Colloid Polym Sci 278:80–83 (2000)

SHORT COMMUNICATION

Y. Yamazaki
T. Tada
S. Kunugi

Effects of acrylic acid incorporation on the pressure–temperature behavior and the calorimetric properties of poly(*N*-isopropylacrylamide) in aqueous solutions

Received: 19 July 1999
Accepted in revised form: 7 September 1999

Y. Yamazaki · T. Tada · S. Kunugi (✉)
Department of Polymer Science
and Engineering
Kyoto Institute of Technology
Matsugasaki, Sakyo
Kyoto 606-8585, Japan

Abstract We studied the effects of pH on the pressure–temperature dependence of coil–collapse transition for aqueous solutions of copolymers of *N*-isopropylacrylamide and acrylic acid (Ac). At low pressures, the transition temperature (T_{tr}) increased with pressure, but T_{tr} decrease with increasing pressure at pressures higher than 50–100 MPa. By increasing the pH, the transition contour shifted to a higher temperature. When the Ac content was increased, the effects of pH became more evident. From a calorimetric study at atmospheric pressure, ΔH_{tr} was found to become smaller by increasing the portion of the ionized residues in the copolymer. The ratio to the van't Hoff enthalpy changes became larger with an increase in pH, which indicated that the production of charge decreased the cooperative domain size.

Key words Poly(*N*-isopropylacrylamide) · Poly(acrylic acid) · Cloud point · Thermoresponsive polymer · High pressure

Introduction

There are several known synthetic polymers [1–3] that carry both hydrogen-bonding properties and hydrophobic interactions in aqueous solutions and show distinct changes in their molecular level states (coil to collapse or globule, in solution) and volume phase transitions (in gel form). We have shown that aqueous solutions of these polymers [4–6] exhibited characteristic pressure–temperature behavior; near atmospheric pressure, the temperature of the coil–collapse (globule) transitions increased with an increase in pressure, but decreased when the pressure increased above about 100 MPa, to give the apparent extremum in the temperature–pressure diagrams. The behavior was explained by a general thermodynamic equation for the pressure- and temperature-induced denaturation of proteins [7, 8]. These results, coupled with those of the effects of salts and solvents, indicated the possibility of using these thermoresponsive polymers as synthetic models of the so-called cold denaturation of proteins in a reversible manner [9, 10].

In real proteins, however, another type of the distinct interacting forces is at work, namely electrostatic interactions, and several studies have been reported on the effects of anionic (or cationic) charge incorporation on the thermoresponsive properties of these polymers and their gel forms [11–14]. We take the copolymer of *N*-isopropylacrylamide (NIPAM) and acrylic acid (Ac) as a model in this report, and study the effects of anion generation, by changing pH, on the pressure–temperature behavior of the coil–globule transition of the polymer in the pressure–temperature plane (10–50 °C/0.1–400 MPa) as well as on their calorimetric properties at atmospheric pressure.

Materials and methods

NIPAM monomer was donated by Kojin Co. (Tokyo, Japan) and was purified from a benzene/hexane system. The homopolymer and the copolymer were polymerized by a radical initiator 2,2′-Azobis(isobutyronitile) and were purified by precipitation from diethyl ether. The molecular weights and molecular–weight distributions of the polymers obtained were determined by gel permeation chromatography (GPC) [TSK-gel super-H 3000 and

4000 columns with a *N*,*N*-dimethylformide solution (containing 10 mM LiBr); M_n was based on commercial poly(ethylene glycel) poly(ethylene oxide) standards; the equipment used was a Shimadzu 10A GPC system with a refractive index detector]. The content of the Ac monomer was determined by IR spectrometry. The Ac content was also certified by a reverse titration with dilute NaOH solutions by using conductometry and by pH titration. The estimated Ac content, M_n and M_w/M_n of the three copolymers were 5.4 (monomer mol/mol)%, 330 kDa, and 2.8, 37.9%, 340 kDa, and 3.0, and 75.0%, 94 kDa, and 1.8.

