Phase transition of aqueous poly-(*N*-isopropylacrylamide) solutions: a study by non-radiative energy transfer

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The thermoreversible phase separation of aqueous solutions of poly-(*N*-isopropylacrylamide) (PNIPAM) was investigated by fluorescence experiments that probe transfer of electronic excitation between donor and acceptor chromophores attached to the same polymer chain. Doubly labelled polymers were prepared by reaction of a copolymer of *N*-isopropylacrylamide and *N*-acryloxysuccinimide first with 4-(1-pyrenyl)-butylamine, then with 1-naphthylethylamine. Samples were prepared and characterized. They differ in total chromophore incorporation, on average 1 chromophore per 170 and 416 *N*-isopropylacrylamide units, and in pyrene to naphthalene molar ratio, 3.8 and 7.3, respectively. Non-radiative energy transfer between excited naphthalene and pyrene occurred in solutions of both polymers in water, but not in methanol. The energy transfer efficiency was monitored as a function of solution temperature for the more lightly labelled polymer. The value increased gradually from c. 15% at 15°C to c. 75% for solutions heated above their lower critical solution temperature (LCST). Results are interpreted in terms of a mechanism of phase separation initiated by a gradual shrinking of solvated polymer coils into a collapsed state, followed by aggregation of individual chains into larger particles.

(Keywords: poly-(*N*-isopropylacrylamide); PNIPAM; energy transfer; phase separation)

INTRODUCTION

The heat-induced phase separation of aqueous polymer solutions is an intriguing phenomenon. Though common among water-soluble polymers[1,2], the phenomenon is still not well understood. Most experimental techniques employed to examine polymer solutions heated through their phase separation temperature monitor macroscopic changes in solution properties. They include calorimetry, viscometry, turbidimetry, and light scattering. Calorimetry provides information on the thermodynamics of phase separation, and viscosity about the hydrodynamic consequences of aggregation. A wealth of data has been gathered from such experiments. The temperature of phase separation has been determined for many polymers[1,2] and its relation to polymer structure and molecular weight is known with great detail[3]. The effects of additives, especially salts[4,5] and surfactants[6,7], are also well documented. However, questions related to the molecular aspects of the phenomenon are still not answered satisfactorily. Is the phase separation triggered by a collapse of individual chains upon themselves, or rather by hydrophobically-driven interactions among polymer chains? What are the structure and composition of the polymer-rich phase separated above the critical temperature?

Techniques with a spatial resolution in the 2 to 100 Å range are needed to observe the phenomenon on the molecular level. Experiments based upon neutron and X-ray scattering or upon fluorescence quenching measurements offer this capability. To monitor phase separation through fluorescence one needs to attach small amounts of dyes to the polymer chain. The dyes then report on fluctuations in polymer conformation and in polymer/solvent interactions through changes in their emission. Depending on the spectroscopic property upon which the experiments are based the distances probed vary from a few Å to about 100 Å.

The specific technique used here relies on the photophysical process of direct non-radiative energy transfer between the two chromophores[8,9]. The process originates in dipole–dipole interactions between a donor (D) in its excited state and an acceptor (A) in its ground state. For pairs of chromophores which fulfil the spectroscopic requirements for energy transfer by this mechanism, the probability of energy transfer depends on their relative orientation and on their separation distance R. The distance dependence of the energy transfer efficiency, E, is a well defined function of the distance R between the donor and the acceptor (equation (1))[8,9] where R_0, for rotationally averaged pairs, is the donor/acceptor separation distance for which 50% of energy transfer takes place:

$$E = R_0^6/(R_0^6 + R^6) \qquad (1)$$

In the case of small molecules in fluid solution, non-radiative energy transfer takes place when the two interacting chromophores diffuse towards each other during the lifetime of the donor. When the D and A chromophores are attached to a polymer chain, the distribution of D/A separations is related to the chain dimensions. For immobile chains, there are theoretical treatments of the D/A pair distribution function which allow one to calculate the mean dimensions of the polymer to which the D and A groups are attached.

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Fredrickson, Andersen, and Frank$^{10-12}$ have demonstrated that by attaching chromophore labels at multiple sites on a polymer chain it is possible to assess the characteristic ratio of linear chains^{10,11}, the radius of gyration of macromolecules^{13,14}, and intramolecular segment correlation functions^{15}. These theories break down when segmented diffusion occurs on the same time scale as energy transfer. Under these circumstances measurements of intramolecular energy transfer still provide reliable qualitative information about changes in dimensions.

Factors which swell these dimensions increase the mean separation of A and D groups. Conversely, chain collapse should lead to a pronounced decrease in these distances. As a consequence the efficiency of intramolecular energy transfer should be a sensitive measure of swelling or contraction of chain dimensions, increasing sharply as the chain dimensions decrease (*Figure 1*). This feature was demonstrated in a study of the mechanism of protein folding by means of site-specific fluorescence labelling of polypeptide chains^{16}. It was used also to monitor in a qualitative way the effect of chain length on the cyclization of end-labelled polysarcosine chains^{17}. The technique is applied here to the study of the heat-induced phase transition of aqueous polymer solutions.

The polymer chosen is poly-(*N*-isopropylacrylamide) (PNIPAM), which in water exhibits thermoreversible phase separation at 31°C (ref. 18), its lower critical solution temperature, or LCST. The polymer was labelled randomly with small amounts of naphthalene (N), the energy donor and pyrene (Py), the energy acceptor. The preparation and characterization of polymers labelled with varying amounts of chromophores are described. Energy transfer between naphthalene and pyrene labels was detected in aqueous solutions below their LCST. The solutions were heated above their LCST and the extent of energy transfer between the two chromophores was measured as a function of temperature.

EXPERIMENTAL

Materials and syntheses

All commercial chemicals were purchased from Aldrich Chemical Company (Milwaukee, USA), unless otherwise mentioned. *N*-isopropylacrylamide was obtained from Eastman Kodak Chemicals (Rochester, NY, USA). *N*-Acryloxysuccinimide (m.p. 69–69.5°C; reported m.p.^{19} 68–69°C) was prepared by reaction of acryloyl chloride with *N*-hydroxysuccinimide^{20}. 4-(1-Pyrenyl)-butyl amine (**1**) was prepared from 4-(1-pyrenyl)-butanoic acid by the following sequence of reactions: conversion to the acid chloride by treatment with oxalyl chloride^{21}; reaction of 4-(1-pyrenyl)-butyroyl chloride with aqueous ammonia to yield 4-(1-pyrenyl)-butyramide (m.p. 181–182°C; reported m.p.^{22} 180.5–181°C); reduction with $LiAlH_4$ in dry THF. It was isolated as the hydrochloride recrystallized from methanol (m.p. 262– 264°C; reported m.p.^{22} 252–256°C). For the syntheses, reagent grade solvents were used without further purification, except for tetrahydrofuran (THF) which was dried by distillation from sodium/benzophenone. Water was deionized with a Millipore Milli-Q (Bedford, MA, USA) water purification system. Spectroscopic grade solvents were used for all spectroscopic measurements.

