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Solution Properties of Hydrophobically-Modified Copolymers of *N*-Isopropylacrylamide and *N*-L-Valine Acrylamide. A Study by Fluorescence Spectroscopy and Microcalorimetry

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ABSTRACT: Copolymers of *N*-(isopropylacrylamide) (NIPAM, 90 mol%) and *N*-L-valine acrylamide (Val, 10 mol%), as well as their hydrophobically-modified copolymers, namely a copolymer of NIPAM, Val, and *N*-*n*-octadecylacrylamide (PNIPAM-Val-C_{18}, 1 mol%) and a copolymer of NIPAM, Val, and *N*-[4-(1-pyrenyl)butyl]-*N*-*n*-octadecylacrylamide (PNIPAM-Val-C_{18}Py, 1 mol%) were prepared by free radical copolymerization of the respective monomers. Studies by turbidimetry, microcalorimetry, dynamic light scattering, and fluorescence spectroscopy indicated that the hydrophobically-modified copolymers form polymeric micelles in water (effective hydrodynamic diameter: 25 nm ± 5 nm). Solutions of all the copolymers underwent pH-dependent phase separation upon heating, but the pH- or temperature-stimulated coil-to-globule collapse/chain expansion did not result in complete disruption of the hydrophobic microdomains.

KEY WORDS *N*-Isopropylacrylamide Copolymer / Polymeric Micelle / Cloud Point / Pyrene / Amphiphilic Polyelectrolyte / Microcalorimetry /

There has been continuing interest over the years in the solution properties of polyelectrolytes.[1] The motivation for these studies stems from the complexity of the conformation of flexible chains carrying a large number of charges and from the numerous practical applications these polymers have found in industrial and household fluids. A particularly intriguing case is that of weak amphiphilic polyelectrolytes. Their conformation in solution is determined by a balance between hydrophobic cohesive interactions and repulsive electrostatic interactions.[2,3] At low ionization degree, the cohesive interactions between the hydrophobic moieties are predominant, and the polymer takes a compact, coiled conformation in which the hydrophobic groups are clustered in microdomains that tend to exclude water molecules. As the degree of ionization increases, the repulsive electrostatic interactions prevail, leading to the unfolding of the compact coil into a more expanded conformation. The first studies of weak polyelectrolytes have focussed on the solution properties of poly(acrylic acid) and poly(methacrylic acid).[4,5] Polyelectrolytes obtained from acrylic or methacrylic derivatives of amino acids have been synthesized more recently, in order to assess the relationship between the hydrophobicity of the amino acid residue and the solution properties of the polymer.[6,7] Thus, it was shown that poly(methacrylamides) bearing glycine residues in their side chains behave as normal weak polyelectrolytes, while poly(methacrylamides) bearing phenylalanine residues adopt a compact conformation at low pH, stabilized by the hydrophobic attraction of the phenyl groups.[8]

Casolaro and coworkers have reported the preparation of copolymers of *N*-isopropylacrylamide (NIPAM) and acrylamides or methacrylamides containing L-valine or L-leucine residues .[9,10,11] Other copolymers of acrylamides bearing amino acid residues and *N*-alkylacrylamides have been described,[12,13,14] but copolymers of NIPAM are unique, since the properties of their aqueous solutions depend not only on pH, but also on temperature. Indeed, graft and random copolymers of NIPAM and acrylic acid have been shown to be responsive to both pH and temperature.[15—21] Similar phenomena have been observed in the case of copolymers of NIPAM and *N*-glycine acrylamide,[22,23] and terpolymers of NIPAM, butylacrylate and (*N*,*N*-diethylamino)ethyl methacrylate.[24]

The amphiphilic character of NIPAM copolymers can be controlled by incorporation of *N*-alkylacrylamides with alkyl moieties ranging in length from decyl to octadecyl. Such copolymers, known as hydrophobically-modified (HM) copolymers, form micellar assemblies in water at all temperatures, even below the macroscopic cloud point of their solution. The micellar structures consist of hydrophobic microdomains, formed *via* association of the alkyl side chains, surrounded by a corona of hydrated PNIPAM chains.[25,26] Aggregation of the micelles below the cloud point is prevented by steric repulsion of the extended hydrated polymer chains. The heat induced collapse of these chains prompts aggregation of the micelles and macroscopic phase separation. Individual polymer micelles are disrupted with concomitant intermixing of the hydrophobic micellar domains in the macroscopically separated polymer-rich phase.[27] In contrast, polymer micelles formed in solutions of hydrophobically-modified copolymers of NIPAM and *N*-glycineacrylamide are not disrupted during the heat-induced phase transition of their aqueous solutions.[23] The micelles also preserve their integrity in solutions of pH ranging from 2 to 8 and in solutions of high ionic strength. This unexpected structural stability, related to