The cloud points of the aqueous solutions of these polymers (normally 0.1 wt/v%) were determined by observing the light transmission. A high-pressure optical cell, with two sapphire windows [15] (Teramecs Co., Kyoto, Japan), was placed between the light source (Xe lamp) and the monochrome/photomultiplier (Otsuka Electronics Co., Hirakata, Japan) via optical fibers. The apparent transmittance at 500 nm was recorded by changing either the temperature at a constant pressure or the pressure at a constant temperature. The temperature of the cell was controlled by a Peltier-type thermoregulator and detected by a Pt resistance thermometer. The extraneous pressure was applied by a high-pressure hand pump equipped with an intensifier (ratio 8.5:1) (Teramecs Co.), and the pressure medium was deionized water [15]. The pressure was measured by a Bourdon-tube-type pressure gage. The transition temperature or pressure was determined as the peak of the 1st derivative of the transmittance–temperature (or pressure) curve.

Differential scanning calorimetry (DSC) measurements were performed using a high-sensitivity differential scanning calorimeter (Nano-DSC model 5100; Calorimetry Science Co., Utah, USA). About 1 ml 0.1% aqueous solution of polymer was introduced to the sample tube of the apparatus and the temperature scanning rate was usually 1 °C/min. The reference solution was the equilibrium solution in the dialysis of each sample overnight. The pH of the solution was adjusted by adding an appropriate amount of NaOH solution before dialysis and the pH value of the sample solution was recorded after the DSC measurement.

Results and discussion

Effects of pH on the pressure-temperature dependence of the transition

The cloud points of aqueous solutions of three copolymers of NIPAM containing 5, 38, and 75 (monomer mol)% of Ac (pNIPAM$_x$-*co*-Ac$_y$), equilibrated at various pH, were measured by scanning the temperature under various constant pressures or scanning the pressure at various constant temperatures. The results are shown in Fig. 1. For pNIPAM$_{95}$-*co*-Ac$_5$, the curve obtained is more or less similar to that for the homopolyNIPAM solution at pH 2 or 3: the overall shape is circular or ellipsoidal and the extremum was observed at about 50 MPa. At lower pressures, the transition temperature (T_{tr}) increased with pressure, but T_{tr} decreased with increasing pressure when the pressure became much higher. By increasing the pH, the transition contour shifted to a higher temperature and it was practically invisible above 100 MPa at pH 4 or at all pressures at a pH above 5. When the Ac content was increased, the effects of pH became more evident; for pNIPAM$_{62}$-*co*-Ac$_{38}$ the contour shifted at pH 3 and the transition pressure (P_{tr}) at the same temperature shifted to a higher pressure. Above this pH, the transition of pNIPAM$_{62}$-*co*-Ac$_{38}$ was practically invisible at any pressure applicable in our system. For this copolymer, T_{tr} at pH 2 and atmospheric pressure was slightly lower than that for pNIPAM$_{95}$-*co*-Ac$_5$, while the latter value was almost identical to the T_{tr} of homopolyNIPAM of the corresponding M_n. The copolymer having a much larger portion of Ac (pNIPAM$_{25}$-*co*-Ac$_{75}$) showed a much larger deviation of the transition point with respect to pH. At pH 3, T_{tr} increased monotonously with increasing pressure and no extremum was observed within the measured range of pressure and temperature. At pH > 3, the transition became invisible at any pressure. Moreover T_{tr} of this copolymer at pH 2 and 0.1 MPa was much lower than that for pNIPAM$_{95}$-*co*-Ac$_5$ or pNIPAM$_{62}$-*co*-Ac$_{38}$. It is known that the H-form (acidic form) of homopoly Ac of relatively large molecular weights shows an exothermic dissolution phenomenon at around room temperature [17–19], and pNIPAM-*co*-Ac having very high contents of Ac may show some similarities to such a homopolymer.