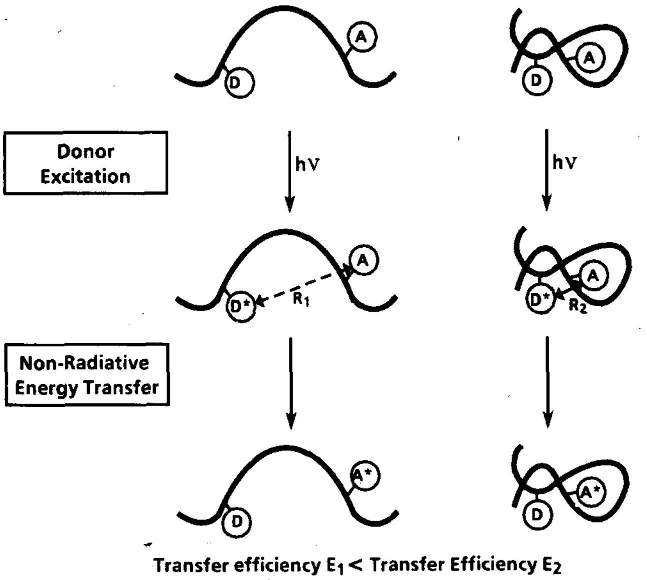

Figure 1 Influence of polymer mean dimensions on the efficiency of energy transfer (*E*) between donors and acceptors labels

N-*isopropylacrylamide*–N-*acryloxysuccinimide copolymer (PNIPAM–NASI)*

N-isopropylacrylamide (5.0 g, 0.044 mol) and *N*-acryloxysuccinimide (0.150 g, 0.88 mmol) were dissolved in *tert*-butanol (30 ml) at 70°C under nitrogen. AIBN (30 mg) in *tert*-butanol (1 ml) was added to the solution. The reaction mixture was stirred at 70°C for 20 h. It was cooled to room temperature. The solvent was evaporated *in vacuo*. The polymer (2.2 g) was isolated by successive precipitations from a THF solution into hexane and from a methanol solution into dry diethyl ether; i.r. (KBr) 3435, 2970, 2924, 1814, 1784, 1734, 1652, 698 cm^{-1}.

Pyrene-labelled PNIPAM (PNIPAM–Py/200)

Triethylamine (10 mg, 0.08 mmol) was added to a solution of 4-(1-pyrenyl)-butyl amine hydrochloride (5 mg, 0.016 mmol) and PNIPAM–NASI (0.5 g) in THF (5 ml). The solution was stirred at room temperature for 17 h. Then *N*-isopropylamine (10 mg, 0.17 mmol) in THF (0.5 ml) was added. The reaction mixture was stirred for an additional 2 h period. The polymer was isolated by precipitation in hexane (500 ml). It was purified further by two precipitations from a methanol solution (15 ml) into dry diethyl ether (400 ml). It was dried *in vacuo* for 24 h (380 mg); u.v. (MeOH) λ_{max} 264, 275, 325, 341 nm.

Naphthalene-labelled PNIPAM (PNIPAM–N/27)

1-Naphthyl ethylamine (30 mg, 0.17 mmol) was added to a solution of PNIPAM–NASI (0.5 g) in THF (5 ml). The solution was stirred at room temperature in the dark for 21 h. Then *N*-isopropylamine (0.1 ml) was added to the solution. The solution was stirred for an additional hour. The polymer was isolated by precipitation in hexane (500 ml). It was purified further by two precipitations from a THF solution (15 ml) into hexane (400 ml). It was dried *in vacuo* for 24 h (380 mg); u.v. (MeOH) λ_{max} 270, 280 nm; i.r. (KBr) 3440, 2920, 1734, 1653, 843, 698 cm^{-1}.

Pyrene/naphthalene-labelled PNIPAM

PNIPAM–Py/135–N/35. Triethylamine (10 mg, 0.08 mmol) was added to a solution of 4-(1-pyrenyl)-butyl amine hydrochloride (5 mg, 0.016 mmol) and PNIPAM–

NASI (0.5 g) in THF (5 ml). After a period of 2.5 h at room temperature the reaction mixture was treated with 1-naphthylethylamine (20 mg, 0.12 mmol). The mixture was stirred at room temperature in the dark for 17 h, then *N*-isopropylamine (0.1 ml) was added. The reaction mixture was stirred for an additional 2 h period. A work-up procedure identical to that described for PNIPAM–N/27 gave the labelled polymer PNIPAM–Py/135–N/35 (410 mg); u.v. (MeOH) λ_{max} 264, 275, 312, 325, 342, 376 nm.

PNIPAM–Py/366–N/50. The polymer was prepared following the procedure described for PNIPAM–Py/135–N/35, starting with PNIPAM–NASI (0.5 g), 4-(1-pyrenyl)-butyl amine hydrochloride (2.5 mg, 8×10^{-3} mmol) and 1-naphthylethylamine (10 mg, 0.12 mmol); pyrene content: 2.4×10^{-5} mol g^{-1} or 1 pyrene per 366 NIPAM units; naphthalene content: 1.74×10^{-4} mol g^{-1} or 1 naphthalene per 50 NIPAM units.

Instrumentation and measurement techniques

U.v. spectra were recorded with a Hewlett-Packard 8480A diode array spectrometer. Temperature-controlled experiments were done with a Hewlett-Packard 89100A temperature control accessory consisting of a digitally controlled thermoelectrically heating and cooling cell holder with sample stirring capability and programmed temperature ramping. The temperature of the sample fluid was measured with a Hewlett-Packard 89102A teflon-coated temperature sensing probe immersed in the sample fluid. Heating and cooling rates were chosen between 0.2°C min^{-1} and 1°C min^{-1}. Gel permeation chromatography (g.p.c.) was performed with a system consisting of a Shimadzu SPD-6A u.v.-visible spectrophotometric detector, a Shimadzu RID-6A differential refractive index detector and a Shimadzu-Chromatopac data analysis system (Shimadzu Corp., Kyoto, Japan). Two Progel columns (G5000PW and G3000PW) from Toyo-Soda were used. Deionized water was used as the eluent at a flow rate of 1.0 ml min^{-1}. Solution viscosities were measured at 30°C with a Viscotek Model 100 differential viscometer (Viscotek, Porter, TX, USA). The polymers were dissolved in THF at concentrations of c. 50 ppm. Cloud points were determined by spectrophotometric detection of the changes in turbidity of solutions heated at a constant rate in a magnetically stirred UV cell, as described previously[23].