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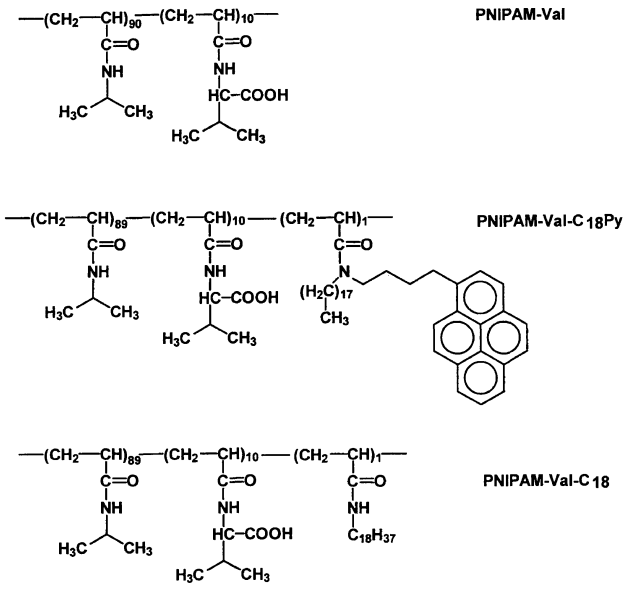

Figure 1. Structure of the polymers used in this study.

the presence of ~20 mol% glycine along the polymer chain, offers new opportunities in the design of delivery systems for drugs or other hydrophobic compounds. The present study was initiated to assess the importance of the structure of the amino acid on the properties of the corresponding HM-copolymers.

The polymers of concern here are hydrophobically-modified copolymers of NIPAM and *N*-L-valine acrylamide (Val). The isopropyl group of the valine residue imparts to this amino acid a substantial hydrophobic character, compared to glycine, the simplest amino-acid. Three NIPAM/Val copolymers were prepared (Figure 1): a copolymer of NIPAM and Val (PNIPAM-Val); a copolymer of NIPAM, Val, and *N-n*-octadecylacrylamide (PNIPAM-Val-C_{18}); and a copolymer of NIPAM, Val, and *N*-[4-(1-pyrenyl)-butyl-]-*N-n*-octadecylacrylamide (PNIPAM-Val-C_{18}Py). In all copolymers, the level of Val incorporation was kept constant (Val/NIPAM 10/90 mol%) and the degree of hydrophobic substitution was set at 1—2 mol%, relative to the sum of NIPAM and Val units. Aqueous solutions of these copolymers were analyzed by turbidimetry to determine their cloud points and by microcalorimetry to assess the thermodynamic characteristics of the phase transition. Dynamic light scattering studies were performed to detect the occurrence of polymeric micelles in solutions of the copolymers. Fluorescence spectroscopy was used to probe the fate of the polymeric micelles during pH- or temperature-induced phase separation. Results are discussed in terms of the pH- and temperature-dependent formation and disruption of micellar assemblies in aqueous solutions of the various NIPAM/Val copolymers.

EXPERIMENTAL

Materials

Water was deionized with a Barnstead NANOpure water purification system. Reagent grade solvents (Caledon) were used without further purification, except for dioxane, which was distilled from sodium under nitrogen. Acryloyl chloride, L-valine ethyl ester hydrochloride, and *n*-octadecylamine were obtained from Aldrich Chemical Company Inc. *N,N*-azobis-isobutyronitrile (AIBN) was purchased from Spectrum Chemicals. *N-n*-octadecylacrylamide and *N*-[4-(1-pyrenyl)butyl]-*N-n*-octadecylacrylamide were prepared as previously described.[25] *N*-isopropylacrylamide NIPAM (Acros Chemicals) was recrystallized from toluene:hexane (1:1, v/v). Buffers were prepared from 0.1 M citric acid and 0.1 M NaOH, except if otherwise stated. Ionic strength was adjusted by addition of NaCl (0.1 M).

Monomer and Polymer Synthesis

Synthesis of N-Valine Acrylamide Ethyl Ester. A solution of acryloyl chloride (0.905 g, 0.01 mol) in dichloromethane (10 mL) was added dropwise to a solution of L-valine ethyl ester (1.817 g, 0.01 mol) and triethylamine (2.024 g, 0.02 mol) in dichloromethane (60 mL) kept at −5℃ under nitrogen. The mixture was stirred at −5℃ for 2 h at the end of the addition. It was stirred overnight at room temperature. The reaction mixture was filtered to remove triethylamine, HCl. The filtrate was washed twice with water (10 mL) and twice with brine (10 mL). The solvent was evaporated to yield a colorless oil; ^{1}H NMR: 0.95 ppm, d, 6H: $-CH(CH_3)_2$; 1.3 ppm, t, 3H: $-COO-CH_2-CH_3$; sept, 2.2 ppm, 1H, $-CH(CH_3)_2$; quad, 4.2 ppm, 2H, $-COO-CH_2-CH_3$; quad, 4.65 ppm, 1H, $-NH-CH-CH-$; d, 5.65 ppm, 1H, $H_2C=CH-CO$; broad m, 6.1—6.3, 3H.