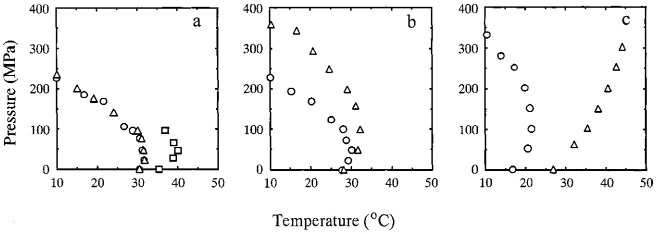

Fig. 1a–c Temperature and pressure dependence of the cloud point of copolymers of *N*-isopropylacrylamide (*NIPAM*) and acrylic acid (*Ac*) in aqueous solutions. pH 2 (○); pH 3 (△); pH 4 (□). Copolymer concentration of 0.1 wt/v%. *a* pNIPAM$_{95}$-*co*-Ac$_5$, *b* pNIPAM$_{62}$-*co*-Ac$_{38}$, *c* pNIPAM$_{25}$-*co*-Ac$_{75}$

The carboxylic acid pendant performs proton dissociation at higher pH affecting the transition at a much lower stage of dissociation well below the apparent half-neutralization pH of the copolymer (about 5.2 for these three polymers; data not shown). This effect is more evident when the carboxylic acid content becomes higher.

The transitions of the thermoresponsive polymers in aqueous solutions occur by a dehydration process and a strengthening of hydrophobic interactions among the side chains. The results for the ionic copolymers are primarily explained by considering the electrostatic repulsive forces in the dehydration process, the strengthening of the hydration around the side chains through ion–dipole interactions, and the weakening of hydrophobic interactions among the side chains. In the case of carboxylic acid copolymers, pressure will affect the acid dissociation process to a large extent, since it is known that carboxylic acids such as acetic acid show large and

Table 1 Thermodynamic parameters of the transition of poly(*N*-isopropylacrylamide) (*pNIPAM*)-*co*-acrylic acid (*Ac*) in aqueous solution as determined by differential scanning calorimetry (*DSC*) measurement. 0.1% aqueous solution of polymer. The temperature scanning rate was 1 °C/min. The reference solution was the equilibrium solution in the dialysis of each sample overnight. The pH of the solution was adjusted by adding the appropriate amount of NaOH solution before dialysis and the pH value of the sample solution was recorded after the DSC measurement

Co-polymer	M_n kDa	pH	T_{tr} °C	ΔH_{tr} kJ/mol	ΔC_p J/mol/K	ΔH_{vH} kJ/mol	$\Delta H_{tr}/\Delta H_{vH}$
pNIPAM_{95}-*co*-Ac_5	330	2	34.2	20661	−532	3694	5.6
		3	34.3	24613	−463	3602	6.7
		4	36.2	20045	−182	2336	8.3
		5	38.0	19209	−408	1182	16.1
		6	39.4	14344	−163	928	15.4
pNIPAM_{62}-*co*-Ac_{38}	340	5	42.1	6588	−79	1271	5.3
pNIPAM_{25}-*co*-Ac_{75}	94	5	47.1	260	−10	3364	0.08

negative ΔV (i.e., −11 ml/mol for acetic acid at 25 °C) [16]. The dissociation (anion formation) is facilitated under high pressure, and the shift of the transition curves in carboxylate copolymers is enhanced under higher pressure; this might be one reason why the pH and hence the acid dissociation affected the pressure response to a larger extent than the temperature response.

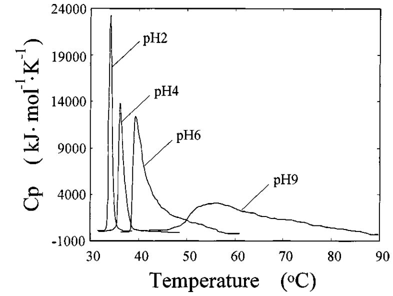

Fig. 2 Differential scanning calorimetry thermogram of pNIPAM_{95}-*co*-Ac_5 at various pHs under atmospheric pressure. Copolymer concentration of 0.1 wt/v%. Temperature scanning of 1 °C/min

Effects on the calorimetric properties at atmospheric pressure

The DSC thermogram of pNIPAM_{95}-*co*-Ac_5 at different pH is shown in Fig. 2. At pH 2, an endothermic peak similar to that of homopolyNIPAM was observed, though its peak position is somewhat higher and its peak height is slightly lower than the latter. At pH 4, the peak position shifted to a higher temperature an the peak height became smaller. By increasing the pH further, the peak shifted to a higher temperature and it became broader and rather asymmetric. The peak position (approximately equal to the transition temperature, T_{tr}) and the enthalpy change (ΔH_{tr}), as calculated by integrating the peak and the ΔC_p values are shown in Table 1.

