Polymer Vol. 38 No. 2, pp. 483–486, 1997
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0032-3861/97/$17.00 + 0.00

S0032-3861(96)00636-2

polymer communications

Time-resolved fluorescence anisotropy studies of the temperature-induced intramolecular conformational transition of poly(*N*-isopropylacrylamide) in dilute aqueous solution

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(Revised 4 June 1996)

Time-resolved fluorescence anisotropy measurements have been performed upon an acenaphthylene-labelled (0.5 mol%) sample of poly(*N*-isopropylacrylamide), PNIPAM, in dilute solutions in both methanol and water as solvents. In methanol, segmental relaxation of PNIPAM follows an Arrhenius dependence upon temperature over the range investigated (279–323K) characterized by an 'activation energy' of 13.4(±0.5) kJ mol^{-1}. This is only slightly greater (by *ca* 2.4 kJ mol^{-1}) than that of solvent flow and it is likely that specific dipolar interactions between the PNIPAM and the methanol determine the macromolecular dynamics in this solvent. In aqueous solution, the segmental mobility of PNIPAM exhibits a dramatic thermoreversible discontinuity at *ca* 32°C. This change in conformational behaviour occurs at the polymer's lower critical solution temperature in aqueous media. This observation, supports the proposition (Winnik, F. M. *Polymer* 1990, **31,** 2125) that the thermally-induced separation in this system occurs by a 'dual mode' mechanism wherein intermolecular aggregation is preceded by intramolecular conformational shrinkage of the polymer coils. Copyright © 1996 Elsevier Science Ltd.

(Keywords: *N*-isopropylacrylamide; fluorescence; anisotropy)

Introduction

Poly(*N*-isopropylacrylamide), PNIPAM, exhibits thermoreversible phase-separation behaviour in aqueous solution[1]: the PNIPAM/water system is characterized by a lower critical solution temperature (LCST) of 31–32°C[1,2].

LCST behaviour is regarded as an unusual phenomenon in low molar mass media. It requires the existence of specific interactions between the solute and the solvent which result in *both* a negative enthalpy change, ΔH_m, upon mixing and a negative entropy change, ΔS_m. In addition, the relative magnitudes of ΔH_m and ΔS_m must be such as to reverse the sign of the free energy change, ΔG_m, for the mixing process, within the range of temperatures over which the solvent remains a liquid. In low molar mass media, the nicotine/water[3] system is regarded as a classic example of a phase-separating combination showing LCST behaviour.

In water-soluble polymers, the principles involved in achieving LCST behaviour are well-established[4]. However, the number of polymer systems which have been cited as confirming these predictions is limited (see ref. 4 and refs therein). PNIPAM and modifications of PNIPAM have provided the prime focus of attention for studies of LCST behaviour in water-soluble polymers. (See, for example, refs 1, 2, 4 and 5–10, and refs therein.)

The LCST behaviour of PNIPAM has promoted interest in generation of membranes, capsules and gels designed to evince temperature-sensitive permeability characteristics. (See, for example, refs, 2, 4, 11, 12 and refs therein.)

Thermoresponsive polymers are an integral part of an emerging drive to develop classes of 'smart' materials which will respond, in a dramatic fashion, to changes in, for example, the temperature, salinity or pH of their environment. As part of a continuing programme seeking to develop such 'smart', polymer-based systems, we have initiated studies upon thermoresponsive water-soluble polymers. Systems of this type are of obvious technological importance[11] and this communication is the first of a series of reports on polymers which exhibit temperature-induced phase separation in aqueous media. In earlier publications (see, for example, refs 13–15) we have described investigations of water-soluble polymers, the conformational behaviours of which are controlled by pH. In these studies, we were particularly reliant upon fluorescence techniques to provide information, at the molecular level, regarding macromolecular behaviour in dilute, aqueous solution.