Poly(N-isopropylacrylamide-co-N-valine acrylamide) (PNIPAM-Val). A solution of NIPAM (1.017 g, 9 mmol) and *N*-valine-acrylamide ethyl ester (0.199 g, 1 mmol) in dioxane (15 mL) was degassed with nitrogen at 25℃ for 20 min. It was heated to 60℃ under nitrogen. A solution of AIBN (30 mg) in dioxane (2.0 mL) was added at once to the solution. The polymerization mixture was kept at 60℃ for 20 h. The solution was then cooled to room temperature. The polymer was isolated by precipitation into diethyl ether. It was purified further by two precipitations from methanol into diethyl ether. A solution of this polymer (0.76 g) in THF/water (20 mL, 4/1 v/v) was treated with a solution of NaOH (0.05 g) in THF/water (10 mL, 4/1 v/v). The hydrolysis mixture was stirred at room temperature for 15 h. The solution was then brought to pH 3.0 *via* dropwise addition of concentrated HCl. The mixture was dialysed against deionized water. The purified polymer was isolated by lyophilization (0.66 g); ^{1}H NMR ($CDCl_3$, δ) 0.84 ppm (br, $-CH_3$ Val), 1.03 (br, $-CH_3$ NIPAM), 1.45 ppm and 1.88 ppm (br, $-CH_2-CH-$, backbone), 3.83 ppm (br, NIPAM methine proton), 4.05 ppm (br, Val methine proton), 7—7.02 ppm (br, N–H); UV (THF) 242, 266, 276, 312, 326, and 342 nm.

Poly(N-isopropylacrylamide)-(N-valine-acrylamide)-(N-n-octadecylacrylamide) (PNIPAM-Val-C_{18}). The polymer (0.63 g) was prepared following the procedure described for PNIPAM-Val, starting form NIPAM (0.995 g, 9.0 mmol), *N*-valine-acrylamide ethyl ester (0.199 g, 1.0 mmol) and *N-n*-octadecylacrylamide (64 mg, 0.2 mmol), ^{1}H NMR ($CDCl_3$, δ): 0.84 ppm (tr, terminal $-CH_3$ of $C_{18}H_{37}$), 0.91 ppm (br, $-CH_3$ Val), 1.08 ppm (br, $-CH_3$ NIPAM), 1.45 ppm and 1.88 pm (br, $-CH_2-CH-$ backbone), 3.85 ppm (br, NIPAM methine proton), 4.1 ppm (br, Val methine proton), 7—7.2 ppm (br, –NH).

Poly(N-isopropylacrylamide)-(N-valine-acrylamide)-(N-[4-(1-pyrenyl)-butyl]-n-octadecyl-acrylamide) (PNIPAM-Val-C_{18}Py). The polymer (0.63 g) was prepared following the procedure described for PNIPAM-Val starting with NIPAM (0.49 g, 4.4 mmol), *N*-valine-acrylamide ethyl ester (0.092 g, 0.5 mmol) and *N*-[4-(1-pyrenyl)-butyl]-*n*-octadecylacrylamide (57.9 mg, 0.1 mmol); ^{1}H NMR ($CDCl_3$, δ): 0.87 (tr, $-CH_3$ of $C_{18}H_{37}$), 0.95 (br, $-CH_3$ Val), 1.08 (br, $-CH_3$ NIPAM), 1.65 and 1.80 (br, $-CH_2-CH-$ backbone), 3.95 (br, NIPAM and Val methine protons), 6.0—7.0 (br,–NH), 8.0—8.5 (br, aromatic protons Py); UV (methanol) 234, 242, 266, 276, 312, 326, and 342 nm.

Instrumentation

^{1}H NMR spectra were recorded on Bruker 200 MHz and 600 MHz spectrometers. UV spectra were measured with a Hewlett Packard 8452A photodiode array spectrometer, equipped with a Hewlett Packard 89090A temperature controller. Potentiometric titrations were performed using a PC Titrate system (Mantech associates Inc.) consisting of a Burivar Buret Module and PC Titration electrode. Dynamic light scattering was performed on a Brookhaven BI9000 AT instrument equipped with an argon laser (λ = 514.5 nm, scattering angle: 90°). The cell was thermostated with a Neslab RTE 111 circulating bath, and measurements were performed using polymer solutions (concentrations from 0.05 to 1 g L^{-1}) filtered through a 0.45 μm membrane prior to measurements. Data were analyzed using the software provided by the manufacturer (CONTIN calculations). Dilute solution viscosities were determined from solutions in THF at 27℃ with a Ubbelhode viscometer.

Potentiometric Titrations

A known amount of standard sodium hydroxide solution was added to a solution of a polymer aliquot (*ca.* 10 to 30 mg) in aqueous NaCl (25 mL, 0.01 M). The solution was titrated with aqueous HCl (0.1 N). The end point of the titration was determined at the inflection point of the pH curve. A blank solution prepared under identical condition, but without added polymer, was titrated as well to calculate the excess of sodium hydroxide equivalents.

Fluorescence Measurements

Fluorescence spectra were recorded on a SPEX Industries Fluorolog 212 spectrometer equipped with a GRAMS/32 data analysis system. Temperature control of the samples was achieved using a water-jacketed cell holder connected to a Neslab circulating bath. The temperature of the sample fluid was measured with a thermocouple immersed in a water filled cuvette placed in one of the four cell holders in the sample compartment. Solutions of the pyrene-labeled polymer were prepared from stock solutions (1.0 g L^{-1}) in water or methanol. The slits setting ranged from 0.5 to 2.5 mm (emission) and 1.0 to 2.0 mm (excitation), depending on the polymer concentration. The excitation wavelength was 342 nm (methanolic solutions) or 346 nm (aqueous solutions). Aqueous solutions were not degassed, whereas samples in methanol were purged during 5 min with nitrogen The pyrene excimer to monomer ratio (I_E/I_M) was calculated by taking the ratio of the intensity (peak height) at 480 nm to the half sum of the intensities at 377 and 397 nm. Excimer emission requires that an excited pyrene (Py*) and a pyrene in the ground state come in close proximity during the Py* lifetime.[28] Excimer formation occurs in concentrated Py solution or under circumstances where microdomains of high local pyrene concentration form, even though the macroscopic pyrene concentration is low. In salt solutions of PNIPAM-Val-PyC_{18} the excimer emission is strong, indicating the formation of polymer micelles.