With an increase in the portion of the ionic residues in the copolymer by increasing the pH, T_{tr} shifted to higher values and ΔH_{tr} became smaller. These changes in the thermodynamic parameters are also explained by the charge production factor in the side chains, the electrostatic repulsive forces in the dehydration process, and the strengthening of the hydration. The latter contribution is even achieved only by introducing the noncationized acid groups in the main chain. The effect of Ac content on the T_{tr} of the NIPAM–Ac copolymer was measured at pH 5, and the result is also compiled in Table 1. With an increase in the Ac content. T_{tr} shifted to higher temperatures and ΔH_{tr} became smaller.

From these thermograms and the derived thermodynamic parameters, approximate van't Hoff enthalpy changes (ΔH_{vH}) were calculated, assuming the transition is in two states; the calculated values are listed in Table 1, as well as the ratios of $\Delta H_{vH}/\Delta H_{tr}$ [20, 21]. This parameter is related to the number of cooperative domains in one polymer molecule to show the structural transition. A smaller value means the collapse domain is larger. The charge production on the copolymers resulted in an increase in this parameter, which denotes that the cooperative domain size becomes smaller, maybe due to the division of the domain by the interference from charged groups.

References

1. Heskins M, Guillet JE (1968) J Macromol Sci Chem 2:1441
2. Schild HG (1992) Prog Polym Sci 17:163
3. Shibayama M, Tanaka T (1993) Adv Polym Sci 109:1
4. Kunugi S, Takano K, Tanaka N, Suwa K, Akashi M (1997) Macromolecules 30:4499
5. Suwa K, Yamamoto K, Akashi M, Takano K, Tanaka N, Kunugi S (1998) Colloid Polym Sci 276:529
6. Kunugi S, Yamazaki Y, Takano K, Tanaka N, Akashi M (1999) Langmuir 15:4056
7. Hawley SA (1971) Biochemistry 10:2436
8. Smeller L, Heremans K (1997) In: Heremans K (ed) High pressure research in bioscience and biotechnology. Leuven University Press, Leuven, pp 55–58
9. Tiktopulo EI, Bychkova VE, Ricka J, Ptitsyn OB (1994) Macromolecules 27:2879
10. Tiktopulo EI, Uversky VN, Lushchik BB, Klenin SI, Bychkova VE, Ptitsyn OB (1995) Macromolecules 28:7518
11. Chen G, Hoffman AS (1995) Makromol Chem Phys 196:1251
12. Seida Y, Nakano Y (1993) J Chem Eng Soc Jpn 26:328
13. Shibayama M, Ikkai F, Inamoto S, Nomura S, Han CC J Chem Phys 105:4358
14. Kawasaki H, Sasaki S, Maeda H (1997) J Phys Chem 10:5089
15. Morishita M, Tanaka T, Kawai S (1997) In: Suzuki A, Hayashi R (ed) High pressure bioscience and technology; San-ei Publishing, Kyoto, pp 187–192
16. Kunugi S (1993) Prog Polym Sci 18:805
17. Safronov AP, Tager AA, Klyuzhin ES, Adamova LV (1993) Polym Sci USSR Ser A 35:818
18. Adamova LV, Klyuzhin ES, Safronov AP, Nerush NT, Tager AA (1993) Polym Sci USSR Ser B 35:982
19. Tager AA, Klyuzhin ES, Adamova LV, Safronov AP (1993) Polym Sci USSR Ser B 35:1125
20. Privalov PL (1979) Adv Protein Chem 33:167
21. Privalov PL (1982) Adv Protein Chem 35:1