Steady state fluorescence spectra were recorded on a SPEX Fluorolog 212 spectrometer equipped with a DM3000F data system (SPEX Industries Inc., Edison, NJ, USA). The temperature of the water-jacketed cell holder was controlled with a Neslab circulating bath. The temperature of the sample fluid was measured with a thermocouple immersed in the sample fluid. For measurements at different temperatures samples were allowed to equilibrate for 10 min at a given temperature. The heating rate corresponded to approximately 0.2°C min^{-1}. The excitation spectra were measured in the ratio mode. The emission spectra were not corrected, except for those used in quantum yield determinations. A calibrated tungsten lamp (NBS, Washington, DC) was used to determine the correction factors. Slit widths ranging from 0.9 to 2.0 nm were chosen, depending on chromophore concentration.

Solutions in methanol were degassed by vigorous bubbling with solvent-saturated argon for 1 min. Solutions in water were not degassed, because it was determined that for aqueous solutions the fluorescence intensities of pyrene in PNIPAM/Py were not affected significantly by degassing. Labelled polymer solutions were prepared at room temperature. They were allowed to stand for 24 h before they were diluted to a known volume (*Table 1*). They were kept at room temperature for at least 2 h prior to the measurements. In all solutions the concentration of polymer was such that the absorbance at the excitation wavelengths (290 nm or 328 nm) was lower than 0.05.

Quantum yields were calculated by integration of peak areas of corrected spectra in wave number units, using as standards either 2-aminopyridine in 0.1 N H_2SO_4 ($\Phi = 0.60$, $\lambda_{exc} = 290$ nm, 25°C)[24] or quinine sulphate in 1 N H_2SO_4 ($\Phi = 0.546$, $\lambda_{exc} = 328$ nm, 25°C)[25]. Beer's law corrections were applied for optical density changes at the excitation wavelengths. Corrections were made as well for refractive index differences. The excimer to monomer ratios were calculated by taking the ratio of the emission intensity at 480 nm to the half-sum of the emission intensities at 376 nm and 396 nm. It was verified that peak areas and peak heights were proportional. For determination of energy transfer efficiencies fluorescence intensities were obtained by integration of corrected spectra in wave number units, from 310 nm to 360 nm for the naphthalene emission and from 360 to 560 nm for the pyrene emission. For the pyrene spectra obtained with an excitation wavelength of 290 nm, the contribution due to naphthalene emission was eliminated by subtraction of the spectrum of PNIPAM–N/27 normalized at 338 nm. For calculations of the extent of naphthalene quenching spectra were integrated from 310 to 360 nm.

Calculation of spectroscopic parameters

Fraction P *of the total light absorbed by the naphthalene in the presence of pyrene.* Fraction *P* was calculated at the excitation wavelength λ_1 ($\lambda_1 = 290$ nm) using equation

Table 1 Solutions used in the energy transfer experiments

Polymer (concentration)	Solvent	[Py] mol l^{-1}	[N] mol l^{-1}
Intrapolymeric measurements:			
PNIPAM–Py/366–N/50 (44 ppm)	H_2O	1.1×10^{-6}	7.7×10^{-5}
PNIPAM–Py/135–N/35 (37 ppm)	H_2O	1.6×10^{-6}	6.4×10^{-5}
PNIPAM–Py/366–N/50 (130 ppm)	MeOH	3.2×10^{-6}	2.3×10^{-5}
PNIPAM–Py/135–N/35 (125 ppm)	MeOH	8.1×10^{-6}	3.2×10^{-5}
Interpolymeric measurements:			
PNIPAM–Py/200 (24 ppm) + PNIPAM–N/27 (12 ppm)	H_2O	1.1×10^{-6}	3.8×10^{-5}

(2)[26]

$$P = (1 - 10^{-A_N(\lambda_1)})/(2 - 10^{-A_N(\lambda_1)} - 10^{-A_{Py}(\lambda_1)}) \qquad (2)$$

where $A_N(\lambda_1)$ and $A_{Py}(\lambda_1)$ are the absorbances at λ_1 of naphthalene and pyrene, respectively. It was assumed that P does not vary with solution temperature in the 4 to 40°C range.

Critical energy transfer distance R_0 *for the naphthalene and pyrene labels on PNIPAM.* R_0 is given by the expression[8,9]:

$$R_0 = [9000(\ln 10)\kappa^2\Phi_N J/(128\pi^5 n^4 N)]^{1/6} \qquad (3)$$

where Φ_N is the naphthalene quantum yield in the absence of energy transfer, κ^2 is an orientation factor assumed to be equal to 2/3, n is the solvent refractive index, N is Avogadro's number, and J is the overlap integral between the normalized donor fluorescence intensity $I(\lambda)$ and the acceptor extinction coefficient $\varepsilon(\lambda)$:

$$J = \int_0^\infty \lambda^4 I(\lambda)\varepsilon(\lambda)\,d\lambda \qquad (4)$$

where

$$\int_0^\infty I(\lambda)\,d\lambda = 1$$

R_0 was determined experimentally from the fluorescence and absorption spectra of solutions of PNIPAM–N/27 and PNIPAM–Py/200 in methanol. Its value, 29.1 Å ($J = 16.87 \times 10^{-15}\,\text{cm}^6\,\text{mol}^{-1}$) is in good agreement with the reported value for the 1-methylnaphthalene/pyrene donor/acceptor pair ($R_0 = 28.64$ Å, $J = 16.5979 \times 10^{-15}\,\text{cm}^6\,\text{mol}^{-1}$)[27].

Energy transfer efficiency (X). Values of X were calculated by[28]:

$$X/(1 - X) = \Phi_N I_{Py}/\Phi_{Py} I_N \qquad (5)$$

where Φ_N is the fluorescence quantum yield of naphthalene in PNIPAM–N/27 and Φ_{Py} is the quantum yield of pyrene emission in the doubly labelled polymer upon direct excitation ($\lambda_{exc} = 328$ nm), I_N and I_{Py} are the areas of the emission spectrum of doubly labelled polymer corresponding to naphthalene and pyrene, respectively.

Naphthalene quenching efficiency (E_N). Values of E_N were calculated by[26]:

$$E_N = 1 - (I_D^N A_S / I_S^N A_D) \qquad (6)$$

where I_D^N and I_S^N are the fluorescence intensities of naphthalene from doubly and singly labelled polymers, with absorbances A_D and A_S at the excitation wavelength (290 nm), respectively.

