $D_1 \ll D_3$. Within this simplified model the reorientational diffusion is formally as simple as the diffusion of an ellipsoid of revolution. Correspondingly the anisotropy $r(t)$ can be expressed in terms of three exponentials, their contributions depending on the orientation of the absorption and emission dipoles with respect to the principal axes (1, 2, 3) of the diffusion tensor. The number of exponentials reduces further to two when one of the transition moments is aligned with one of the diffusion axes. There are indications that such fortunate simplification applies at least approximately to our system: Though there are no reliable data on the orientation of the transition moments of dansyl, it appears reasonable to assume that one of them is parallel to the long axis of the naphthalene group (such as would be the case for the main absorption dipole of naphthalene), as indicated in the schematic drawing in Figure 4. Looking now at a space-filling model of the copolymer label structure, we see that steric hindering allows only label rotations around an axis perpendicular to the long axis of the naphthalene. We denote this rotation axis by 3 and associate it with the "fast" diffusion constant D_3. In other words, the long axis of the naphthalene group and thus one of the transition moments coincides indeed with one of the remaining axes (1 or 2) of the diffusion tensor. In Figure 4 we have chosen somewhat arbitrarily the emission dipole **B** to be aligned with axis 2 but we could as well align the dipole **A** without changing the final result. Introducing the angles θ and ϵ as defined in Figure 4, the anisotropy $r(t)$ can be calculated (e.g., from eq 17 of ref 14) as

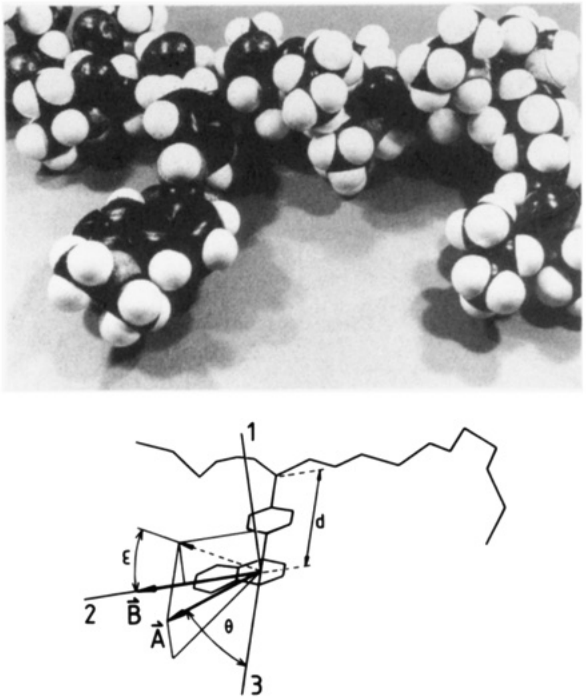

Figure 4. Model of the polymer label configuration: upper half, space-filling model of a short part of the polymer with one label; lower half, geometric definitions of the molecule-fixed coordinate system (1, 2, 3), of transition moments **A** and **B**, and of angles ϵ and θ.

$$r(t) = -0.1(3\cos^2\theta - 1)\exp(-6D_1t) + 0.3(\cos(2\epsilon)\sin^2\theta)\exp((-2D_1 + 4D_3)t) \quad (6)$$

We can now identify the parameters β_1, β_2, k_{r1}, and k_{r2} (eq 3) of the empirical evaluation of the data: First the parameters β_1 and β_2 can be expressed in terms of the angles ϵ and θ

$$\beta_1 = -0.1(3\cos^2\theta - 1) \qquad \beta_2 = 0.3(\cos(2\epsilon)\sin^2\theta) \quad (7)$$

From the measured values of β_1 and β_2 (Table I) we obtain almost temperature-independent values of $\theta = 58°$ and $\epsilon = 28°$. These values account for the rather low average anisotropy $\bar{r}$. Second, the relaxation rates k_{r1} and k_{r2} can be expressed in terms of the diffusion coefficients from eqs 4 and 5:

$$k_{r1} = 6D_1 = \frac{kT}{\eta}\frac{1}{(p + d)^2\pi a} \quad (8)$$

$$k_{r2} = 2D_1 + 4D_3 = \frac{kT}{\eta}\left(\frac{1}{(p + d)^2(3\pi a)} + \frac{1}{2\pi a^3}\right) \quad (9)$$