Turbidimetry

Cloud points were determined by spectrophotometric detection of the changes in turbidity (λ = 600 nm) of aqueous polymer solutions (1.0 g L^{-1}) heated at a constant rate (0.5℃ min^{-1}) in a magnetically stirred UV cell. The value reported is the temperature corresponding to a solution transmittance of 80%.

Microcalorimetry

Calorimetry measurements were performed on a VP-DSC (Microcal Instruments) at an external pressure of 26 psi. The cell volume was 0.516 mL. The heating rate was 90℃ h^{-1}. The solutions for analysis had a polymer concentration of 0.5 g L^{-1}. They were prepared from well-equilibrated stock solutions kept at 10℃ for 24 h and degassed prior to analysis. Three measurements were performed on each sample to ensure reproducibility of the data.

RESULTS AND DISCUSSION

Preparation and Characterization of the Polymers

The copolymer PNIPAM-Val was prepared by a two-step procedure that involved copolymerization of NIPAM and *N*-valine-acrylamide ethyl ester, followed by hydrolysis of the ethyl ester groups of the intermediate copolymer. Free radical polymerization of the two monomers was performed in dioxane using azobis (isobutyronitrile) as initiator. This method had been employed in the preparation of copolymers of NIPAM and *N*-glycine-acrylamide ethyl ester.[23] We had observed that these conditions lead to statistical distribution of the two monomers along the polymer chain and that the copolymer composition closely matches the initial monomer feed ratio. The composition of PNIPAM-Val(ethyl ester) was determined by ^{1}H NMR analysis of solutions in chloroform-d, taking the ratio of signals at 0.92 ppm and 1.05 ppm, attributed to the resonance of the methyl protons of the isopropyl groups of NIPAM and Val, respectively.[10] Potentiometric titration of the carboxylic acid groups in the hydrolyzed copolymer lent support to the ^{1}H NMR analysis.

Copolymers of NIPAM and Val containing *N*-4-(1-pyrenyl)butyl-*N*-*n*-octadecyl groups or *N*-*n*-octadecyl groups, were obtained *via* the same two-step synthetic scheme (Figure 2). This protocol allowed us to carry out the copolymerization in homogenous solution, an important factor if one desires to attain random distribution of the hydrophobic substituents when preparing HM-copolymers. The level of incorporation of the hydrophobic groups, either *n*-octadecyl or *N*-4-(1-pyrenyl)butyl-*N*-

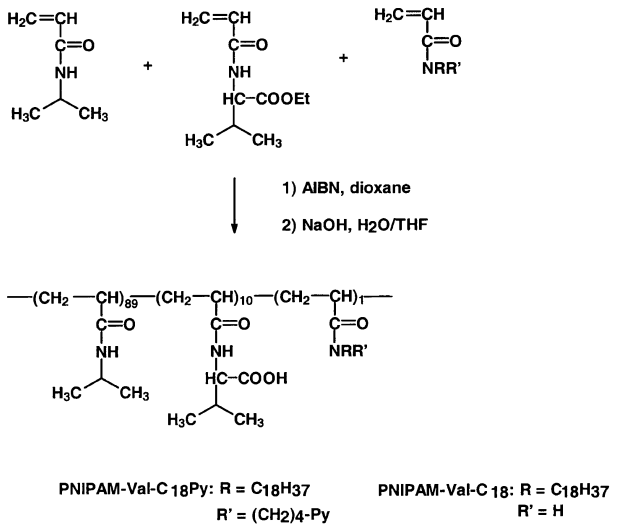

Figure 2. Synthetic scheme for the preparation of the copolymers of *N*-isopropylacrylamide and *N*-valine acrylamide.

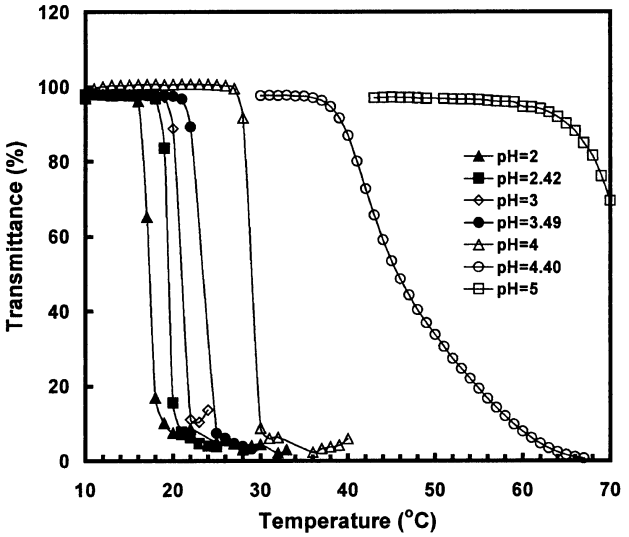

Figure 3. Changes in transmittance ($\lambda = 600$ nm) as a function of temperature for solutions of PNIPAM-Val-C_{18}Py of pH 2.00, 2.42, 3.00, 3.49, 4.00, 4.40, and 5.00; citric acid bufffers, [NaCl] = 0.1 M.