Winnik and coworkers[2,5,6–9,16] have used fluorescence spectroscopic methods, to great effect, in the study of the thermoresponsive behaviour of PNIPAM and certain of its hydrophobically-modified forms in aqueous media. It has been proposed, largely on the basis of intramolecular energy transfer data[16], that the temperature-induced phase separation of PNIPAM in water occurs as a result of a two-stage mechanism: first the polymer coils, to shrink into a collapsed state, and this is followed by aggregation of individual chains into larger particles.

In the current communication, we report on the use of time-resolved (fluorescence) anisotropy measurements, TRAMS, in studying the temperature-induced conformational transition of PNIPAM in water. The results demonstrate, clearly, for the first time, the dramatic effect that this transition has on the dynamics of the macromolecule in dilute aqueous solution. The data

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support Winnik's[16] proposal of a two-stage mechanism for the phase-separation process: the intramolecular coil collapse which occurs during the conformational transition has a significant effect upon the polymer's segmental relaxation behaviour. The change in segmental dynamics is comparable to that brought about by the collapse from open-coil to 'hypercoiled' structure which may be induced in poly(methacrylic acid) upon lowering the pH of the system. (See, for example, refs 13 and 14 and refs therein.)

Experimental

All materials were supplied by Aldrich, unless otherwise stated. Acenaphthylene (ACE) was triply recrystallized from methanol and triply sublimed. Dioxane (99.9%; h.p.l.c. grade) was used as received. *N*-Isopropylacrylamide (NIPAM) was used as received. Diethylether (May and Baker) was fractionally distilled. Methanol (spectroscopic grade) was used without further purification. Water was doubly distilled. Fluorescently labelled PNIPAM was prepared by copolymerization of NIPAM with *ca* 0.5 mol% of ACE in dioxane solution, under high vacuum using AIBN as initiator. Polymerization was terminated at *ca* 5% conversion to polymer. The polymer was purified by multiple reprecipitation from dioxane into diethylether. All spectroscopic samples contained 10^{-2} wt% of polymer in methanol and 10^{-3} wt% of polymer in aqueous solution.

Characterization. Estimates of the molar mass of the labelled polymer were obtained using matrix-assisted laser-desorption ionization time-of-flight (MALDI-TOF) mass spectrometry. These estimates were kindly performed by Thermobioanalysis Limited. Values of $\bar{M}_n = 40\,000$ and $\bar{M}_w = 62\,000$ were obtained for the PNIPAM/ACE sample.

Instrumentation. Excitation of fluorescence was achieved using radiation from the Synchrotron Radiation Source, SRS (EPSRC, Daresbury Laboratory, UK). Collection of the fluorescence intensities transmitted by a polarizer, analysing in planes parallel $[I_{\parallel}(t)]$ and perpendicular $[I_{\perp}(t)]$ to that of the polarized excitation, was achieved by means of a 'toggling procedure': the analyser was rotated sequentially through 90° while memory quarters in the MCA (Canberra) were switched simultaneously. Excitation and emission wavelengths of 290 and 340 nm, respectively, were employed.

A Philips XP4278b small photocathode photomultiplier tube was used as the detector and resulted in instrument response functions of less than 330 ps at full width half maximum. Details of this time-resolved single photon counting spectrometer have been previously published[17].

Results and discussion

TRAMS have been made over a range of temperatures, upon dilute PNIPAM/ACE solutions both in methanol and in water.

In the case of an ideal relaxation involving a spherical chromophoric rotor, the anisotropy $r(t)$ would decay exponentially as in equation (1);

$$r(t) = r_0 \exp(-t/\tau_c) \tag{1}$$

where r_0 is the intrinsic anisotropy of the fluorescent molecule and τ_c the correlation time which characterizes its rotational reorientation: the faster the tumbling of the chromophore, the shorter is τ_c. The observed time-dependent anisotropy, $R(t)$, may be constructed from its fluorescence components via the relationship