RESULTS AND DISCUSSION

Synthesis and characterization of the labelled polymers

Attachment of the labels to the PNIPAM backbone was achieved by reacting a copolymer of *N*-isopropylacrylamide and *N*-acryloxysuccinimide (PNIPAM–NASI) with chromophores bearing a short amino-terminated alkyl chain. Specifically, the copolymer was labelled with naphthalene groups (N) by reaction with 1-naphthylethylamine, with pyrene groups (Py) by reaction with 4-(1-pyrenyl)-butylamine, and with Py and N by reaction first with 4-(1-pyrenyl)-butylamine, then with 1-naphthylethylamine. In all syntheses the unreacted *N*-acryloxysuccinimide groups were converted to isopropyl groups by quenching with *N*-isopropylamine (*Figure 2*). Four labelled polymers were prepared: PNIPAM–Py/200 labelled with pyrene only, PNIPAM–N/27, labelled with naphthalene only, and two doubly labelled polymers, PNIPAM–Py/135–N/35 and PNIPAM–Py/366–N/50. The digits in the polymer designations refer to the average number of *n*-isopropyl units per chromophore, e.g. PNIPAM–Py/200 has on average 1 pyrene per 200 *N*-isopropyl groups, a value determined from the u.v. absorption of the polymer (see below).

The characterization of the labelled polymers is based on three techniques: viscosity measurements for molecular weight determination; u.v. absorption for calculation of the level of labelling; and g.p.c. analysis for assessment of polymer purity. Viscosity-averaged molecular weights were estimated from intrinsic viscosities of polymer solutions in THF, using the viscometric relationship[29]: $[\eta] = 9.59 \times 10^{-3}\,\text{Mv}^{0.65}$ (*Table 2*). The amount of pyrene and naphthalene incorporation was calculated from u.v. absorption data of polymer solutions in methanol, using 4-(1-pyrenyl)-butyl amine ($\varepsilon_{342} = 32\,800$)[22] and 2-naphthylethanol ($\varepsilon_{282} = 6740$)[30] as model compounds, respectively. For the doubly labelled polymers, the pyrene content was determined first; the naphthalene content was then calculated from the absorption at 290 nm, after correction for the pyrene absorption at this wavelength (*Figure 3*). Through the use in tandem of a u.v. detector and a refractive index detector for the g.p.c. analysis it was ascertained that the polymers do not contain low molecular weight u.v.-absorbing impurities; the chromophores are covalently bound to the polymer (*Figure 4*); and the molecular weight and molecular weight distribution of PNIPAM–NASI are not altered significantly by the chemical transformations and the polymer purification procedures. The elution times of PNIPAM–NASI for g.p.c. analyses in aqueous solutions were slightly longer than those of the labelled polymers (*Figure 5*). This observation seems to contradict the viscosity data, from which the calculated molecular weights were higher for PNIPAM–NASI than for all the labelled polymers. The differences in g.p.c. elution times may be attributed to contributions of hydrophobic interactions between the labelled polymers and the column packing material, in addition to the size exclusion phenomena. Such effects have been reported for other hydrophobically labelled water-soluble polymers[31,32].

FLUORESCENCE SPECTROSCOPY OF THE LABELLED POLYMERS

The situation in methanol and in water at 25°C

Singly-labelled polymers. Emission and excitation spectra of PNIPAM–N/27 were measured in methanol and in water. The fluorescence of this polymer in methanolic solutions exhibits an emission due exclusively to locally excited naphthalenes, with the [0, 0] band located at 323 nm. In water the polymer presents a weak structureless emission (350 to 460 nm), attributed to naphthalene excimers, in addition to the structured naphthalene emission (310 to 360 nm). The emission spectrum of PNIPAM–Py/200 in methanol shows an emission due