Table I. Physical properties and composition of the polymer

Polymer	Composition/mol%				M_{vis}[c]
	NIPAM	Val	C_{18}	Pyrene[b]	
PNIPAM-Val	86.6[a]	13.4[a]	—	—	96000
PNIPAM-Val-C_{18}	86.4[a]	12.5[a]	1.1[a]	—	120000
PNIPAM-Val-C_{18}Py	88 89[d]	10.0 9.0[d]	1.45[a]	1.21×10^{-4} mol g^{-1}	110000

[a] From analysis of ^{1}H NMR spectra (see text). [b] From UV absorbance spectrum (see text). [c] From viscosity measurements in THF {$[\eta] = 5.8\times10^{-5} M_{vis}^{0.78}$, see F. Ganachaud, M. J. Monteiro, R. G. Gilbert, M. A. Diurges, S. H. Thang, and E. Rizzardo, *Macromolecules*, **33**, 6738 (2000)}. [d] Determined by potentiometric titration.

n-octadecyl, was derived from the ^{1}H NMR spectra of the copolymers, using the ratio of the area of the triplet at 0.84 ppm to that of the broad signal at 3.85 ppm attributed to the NIPAM methine protons. In the case of the Py labeled copolymer, the extent of hydrophobic modification was confirmed by analysis of the UV absorption spectrum of the copolymer in methanol, using 4-(1-pyrenyl)butylamine ($\varepsilon_{342} = 32800$ mol^{-1} L cm^{-1}) as reference compound. The molar ratio of NIPAM/Val units was obtained from the ratio of the broad resonances at 1.03 and 0.88 ppm, respectively. Potentiometric titrations performed with the hydrolyzed copolymers, confirmed the carboxylic acid group content of the copolymers. The composition of the copolymers is given in Table I, together with their molecular weights determined form viscosity measurements of polymer solutions in THF.

Temperature Dependence of the Properties of the Copolymers in Aqueous Solution. Cloud Point Determinations and Microcalorimetric Studies

All the copolymers discussed here were soluble in water at or below room temperature, independent of the pH of the solutions,[29] but their solutions became turbid when heated above a certain temperature. This temperature can be determined for polymer solutions of various pH by a simple spectrophotometric method based on the detection of changes in solution transmittance at a wavelength of light absorbed by neither the solvent nor the solute ($\lambda = 600$ nm). A series of plots of the changes in transmittance of solutions of PNIPAM-Val-C_{18}Py in citric acid buffers as a function of temperature is presented in Figure 3, for solutions ranging in pH from 2 to 5. The drop in transmittance as a function of temperature is very sharp in the case of very acidic solutions (pH 2.0 to 4.0), when the valine carboxylic acid groups are fully protonated. The cloud points of solutions in the pH range 2.0 to 3.5 (18.5 to 26℃) are lower than those of PNIPAM (31℃) or PNIPAM-C_{18}Py (30.8℃),[25] an indication of the increased hydrophobicity of the copolymer due to the incorporation of valine residues. As the pH of the PNIPAM-Val-C_{18}Py solutions increases, and consequently the valine carboxylic acid groups are progressively deprotonated, the decrease in transmittance takes place over an increasingly broad temperature range, until a pH is reached for which the solution remains clear over the entire temperature range scanned (15 to 60℃). The polymer is then fully ionized and remains soluble in water at all temperature. The cloud points of the copolymer solutions exhibit a marked dependence on ionic strength. For example, solutions of PNIPAM-Val-C_{18}Py of pH 5.90 remain clear at all temperatures for salt concentrations less than 0.5 M, however they possess a cloud point when [NaCl] > 0.5 M. The temperature of the cloud point decreases with increasing salt concentration from 56.5℃ to 29℃ ([NaCl] = 2.0 M). It should be noted that there is some uncertainty associated with the determination of cloud points

Table II. Temperatures of maximum heat capacity (T_{max}) and enthalpies of the transition (ΔH) of aqueous solutions of *N*-isopropylacrylamide-*N*-valine-acrylamide copolymers

pH	T_{max}/℃ (ΔH, kcal mol^{-1} NIPAM)			
	PNIPAM-Val	PNIPAM-Val-C_{18}	PNIPAM-Val-C_{18}Py[a]	
2.49	24.0 (0.67)	19.1 (0.76)	18.2 (0.51)	*19.2*
3.02	24.4 (0.61)	19.5 (0.83)	20.1 (0.47)	*20.5*
3.50	25.3 (0.60)	21.7 (0.95)	24.6 (0.41)	*22.2*
4.00	28.8 (0.60)	26.2 (1.20)	24.7 (0.51)	*28.5*
4.51	32.2 (0.60)	29.9 (1.08)	30.0 (0.45)	*41.0*
5.00	39.5 (0.54)	35.4 (0.56)	44.2 (0.47)	*68.0*

[a] Temperatures in italics correspond to the cloud point determined by turbidimetry.