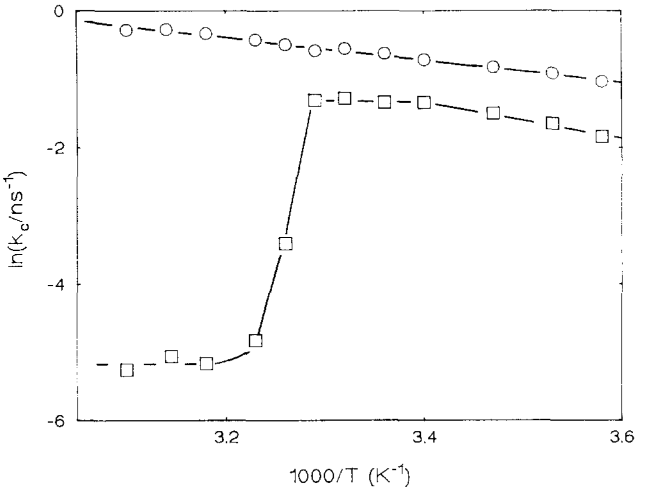

Figure 1 Temperature dependencies of segmental relaxation rates, $k_c[k_c = \tau_c^{-1}]$ of PNIPAM in methanol (○) and water (□)

$$R(t) = \frac{I_{\parallel}(t) - I_{\perp}(t)}{I_{\parallel}(t) + 2I_{\perp}(t)} \tag{2}$$

where $I_{\parallel}(t)$ and $I_{\perp}(t)$ are the intensities of emission detected in planes disposed parallel and perpendicular, respectively, to that of the vertically polarized radiation used for excitation. Recovery of a value of τ_c from $R(t)$ is complicated by the fact that the observed anisotropy contains perturbations caused by the distortion of $I_{\parallel}(t)$ and $I_{\perp}(t)$ by the finite breadth of the excitation pulse. This has the effect that when the width of the excitation pulse is not negligible compared to τ_c, $R(t)$ cannot be analysed directly to yield an estimate of this kinetic parameter in a reliable fashion. Moreover, $R(t)$ is *not* amenable to deconvolution, in a conventional reconvolution approach, such as would be applied to recover the fluorescence lifetime from time-resolved emission intensity data. We have discussed these problems, in some detail, earlier[17,18]. In the current work we have used the method of impulse reconvolution[19,20], in the generation of τ_c data.

Impulse reconvolution analyses of the time-resolved fluorescence from PNIPAM/ACE revealed that the anisotropy behaviour is not strictly described by equation (1), in either methanol or water as solvents. However, modelling the anisotropy decays by single exponential functions of this type *does* lead to values of τ_c which are capable of broad characterization of the dynamic behaviour of the polymer, if not in detail. Data resultant upon this type of analysis are shown in *Figure 1*.

The temperature-dependence of the intramolecular segmental mobility of PNIPAM in dilute methanolic solution is Arrhenius in form. The temperature-dependence of τ_c is characterized by an apparent activation energy, E^*, of 13.4 (±0.7) kJ mol^{-1}. In general, the intramolecular dynamic characteristics of any given polymer in solution will be affected by the solvent and the frictional resistance to segmental reorientation that it generates. Solvent effects upon macromolecular dynamics are commonly considered in the light of Kramers'[21] treatment of the passage of a particle over a potential energy barrier in the presence of frictional resistance, combined with the application, by Helfand and Skolnick[22–24] of this approach to local motions of macromolecules.

According to this treatment, the rotational diffusion of a polymer segment is described, in the limit of high frictional resistance, by

$$\tau_c = B\eta \exp(E_S/RT) \qquad (3)$$

where B is a constant and η is the solvent viscosity. Consequently, the overall activation energy, E^*, is composed of contributions from E_S, the activation energy associated with rotational diffusion over an intramolecular energy barrier, and E_η, the activation parameter associated with solvent flow, by

$$E^* = E_S + E_\eta \qquad (4)$$

E_η for methanol is 11.0 (±0.3) kJ mol^{-1}. Consequently, segmental relaxation of PNIPAM in this solvent would be characterized, in this approach, by a value of E_S of *ca* 2.4 kJ mol^{-1}. This value of E_S is much less than might be expected for segmental motion of a macromolecule and is comparable to the thermal dependence of the Boltzmann term, kT. Although this result might appear surprising, it is not inconsistent with other estimates of E_S for other water-soluble acrylic polymers, such as poly(acrylic acid), poly(methacrylic acid) and poly(dimethylacrylamide) in methanol[25].