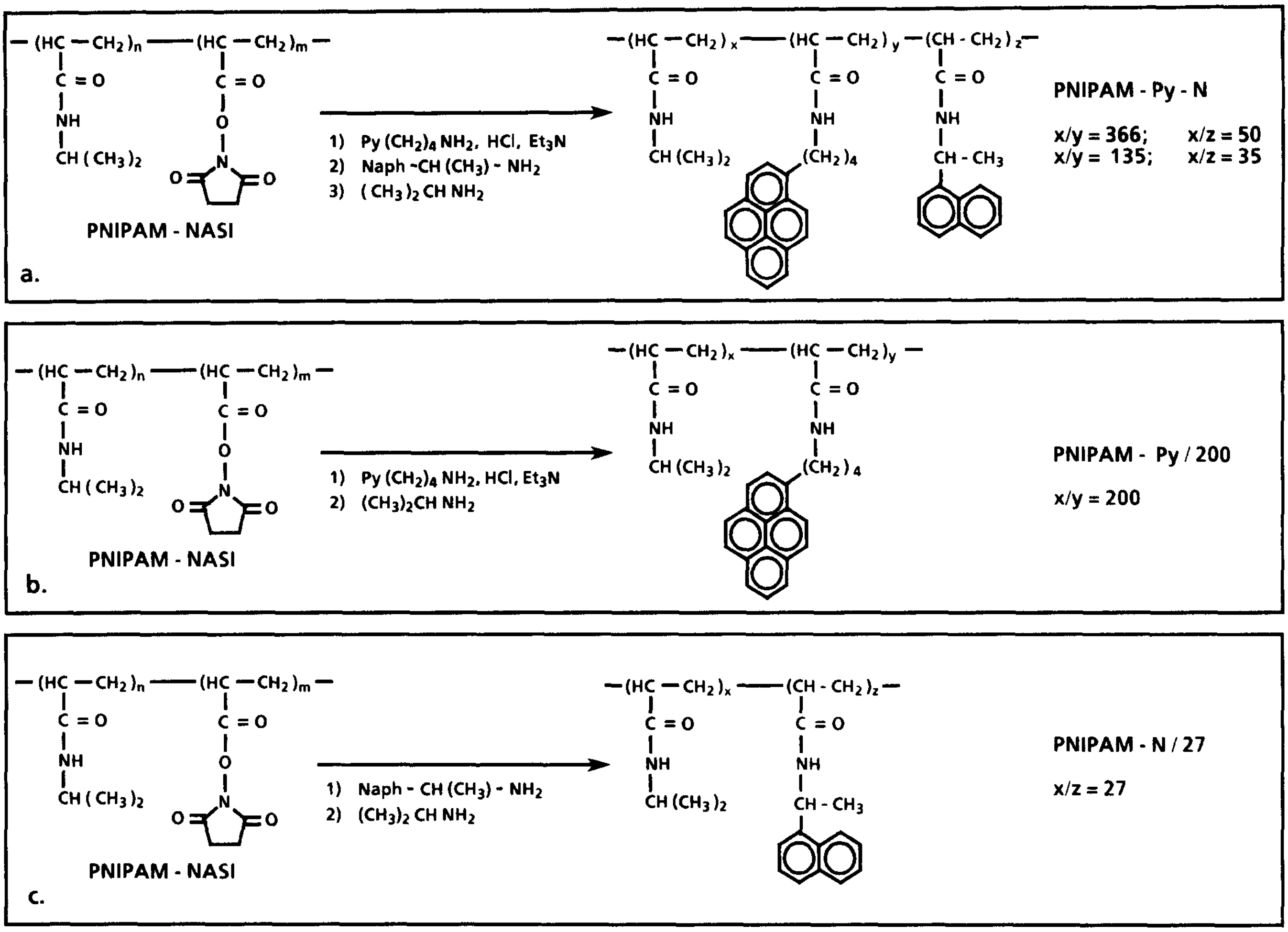

Figure 2 Synthetic scheme for the preparation of labelled poly-(*N*-isopropylacrylamides): (a) doubly labelled polymer; (b) pyrene-labelled polymer; (c) naphthalene-labelled polymer. $Py(CH_2)_4-NH_2$ = 4-(1-pyrenyl)-butyl amine; $Naph-CH(CH_3)-NH_2$ = 1-naphthylethylamine

Table 2 Physical properties of the polymers

Polymer	$[\eta]^a$ ($cm^3\,g^{-1}$)	$M_{viscosity}{}^b$	Pyrene content ($mol\,g^{-1}$)	Naphthalene content ($mol\,g^{-1}$)	Cloud point (concentration, $g\,l^{-1}$)
PNIPAM	72 ± 1	920000	–	–	32.5°C (0.05 to 10)
PNIPAM–NASI	111 ± 1	1.78×10^6	–	–	32.5°C (0.3)
PNIPAM–N/27	87 ± 1	1.23×10^6	–	3.26×10^{-4}	32.5°C (0.05)
PNIPAM–Py/200	103 ± 1	1.1×10^6	4.2×10^{-5}		33°C (0.05)
PNIPAM–Py/366–N/50	84 ± 1	1.2×10^6	2.4×10^5	1.74×10^{-4}	32°C (0.05)
PNIPAM–Py/135–N/35	83 ± 1	1.2×10^6	6.5×10^{-5}	2.5×10^{-4}	32°C (0.05)

[a] From THF solutions
[b] See text

to locally excited pyrenes (intensity I_M, 'monomer' emission) with the [0, 0] band located at 376 nm and a weak broad emission centred at 480 nm, due to pyrene excimer emission (intensity I_E). The spectrum of PNIPAM–Py/200 in aqueous solution exhibits a stronger excimer emission, relative to monomer emission, than in methanolic solution. Moreover this emission originates from excitation of pre-formed pyrene aggregates, and not from excited state complexes formed through encounter of an isolated excited pyrene and a ground-state pyrene. A detailed description of this spectroscopy has been reported elsewhere[33].

Doubly-labelled polymers. Two doubly labelled polymers were examined. They differ not only in the level of total chromophore incorporation (on average 1 chromophore per 170 and 416 NIPAM units), but also in the ratio of pyrene to naphthalene (c. 3.8 and 7.3, in PNIPAM–Py/135–N/35 and PNIPAM–Py/366–N/50). Because the polymers contain two chromophores that absorb light in different wavelength ranges, it ought to be possible to excite directly one chromophore to the exclusion of the other. This is the case for pyrene but not for naphthalene. Light of wavelength longer than 310 nm is absorbed exclusively by the pyrene groups. Thus excitation at 328 nm of solutions of the doubly labelled polymers results in direct pyrene emission with contributions from both monomer and excimer. The excimer contribution

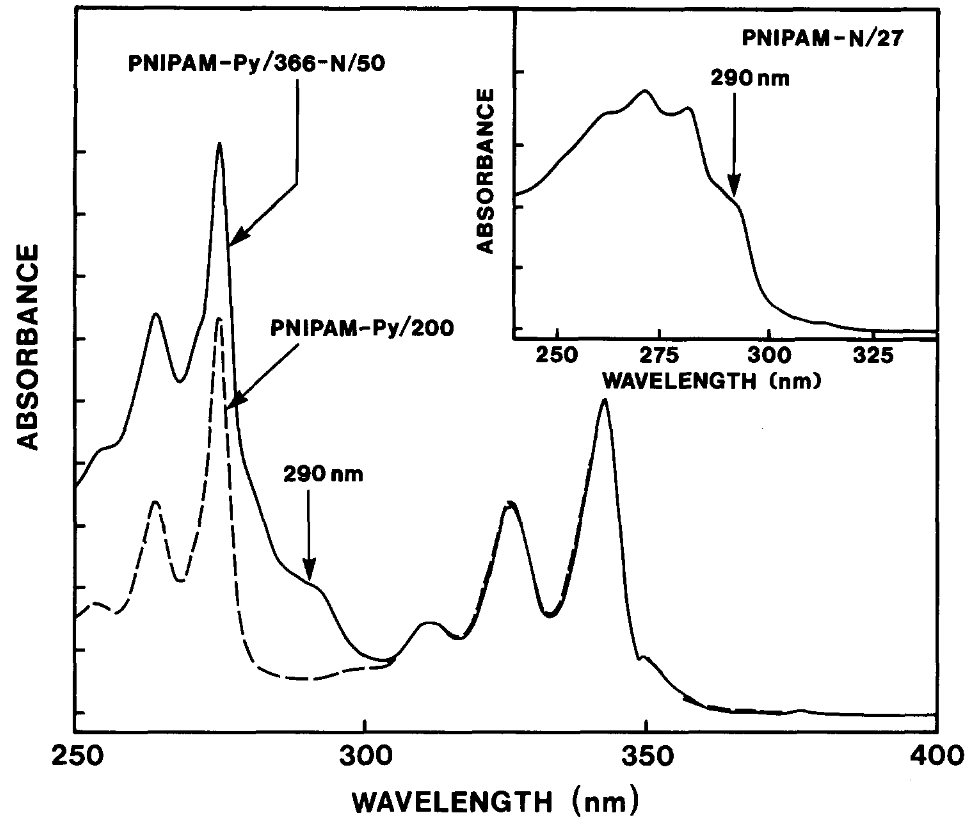

Figure 3 U.v. absorption spectra of methanolic solutions of labelled poly-(N-isopropylacrylamide) samples: PNIPAM-Py/366-N/50 (full line), PNIPAM-Py/200 (dashed line) and PNIPAM-N/27 (inset)