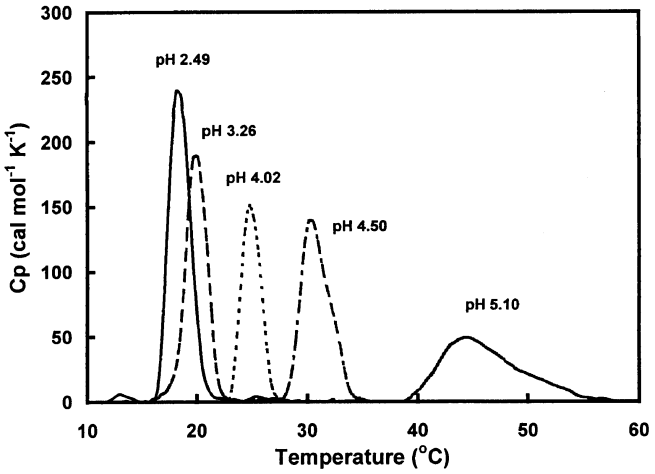

Figure 4. Microcalorimetric endotherms for aqueous solutions of PNIPAM-Val-C_{18}Py (0.5 g L^{-1}) of pH 2.49, 3.26, 4.02, 4.50, and 5.10; citric acid buffers; [NaCl] = 0.1 M; heating rate: 1℃ min^{-1}.

by turbidimetry, in particular when changes in transmittance with temperature occur over a wide range of temperature. Arbitrarily we recorded cloud point values as the temperature corresponding to a 20% decrease in transmittance. This value can be used to compare cloud points measured under the same conditions, but it does not necessarily reflect the true transition temperature. To address this issue it is useful to measure transition temperatures by an alternate method such as microcalorimetry, a sensitive technique which yields in a single measurement the temperature of the transition and the enthalpy associated with the phase transition.[30]

The changes with temperature of the molar heat capacity of aqueous solutions of PNIPAM-Val, PNIPAM-Val-C_{18}, and PNIPAM-Val-C_{18}Py were recorded for solutions of pH ranging from 2.50 to 5.10. The transition temperatures and the enthalpies associated with the transitions are listed in Table II. Representative calorimetric traces obtained with solutions of PNIPAM-Val-C_{18}Py are presented in Figure 4. The value of the transition temperature is identical to the cloud point for solutions of low pH, but not for solutions of pH > 4.0, where the transition temperatures measured by the two technique differ by as much as 24℃ (pH = 5.0, see Table II). The largest discrepancies are noted for the solutions of highest pH, for which the changes of transmittance with temperature occur over several ℃ and for which the endotherms are broad (Figures 3 and 4). The enthalpies of

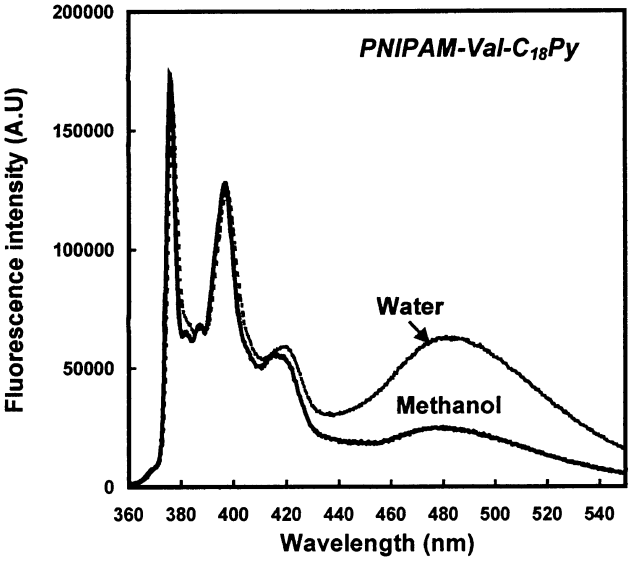

Figure 5. Fluorescence spectra of PNIPAM-Val-C_{18}Py in methanol and in water; T = 25℃; polymer concentration: 0.02 g L^{-1}, λ_{exc} = 342 nm (methanol), 346 nm (water).

transition computed with respect to the concentration of NIPAM units are listed in Table II. For each copolymer, the heat of transition is independent of pH, indicating that the thermodynamics of the phase transition reflect mostly the response of the NIPAM units to changes in temperature. However the value measured for NIPAM-Val copolymers (~0.6 kcal mol^{-1}) is significantly lower than that registered for the phase separation of PNIPAM solutions (~0.9 kcal mol^{-1}) measured by us under the same conditions and reported by Fujishige.[31] The latter value is consistent with a loss of approximately one hydrogen bond per repeat unit.[32] The difference in the heat of phase transition for the two polymers may reflect different structures of the hydration layer for the two polymers. Pressure perturbation calorimetry measurements are being carried out to evaluate this hypothesis.[33]

