

Microcalorimetric Study of Aqueous Solution of a Thermoresponsive Polymer, poly(*N*-vinylisobutyramide) (PNVIBA)

Shigeru KUNUGI,[†] Tomoko TADA, Naoki TANAKA,
Kazuya YAMAMOTO,* and Mitsuru AKASHI*

*Department of Polymer Science and Engineering, Kyoto Institute of Technology,
Matsugasaki, Sakyo, Kyoto 606–8585, Japan*

**Department of Applied Chemistry, Kagoshima University,
Koorimoto, Kagoshima 890–0065, Japan*

(Received March 4, 2002; Accepted April 3, 2002)

ABSTRACT: A temperature-responsive synthetic vinyl polymer with hydrogen-bonding and hydrophobic side residues, poly(*N*-vinylisobutyramide) (PNVIBA), was studied for its calorimetric properties in aqueous solutions. The temperature-responsive behavior was dependent on the polymer concentration at lower range and also on the concentration of added sodium dodecyl sulfate (SDS) at higher range. Thermodynamic parameters and molecular weight dependence were discussed in comparison with the corresponding results from poly(*N*-isopropylacrylamide) (PNIPAM).

KEY WORDS Thermoresponsive Polymer / Poly(*N*-isopropylacrylamide) / Poly(*N*-vinylisobutyramide) / Sodium Dodecylsulfate / Microcalorimetry / Thermodynamics /

Several synthetic vinyl polymers with both hydrogen bonding and hydrophobic properties are known to show changes in their molecular level states (in solution) and volume phase transitions (in gel form) in aqueous media, responding to the changes of the environmental factors such as temperature. Poly(*N*-isopropylacrylamide) (PNIPAM) and other poly(acrylamide) derivatives are the best known examples.^{1–3} These polymers show sharp reversible transitions from extended (coil) state to compact (collapse) state in solution, upon a change in temperature. Several applications such as drug delivery, immobilization of enzymes, and bioseparation have been studied by taking advantage of these characteristic temperature-responsive properties.^{4–6}

Some of the poly(vinylamine) derivatives, such as poly(*N*-vinylisobutyramide) (PNVIBA), also show distinct thermoresponsive properties,^{7–9} and we have studied the effects of pressure, salt, and surfactants on them, and compared with those of poly(acrylamide) derivatives.^{10–13}

PNVIBA and PNIPAM have the side chain amide bonds in inverted directions. Both are isomers of polyleucine and can be considered as a simple but relevant model of proteins.^{14,15} The negative heat capacity changes (ΔC_p) upon transition observed for these polymers have been discussed with relation to the cold denaturation of proteins,¹⁶ and their characteristic elliptic temperature-pressure transition diagrams have been considered to give a general idea of protein denaturations induced by high hydrostatic pressures.¹⁷

PNVIBA has many similarities to PNIPAM, but also shows some differences, such as transition sharpness and higher transition temperature (T_t) and pressure (P_t). In this report, we studied the calorimetric properties of PNVIBA in aqueous solutions by using a high-sensitive differential scanning calorimeter (DSC), as for the polymer concentration dependence, effects of SDS addition, and molecular weight dependence. The results were compared with the corresponding results of poly(*N*-isopropylacrylamide) (PNIPAM) solution, from our own and reported sources.^{14,15,18}

EXPERIMENTAL

Materials

Homopolymers of PNVIBA were synthesized as described previously⁷ and chromatographically fractionated. Their molecular weights and molecular weight distributions were determined by GPC [Shodex AD-80M/S or TSK-gel super-H 3000 and 4000 columns with a DMF solution (containing 10 mM LiBr)]. M_n values indicated below are based on the commercial PEG-PEO standards. The GPC equipment was a Tosoh HLC-8120 GPC or a Shimadzu LC10A system with an RI detector. Three samples of PNVIBA ($M_n = 11$ kDa and $M_w/M_n = 1.4$, $M_n = 66$ kDa and $M_w/M_n = 1.6$, and $M_n = 460$ kDa and $M_w/M_n = 2.4$) and one sample of PNIPAM ($M_n = 49$ kDa and $M_w/M_n = 1.8$) were studied.¹² Sodium dodecyl sulfate (SDS) and other chemical reagents were purchased from Nacalai Tesque (Kyoto, Japan) or Wako Pure Chemicals (Os-

[†]To whom correspondence should be addressed (Tel: +81-75-724-7836, Fax: +81-75-724-7800, E-mail: kunugi@ipc.kit.ac.jp).

aka, Japan).