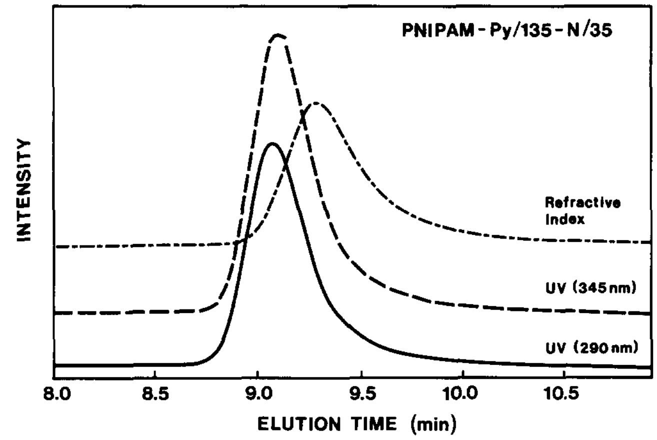

Figure 4 G.p.c. elution profiles of the pyrene/naphthalene labelled polymer (PNIPAM-Py/135-N/35) monitored by refractive index detection and u.v. absorption at 290 nm and 345 nm

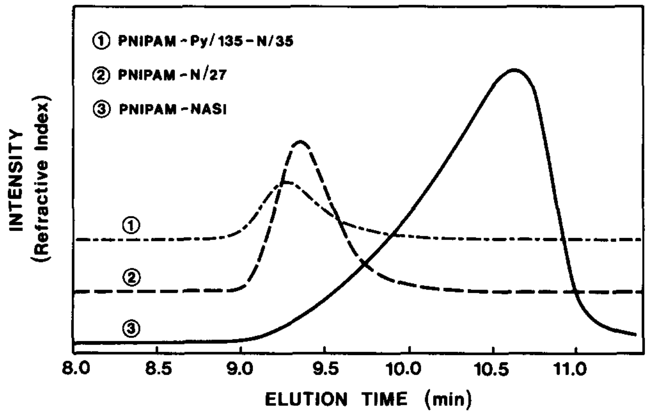

Figure 5 G.p.c. elution profiles of N-isopropylacrylamide-N-acryloxysuccinimide copolymers (PNIPAM-NASI, full line), naphthalene-labelled poly-(N-isopropylacrylamide) (PNIPAM-N/27, dashed line) and pyrene/naphthalene-labelled poly-(N-isopropylacrylamide) (PNIPAM-Py/135-N/35, dotted line) monitored by refractive index detection

to the total emission is greater for the more highly substituted polymer.

An excitation of 290 nm was selected to irradiate N groups preferentially to Py groups. At this wavelength the naphthalenes are responsible for a fraction $P=0.77$ (see Experimental section) of the total light absorbed by the solution. Excitation at 290 nm of solutions of PNIPAM-Py/366-N/50 or PNIPAM-Py/135-N/35 in either methanol or water resulted in a fluorescence composed of naphthalene monomer emission (310-360 nm) and pyrene emission (*Figure 6*). The latter contribution to the total emission intensity cannot be accounted for only by the emission originating from direct pyrene excitation at 290 nm. In all cases it is more intense than the emission of pyrene from a solution of PNIPAM-Py/200 irradiated under identical conditions. This observation is diagnostic of the occurrence of non-radiative energy transfer between the naphthalene and the pyrene chromophores. Further evidence can be derived from the values of quantum yields of the naphthalene emission (*Table 3*). These are lower in the doubly labelled polymers than in PNIPAM-N/27. The decrease is most noticeable in aqueous solutions of the more highly labelled sample.

The extent of energy transfer in a given polymer

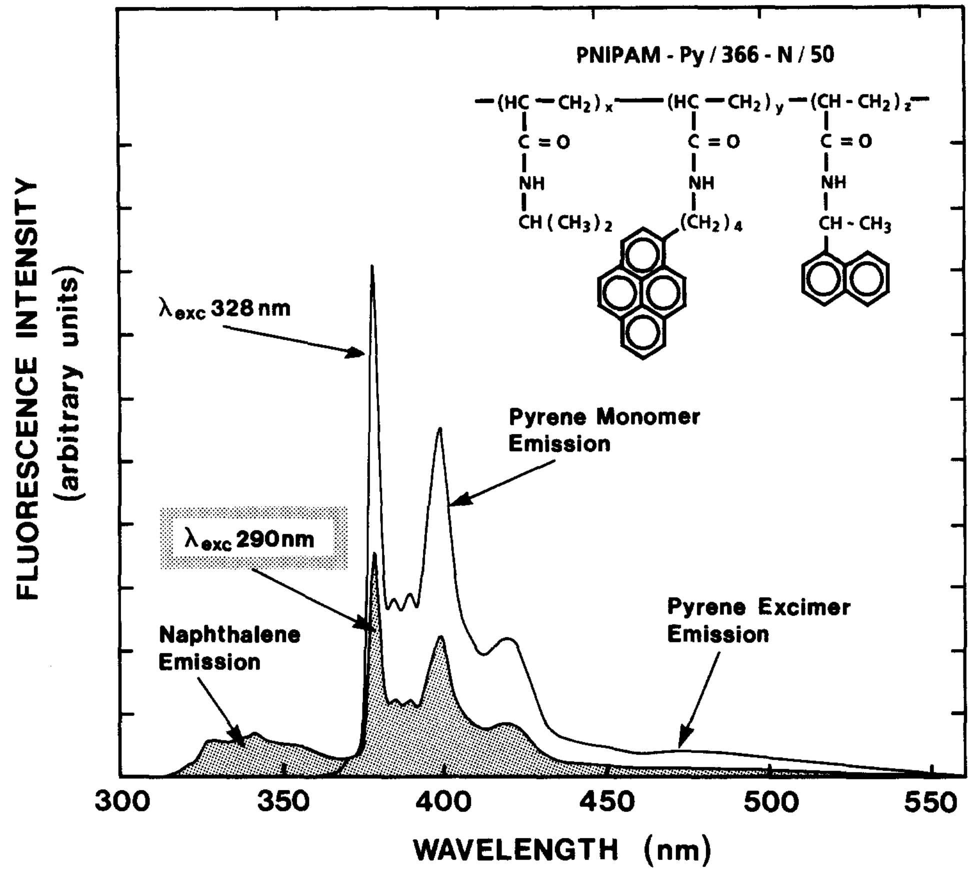

Figure 6 Fluorescence spectra of pyrene/naphthalene labelled poly-(*N*-isopropylacrylamide) (PNIPAM–Py/366–N/50, 44 ppm) in water; excitation wavelength: 328 nm and 290 nm (shaded spectrum)