Spectroscopic Properties of the Pyrene-Labeled Copolymers

The labeled copolymer PNIPAM-Val-C_{18}Py carries pyrene chromophores in the vicinity of the hydrophobic side chains. This labeling site was chosen to allow us to assess by fluorescence the formation of micellar assemblies in aqueous solutions of this polymer. First we compared fluorescence spectra of PNIPAM-Val-C_{18}Py in water and in methanol. The spectrum of PNIPAM-Val-C_{18}Py in water consists of two contributions: an emission due to locally isolated excited pyrenes (intensity I_M, pyrene "monomer" emission) with the [0.0] band located at 376 nm and a strong, broad band centered at 480 nm, due to pyrene excimer emission (intensity I_E) (Figure 5). In contrast, solutions of PNIPAM-Val-C_{18}Py in methanol exhibit only a weak excimer emission, relative to the pyrene monomer emission. Methanol is a good solvent for PNIPAM and its hydrophobically-modified copolymers.[25] In this solvent the polymer adopts an open coil conformation. The observation that the excimer emission from PNIPAM-Val-C_{18}Py methanolic solutions is weak con-

firms that statistical distribution of the comonomers was achieved in the synthesis of PNIPAM-Val-C_{18}Py.

In aqueous solution of PNIPAM-Val-C_{18}Py, however, excimer emission is strong, suggesting that chromophores linked to distant units along the polymer backbone are brought into close proximity, sequestered within aggregates formed *via* interaction of the hydrophobic octadecyl chains. This conclusion was corroborated by scrutinizing the mechanism of pyrene excimer formation. Indeed, according to the mechanism originally proposed by Birks,[34] pyrene excimers form by a dynamic encounter of an excited pyrene and a ground state pyrene. However, it is frequently observed that excimer emission from aqueous solutions of pyrene-labeled polymers originates from preformed ground-state pyrene aggregates. A comparison of the UV absorption spectra and the excitation spectra monitored for the excimer and monomer emissions[28] of polymer solutions in methanol led to the conclusion that in this solvent pyrene excimers form by the dynamic mechanism, whereas in water, the pyrene excimers originate from ground state pyrene aggregates.

We investigated the response of PNIPAM-Val-C_{18}Py in aqueous media to changes in pH by recording the emission of pyrene from solutions of PNIPAM-Val-C_{18}Py in a series of citric acid buffers ranging in pH from 2 to 10. All solutions were of constant ionic strength (0.1 M NaCl) and the measurements were carried out at constant temperature, 15℃, a value selected such that it lies below the phase transition temperature of even the most acidic solution. The overall features of the emission spectrum are maintained for all pH values, but the pyrene monomer emission intensity increases with increasing pH at the expense of the pyrene excimer emission. The effect is illustrated in Figure 6, where the values of the ratio I_E/I_M are plotted as a function of pH. The ratio I_E/I_M decreases slightly for $2.0<pH<4.0$, then it undergoes a sharp drop in a narrow pH range (4.0 to 4.6) which encompasses the pK_a of the polymer (4.4). The decrease in I_E/I_M signals disruption of the pyrene/*n*-octadecyl aggregates induced by the electrostatically-driven expansion of the polymer chain as the valine carboxylic acid substituents are deprotonated. It is noteworthy that, even in strongly alkaline solutions, the excimer contribution to the total fluorescence of PNIPAM-Val-C_{18}Py remains significant, an indication that the micellar structures are only partially disrupted by the expansion of the macromolecule. This result contrasts with previous reports of pH-induced changes in the fluorescence of weak polyelectrolytes that do not carry hydrophobic groups, such as pyrene-labeled poly(acrylic acids)[35,36] and copolymers of NIPAM, *N*-glycine acrylamide, and *N*-1-pyrenylmethyl acrylamide,[23] for which the excimer emission all but disappears in spectra of ionized polymers. Our results may imply that the micellar assemblies in solutions of PNIPAM-Val-C_{18}Py reorganize upon deprotonation of the valine carboxylic groups, but that some level of association of the hydrophobic groups persists at all pH's.

Next, we monitored the temperature-induced changes in the fluorescence of polymer solutions of constant pH. In these experiments, solutions of PNIPAM-Val-C_{18}Py of constant ionic strength ([NaCl]=0.1 M) were prepared

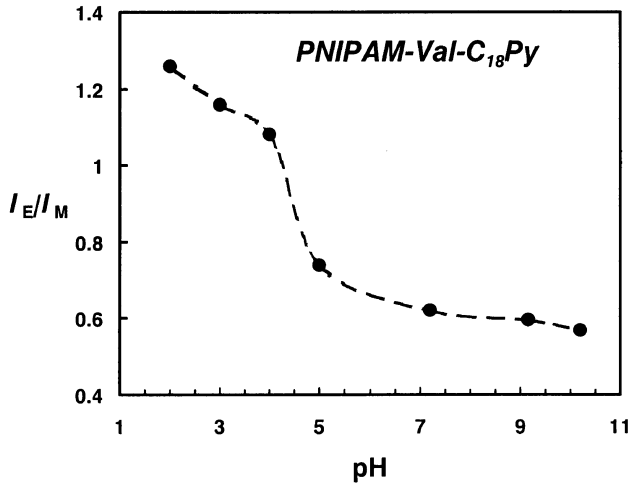

Figure 6. Changes in the ratio, I_E/I_M, of the excimer to monomer emission intensities for solutions of PNIPAM-Val-C_{18}Py in citrate buffers; [NaCl]=0.1 M polymer concentration: 0.02 g L^{-1}; λ_{exc}= 346 nm; T=15℃.