So we see two distinct modes of Brownian reorientation:

The slower relaxation k_{r1} reflects the local flexibility of the polymer backbone and depends on the dynamic persistence length p, whereas the faster relaxation k_{r2} is mainly determined by the free rotation of the label around the axis 3, provided $D_1 \ll D_3$. In Figure 3 we have plotted the reduced relaxation rates μ_1 and μ_2 (see definition 2)

$$\mu_1 = \frac{1}{\pi a(p + d)^2} \tag{10}$$

$$\mu_2 = (1/3)\mu_1 + (2/3)\frac{1}{(4\pi/3)a^3} \tag{11}$$

which do not depend on the temperature but only on the geometry of the model. Note that μ_2 is composed of one-third of the reduced relaxation of the lever arm and two-thirds of the free-label relaxation ($1/\mu_{\text{label}} = 4\pi a^3/3$). Using d = 0.9 nm measured directly on the space-filling model (see Figure 4), we can calculate a and p. The results are given in the last two columns of Table I.

The radius a of the label equivalent sphere is almost temperature independent but it increases at a critical temperature T_c = 30.9 °C. Below T_c its value of 0.7 nm is larger than 0.45 nm from measurements on free dyes. We attribute this difference to the short-range hydrodynamic interactions between the label and the part of the polymer chain in its neighborhood. The increase of the equivalent radius of the label at 30.9 °C coincides with the sharp increase of the fluorescence decay time at this temperature. Both effects can be understood if we think of the shrinking polymer extruding solvent at the transition and thereby decreasing the polarity[6] and increasing the microviscosity of the label environment.

Turning now to the dynamic persistence length p, we observe a gradual increase beginning already before T_c at approximately 30 °C; at T_c it diverges. We can interpret this behavior qualitatively in terms of gradual stiffening of the polymer backbone. This stiffening apparently sets in already before the label senses the direct hydrodynamic interactions due to the polymer shrinking. We thus corroborate the results of Winnik[7] indicating a continuous increase of the transfer efficiency (doubly labeled polymers) between 15 and 30 °C. We have observed such pretransitional conformational changes also in light scattering and viscosimetry measurements on poly(NIPAM) in pure water, and these changes manifest themselves in a gradual shrinking of the polymer molecules, too. Before the onset of the hypothetical stiffening, p amounts to ca. 4 nm (see Table I). We take this value to support the quantitative applicability of our simple model: In our previous work on fluorescently labeled polymers[24,25] we found the value of p to be in good agreement with the length of the Kuhn equivalent segment p', which is a measure of the static persistence of the polymer chain and is defined as

$$p' = 6R_g^2/N_0 l_0 \tag{12}$$

where $N_0 l_0$ is the contour length and R_g is the radius of gyration. Using the value of R_g/R_h = 1.35 found by Meewes[11] and Kubota,[28] we obtain p' = 4.4 nm from our measurement of R_h = 17.5 nm (section 2). The static and dynamic persistence lengths measured below the transition point are thus once again in good agreement, and we regard this finding as a confirmation of the applicability of our model.

We conclude: Using time-dependent fluorescence depolarization, we have monitored local microscopic features of the temperature-induced conformational transition of poly(N-isopropylacrylamide). Within a simple but quantitatively interpretable model we have been able to resolve the anisotropic rotational relaxation of the label dye into two components. One of them reflects the rotation of the label around a free axis and senses the microviscosity of the label environment whereas the other one is coupled to the polymer backbone and provides thus a measure of the chain flexibility. Both modes do "freeze" above the transition temperature. Our findings support the picture of the conformational transition as a transition from the extended coil to a compact globular state while suggesting that the dynamic flexibility of the polymer is affected already before the transition temperature is reached.

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