Table 3 Spectroscopic characteristics of the labelled polymers. The temperature of the solutions was 25°C

	I_E/I_M(Py)[a]		Φ_{Py}[a]		Φ_N[b]		Energy transfer efficiency	
Polymer	MeOH	H_2O	MeOH	H_2O	MeOH	H_2O	X (H_2O)	E_N (H_2O)
PNIPAM–N/27	–	–	–	–	0.19	0.12	–	–
PNIPAM–Py/200	0.10	0.45	0.70	0.41	–	–	–	–
PNIPAM–Py/366–N/50	0.06	0.06	0.79	0.65	0.09[c]	0.04[c]	0.40 ± 0.06	0.56 ± 0.06
PNIPAM–Py/135–N/35	0.15	0.14	0.66	0.64	0.08[c]	0.02[c]	0.73 ± 0.07	0.78 ± 0.07

[a] $\lambda_{exc} = 328$ nm
[b] $\lambda_{exc} = 290$ nm
[c] Values calculated assuming that the naphthalene groups are responsible for 77% of the light absorbed by the solution at 290 nm (see text)

solution was evaluated by monitoring either the quenching of naphthalene emission or the enhancement of pyrene emission as a consequence of non-radiative energy transfer from excited naphthalene to pyrene. The quenching efficiency, E_N, (see equation (6)) of naphthalene emission was evaluated by measuring under identical conditions the naphthalene emission from a singly labelled polymer (e.g. PNIPAM–N/27) and from a doubly labelled polymer. The enhancement of pyrene emission on the other hand allows one to calculate the energy transfer efficiency (X) defined as the ratio between the number of quanta transferred to pyrene groups and the number of quanta absorbed by the naphthalene groups. This value was evaluated using equation (5). The efficiencies calculated by both methods are given in *Table 3* for aqueous solutions of the two doubly-labelled polymers. The values are higher in the more highly labelled polymer, illustrating the effect on energy transfer of changes in the density of energy acceptors around the donors.

The situation in water below and above the LCST

Dilute solutions of the doubly labelled polymer PNIPAM–Py/366–N/50 were heated from 4 to 40°C, and their fluorescence spectra were monitored as a function of temperature. The naphthalene emission ($\lambda_{exc} = 290$ nm) remained constant for solutions in the 4 to 18°C temperature range, and it decreased slowly for higher temperatures. The pyrene emission intensity also remained

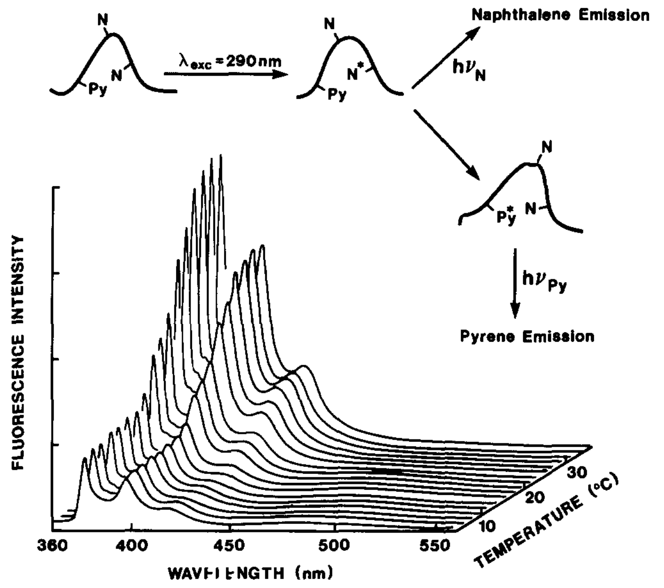

Figure 7 Fluorescence spectra of pyrene/naphthalene-labelled poly-(*N*-isopropylacrylamide) (PNIPAM-Py/366-N/30, 44 ppm) in water measured at several temperatures between 4 and 40°C; excitation wavelength: 290 nm; the 310 to 360 nm range where naphthalene emits is not shown

constant in the 4 to 18°C temperature range, but then it increased with increasing temperature to reach a constant value for solutions heated above 31°C (*Figure 7*). Comparison of spectra from solutions at 15°C and 31°C indicates that the total pyrene emission is enhanced by a factor of c. 3.3 in solutions above their LCST. The simultaneous decrease in total naphthalene emission and increase in total pyrene emission are consistent with an increase in the energy transfer efficiency above the LCST.

As a control experiment, spectra due to direct pyrene excitation ($\lambda_{exc} = 328$ nm) were also recorded as a function of temperature for solutions of doubly labelled polymers (*Figure 8*). An increase of the total pyrene emission was observed with increasing temperature in the 25°C to 31°C range. In this case, however, the amplitude of the enhancement was smaller (*Figure 9*). This increase in total emission reflects an increase of pyrene monomer emission at the expense of pyrene excimer emission, an effect also observed with singly labelled polymers. The effects are small for PNIPAM-N/27 solutions, compared to PNIPAM-Py/200 solutions.

The decrease in excimer emission above the LCST may seem surprising at first. It can be accounted for by the thermodynamics of the pyrene-labelled polymer aqueous solution[33]. Below the LCST pyrene aggregates formation results in a gain in free energy of mixing through hydrophobic interactions between the non-polar groups[34,35]. The non-polar dimers are surrounded by a cage of highly organized water, resulting in a negative entropic contribution. This unfavourable entropic term becomes increasingly important as the temperature of the solution is raised. When a given temperature, the LCST, is reached the pyrene aggregation is precluded, or at least significantly reduced.

Intermolecular interactions below and above the LCST

Above the LCST phase separation occurs. This should lead to polymer association in the polymer-rich phase. In order to assess the role of intermolecular energy transfer under the conditions of these experiments a dilute aqueous solution was prepared of a mixture of PNIPAM-Py/200 and PNIPAM-N/27 (*Table 1*). No energy transfer was detected in this solution below 31°C. Above this temperature a detectable amount of energy transfer occurred, but its efficiency was less than 5% of the value measured from solutions of the doubly labelled polymers. It seems reasonable to conclude that measurements on the doubly labelled polymer, are dominated by intramolecular contributions to the energy transfer process.