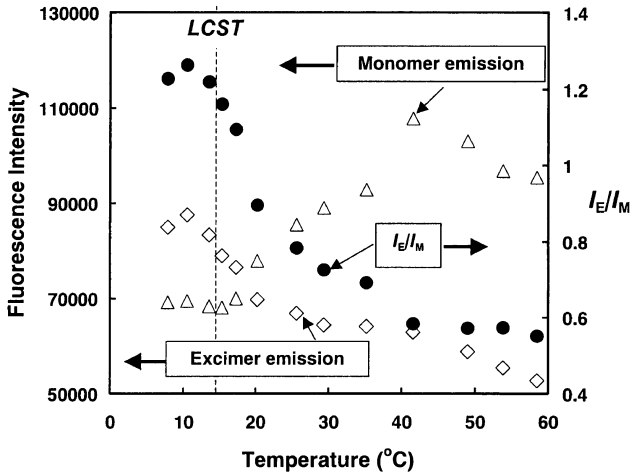

Figure 7. Changes in the pyrene excimer emission intensity, (open diamonds), pyrene monomer emission intensity (open triangles), and in the ratio I_E/I_M of pyrene excimer emission intensity to pyrene monomer emission intensity (full circles) as a function of temperature for a solution of PNIPAM-Val-C_{18}Py of pH 2.0; polymer concentration: 0.02 g L^{-1}; λ_{exc}=346 nm; The dotted line indicates the lower critical solution temperature (LCST, from turbidity measurements) of a PNIPAM-Val-C_{18}Py solution of pH 2.0.

in citric acid buffers of pH 2.0, 3.0, and 4.0. Each solution was heated in the spectrometer sample compartment and emission spectra were recorded as a function of temperature.[37] As the temperature of the solutions was raised, the total emission intensity did not vary significantly, but the relative intensities of monomer and excimer emissions were affected, as represented in Figure 7 for a solution of PNIPAM-Val-C_{18}Py of pH 2.0. In this case, the monomer emission remained constant from 7℃ to ~14℃, while in the same temperature range, the excimer emission increased slightly, an effect best detected by the small increase of the ratio I_E/I_M (full circles, Figure 7). Further heating of the solution resulted in a sharp decrease of the ratio I_E/I_M from 1.25 (14℃) to 0.65 (28℃), corresponding to an increase in monomer emission intensity at the expense of the exci-

mer emission intensity. Continued heating brought no further changes to the ratio I_E/I_M. Identical trends were observed when heating solutions of pH 3.0 and 4.0, except that the temperatures corresponding to the onset of the decrease in I_E/I_M were ~20℃ and ~25℃, respectively, temperatures corresponding to the cloud point of each solution. When solutions of PNIPAM-Val-C_{18}Py of pH 5.0 were heated from 10 to 60℃, the ratio I_E/I_M remained constant (0.70 ± 0.05). Under these conditions the copolymer is fully ionized and responds only sluggishly to changes in temperature, as observed already in the microcalorimetry and turbidity data.

Dynamic Light Scattering Studies

Dynamic light scattering (DLS) measurements performed on aqueous solutions PNIPAM-Val-C_{18} and PNIPAM-Val-C_{18}Py (0.1 g L^{-1}, 15℃, [NaCl] = 0.1 M, pH 2.5 to 6.0) revealed the presence of polymeric micelles with an effective hydrodynamic diameter of 25 ± 5 nm. In contrast, no DLS signal was detected when measurements were performed under the same conditions with solutions of PNIPAM-Val. These preliminary experiments provide further support for the occurrence of polymeric micelles in aqueous solutions of the hydrophobically modified copolymers.

CONCLUSION

Hydrophobically-modified copolymers of NIPAM and *N*-valine acrylamide form polymeric micelles in water as a result of the association of the hydrophobic groups. The copolymers exhibit sensitivity to both changes in temperature, due to the presence of the *N*-isopropylacrylamide residues and to changes in pH, due to the presence of the carboxylic acid groups of the *N*-valine acrylamide units. Evidence from microcalorimetry, dynamic light scattering and fluorescence label studies indicates that 1) in their protonated form, copolymers of NIPAM and Val are less soluble in water than PNIPAM and their solubility depends critically on the level of Val incorporation; 2) it is possible to adjust conditions of pH (between 2.0 and ~6) and of temperature (between 15 and ~50℃) to trigger phase separation in copolymer aqueous solutions; 3) in polymeric micelles, the main chain remains responsive to changes in temperature or pH but the hydrophobic microdomains persist throughout ionization-induced chain expansion or temperature-induced chain collapse, although data from fluorescence label experiments point to some level of reorganization within the hydrophobic microdomains.

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37. Note that fluorescence measurements were performed on solutions far less concentrated than those used to monitor changes in turbidity (0.02 g L^{-1} *vs.* 1.0 g L^{-1}) in order to avert artifacts due to inner filter effects associated with excessive pyrene concentration and undesired scattering of the excitation light by turbid samples. Solutions for fluorescence experiments remained clear to the eye even above the cloud point.