The Kramers[21]/Helfand[22,24] approach to treating macromolecular dynamics assumes that specific interactions between polymer and solvent are absent. This is liable to prove a poor assumption in the PNIPAM/methanol system. Dipolar interactions between the macromolecular solute and its methanolic host are likely to dominate the kinetic behaviour of the polymer. It is to be expected that the macromolecule's reorientational relaxation might be controlled by the solvent-flow characteristics, as observed in our current TRAMS studies.

PNIPAM exhibits a markedly different behaviour in aqueous media which is largely independent of pH[26]. Typical data are shown in *Figure 1*. A discontinuity in the thermal dependence of τ_c occurs at 32°C, the temperature which marks the onset of phase separation in more concentrated solutions. Clearly, this temperature-induced transition in the behaviour of PNIPAM is accompanied by a significant reduction in chain flexibility of the polymer.

Winnik[16], studying intramolecular energy transfer between donor and acceptor chromophores bound to PNIPAM, reported that a marked contraction of the polymer coil occurred at the LCST. The current TRAMS data are the first to demonstrate that this conformational change effects a dramatic change in the macromolecule's dynamics. The thermoreversible collapse of the polymer coil which characterizes PNIPAM's LCST places constraints upon the motion of the polymer segments which are comparable to those encountered[27] in the pH-induced collapse of poly(methacrylic acid), to form its 'hypercoiled' state in acidic media. At temperatures of 28–31°C, just below the LCST, PNIPAM exists as a relatively flexible polymer coil: τ_c is of the order of 3.6–3.7 ns, similar to that of poly(methacrylic acid) in methanol[17]. At a temperature of 45°C, beyond the LCST, the segmental motion of the macromolecule is characterized by a τ_c value of *ca* 160 ns: the relaxation rate of the polymer is reduced, across this temperature range by a factor of over forty!

The TRAMS approach is clearly very useful for studies of thermally-induced conformational transitions in polymers. In this context, the acenaphthylene label is particularly useful since is cannot move independently of the polymer chain. In a previous study of the conformational behaviour of poly(methacrylic acid), it appeared that fluorescent labels, such as 1-vinylnaphthalene, bound to the chain by a flexible covalent linkage, could move independently of the polymer when the chain motion became severely restricted[27]. It is likely that such labels would be less useful in studies of PNIPAM. The TRAMS data presented above offer support for the Winnik[16] mechanism for the thermoreversible behaviour of PNIPAM, in that they provide unambiguous evidence for the 'coil collapse' that she proposes as the first stage in phase separation.

Fluorescence studies of PNIPAM and other polymers showing thermoresponsive behaviours continue in our laboratories[26].

Conclusions

1. The technique of TRAMS is useful in the study of thermoreversible conformational changes of polymers which, in aqueous solution, show lower critical solution behaviour.
2. In the PNIPAM/water system, phase separation, the onset of which occurs at 32°C, is accompanied by a marked conformational transition in the polymer (similar, in significance to the chain dynamics, to that which accompanies[27] the pH-controlled conformational behaviour of poly(methacrylic acid) in aqueous media).
3. The current study provides supporting evidence for the dual-mode mechanism of phase separation in PNIPAM/water, proposed by Winnik[16].
4. In methanol, PNIPAM exhibits a normal Arrhenius thermal dependence of its relaxation behaviour, in which the apparent energy of activation is little more than that of solvent flow. It is likely that the solvent exerts a dominating influence upon the chain mobility through dipolar attractive interactions with the polar substituents of the polymer.

Acknowledgements

The authors gratefully acknowledge financial support from EPSRC and the invaluable help of Dr David Shaw on station 13.1 b at the SRS, Daresbury. Thanks are also due to Thermobioanalysis Ltd for molar mass determinations by MALDI-TOF spectrometry.

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