Conformational effects

A study of the phase separation process of PNIPAM solutions was reported recently by Fujishige, Kubota, and Ando[36]. They measured cloud points of solutions of fractionated PNIPAM ranging in molecular weight from 50 000 to 8.4×10^6. In this range the solution cloud points (31°) were independent of molecular weight. They also reported dynamic and static light scattering measure-

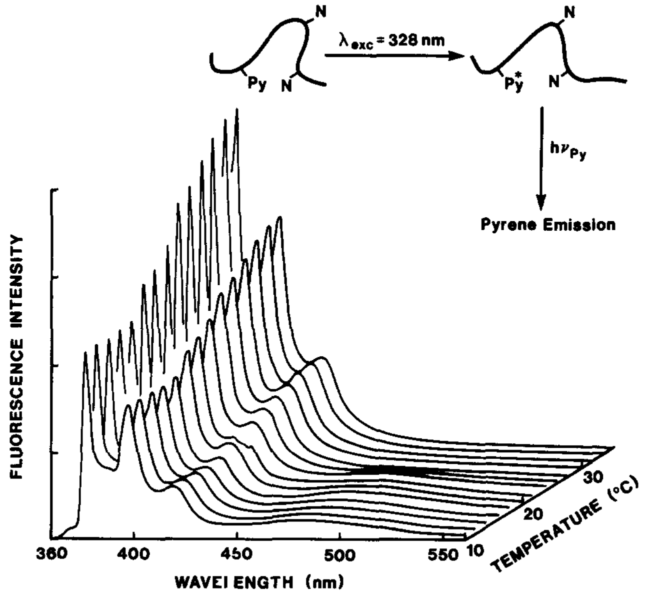

Figure 8 Fluorescence spectra of pyrene/naphthalene-labelled poly-(*N*-isopropylacrylamide) (PNIPAM-Py/366-N/50, 44 ppm) in water measured at several temperatures between 10 and 40°C; excitation wavelength: 328 nm

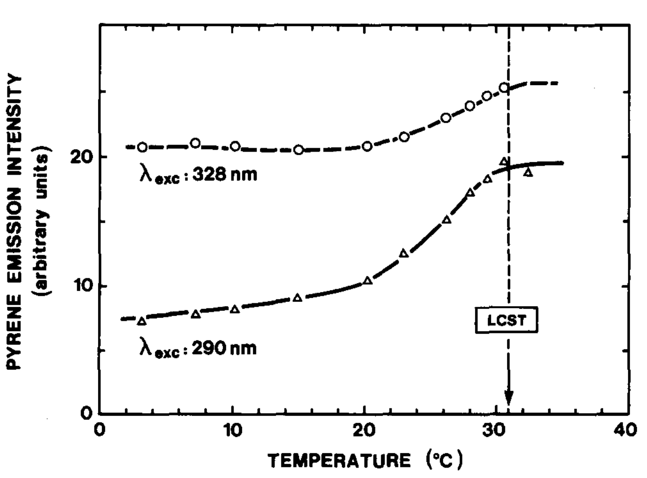

Figure 9 Plot of pyrene emission intensity as a function of temperature for an aqueous solution of pyrene/naphthalene-labelled poly-(*N*-isopropylacrylamide) (PNIPAM-Py/366-N/50, 44 ppm) in water; excitation wavelength: 290 nm (full line); 328 nm (dashed line)

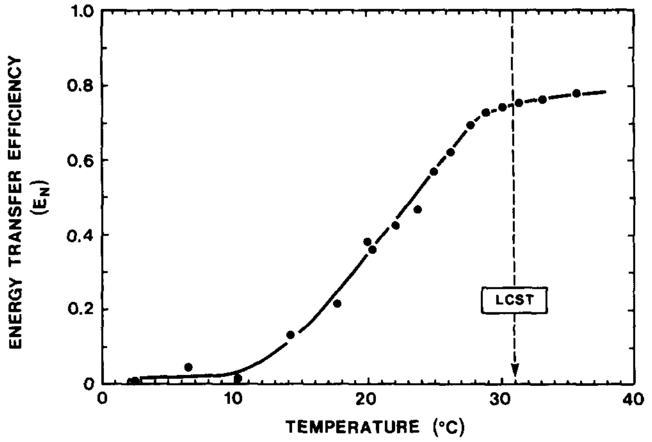

Figure 10 Plot of the non-radiative energy transfer efficiency (E_N) as a function of temperature for an aqueous solution of pyrene/naphthalene-labelled poly-(*N*-isopropylacrylamide) (PNIPAM–Py/366–N/50, 44 ppm); excitation wavelength: 290 nm

ments of very dilute solutions (c. 13 ppm) of fractionated PNIPAM (M_w 8.4×10^6). They detected an abrupt decrease in chain dimensions in the vicinity of the phase transition: the polymer hydrodynamic radius, for example, decreased from c. 1000 Å at 30°C to c. 400 Å at 32°C. From these and previous results[29] they put forward a two-stage mechanism of phase separation, initiated by a sharp coil/globule transition triggered by a change in solution temperature, followed by aggregation of individual chains into larger particles.

The fluorescence experiments described here offer a complementary view on the phase separation process. The labels are blind to changes occurring on the 400 to 1000 Å length scale. They report only on phenomena taking place in the c. 50 Å scale. But, as is the case for light scattering data, the efficiency of energy transfer between excited donors and acceptors is related to polymer dimensions. The experiments present clear evidence for a decrease in polymer dimensions upon heating: both the enhancement of pyrene emission (see *Figures* 7 and 9) and the increase in naphthalene quenching efficiency (*Table 3* and *Figure 10*) are expressions of this phenomenon.

There is an intriguing aspect of the energy transfer experiments that merits further discussion. The changes in energy transfer efficiency as a function of temperature do not occur at the LCST of the solutions, but below this temperature, and over a broad temperature range. This pattern is seen for example in the plot of E_N as a function of temperature (*Figure 10*). It implies that the average distance between the chromophores is gradually reduced as the temperature increases. This may result either from a continuous decrease in the size of the polymer coil, or from an enhancement in the density fluctuation, giving rise to a higher incidence of naphthalene–pyrene contacts. The precise nature of the conformational changes incurred by the polymer chains cannot be described on the basis of energy transfer experiments alone. However the results reported here indicate that conformational reorganization begins well below the LCST.

CONCLUSION

The energy transfer efficiency between donor and acceptor chromophores has been measured for PNIPAM doubly labelled with naphthalene (donor) and pyrene (acceptor). The value is strongly temperature-dependent for solutions of the polymer in water. It increases gradually over a c. 10°C temperature range to reach a constant value above the LCST. This increase in energy transfer efficiency indicates a substantial decrease of the average distance between donor and acceptor chromophores, in agreement with a shrinking of individual polymer chains from an expended coil to a more compact globule. In contrast to the situation found in solutions of other hydrophobic water soluble polymers, such as hydroxypropyl cellulose[37], there is no evidence for interpolymeric aggregation below the LCST. The fluorescence experiments provide a means to observe individual polymer chains. From this perspective the macroscopic phase separation is but the culmination of a large number of small amplitude conformational changes triggered by temperature induced perturbations of the polymer/water interactions.

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