Methods

DSC measurements were performed by a high-sensitive DSC meter, Nano-DSC II Model 6100 (Calorimetry Science Co., UT, USA). About 0.3 mL of an aqueous solution of polymer was introduced to the sample tube of the apparatus and the temperature-scanning rate was usually 1 K min⁻¹. The effects of scanning rate on the obtained thermodynamic parameters were preliminary tested and checked. The parameters obtained from these DSC measurements were calculated on the base of polymer molarity and the M_n was used for the calculations. The van't Hoff enthalpy (ΔH^{vH}) was approximately calculated with the following equation.^{14, 19}

$$\Delta H^{vH} = 4RT_p^2(C_{p,max}/\Delta H),$$

where T_p , $C_{p,max}$ and ΔH are the peak temperature, maximum C_p , and enthalpy change of transition as integrated from the thermogram, respectively. The cooperative number (n) is the ratio of ΔH and ΔH^{vH} and indicates the (average) number of cooperative units or domains in one polymer molecule.

RESULTS

Polymer Concentration Dependence

The thermograms observed for various concentrations of PNVIBA ($M_n = 11$ kDa) are shown in Figure 1. PNVIBA of other molecular weights gave fundamentally similar thermograms. The T_t observed at 0.04 (w/v)% (41.7°C) was almost identical with that observed in turbidimetry.¹² With diluting polymer solutions, the endothermic peak shifted slightly to the higher temperature and the peak was 45.0°C at 0.001 (w/v)%, though the thermogram was rather noisy with such a low concentration of polymer. The thermodynamic parameters calculated from these and other thermograms are compiled in Table I, together with the corresponding results from PNIPAM ($M_n = 49$ kDa). As reported before,^{12, 15} polymers with smaller molecular weight showed higher transition temperature. Both ΔH and ΔS of transition increased with increasing polymer concentration. The van't Hoff enthalpy (ΔH^{vH}) and hence the cooperative number ($n = \Delta H/\Delta H^{vH}$) showed some specific values at the lowest concentration, probably due to the lower quality of the thermograms at such low concentrations, but they did not show significant concentration dependence. To compare the thermodynamic values obtained for polymers of different M_n , the concentration dependence of ΔH and ΔS per monomer unit ($\Delta H_{monomer}$ and $\Delta S_{monomer}$) are shown in Figure 2,

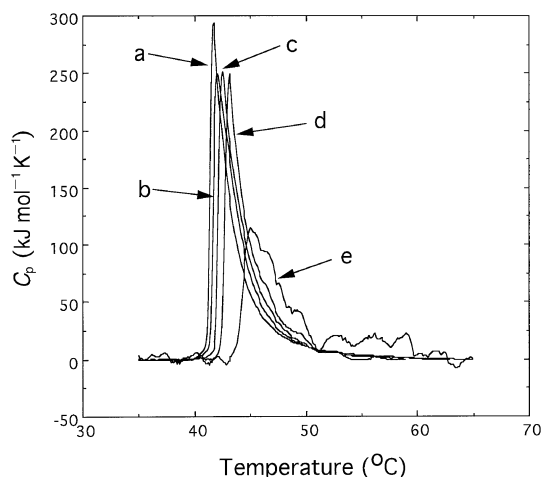


Figure 1. DSC thermograms for various concentrations of PNVIBA aqueous solutions. Polymer concentrations ((w/v)%): a, 0.04; b, 0.02; c, 0.01; d, 0.005; e, 0.001. Scanning rate; 1 K min⁻¹.

together with T_t and cooperative monomer number ($n_m = [\text{degree of polymerization (DP)}]/n$; denotes the (average) number of monomer units involved in one cooperative unit or domain). The concentration dependence of T_t , $\Delta H_{monomer}$ and $\Delta S_{monomer}$ is larger for shorter polymers, although the values of latter two were not aligned with the M_n values. For the polymer of the highest M_n , the concentration dependence was rather small. The shift in T_t for PNIPAM was comparable to PNBIVA, but those for $\Delta H_{monomer}$ and $\Delta S_{monomer}$ were larger for PNIPAM and they were well compensated to show very small shift in T_t . About 400 monomer units are involved in one cooperative domain of collapse transition, except for the shortest polymer, where around 200 monomer units composed a cooperative domain, which corresponds to two polymer chains.

The ΔC_p values for PNVIBA and PNIPAM were always negative. By this fact, the transitions of these and other thermoresponsive polymers are considered as a good model of the cold denaturation of proteins.^{14–17} Usually C_p values are lower in the native structures of proteins and both thermal and cold denaturation bring about an increase in ΔC_p ; as a result the inverse process of so-called cold denaturation (changes caused by increasing temperature from the cold-denatured state) is associated with a negative ΔC_p .¹⁶

Effects of SDS Addition

The thermograms of PNVIBA ($M_n = 11$ kDa) solutions in the presence of various concentrations of SDS are shown in Figure 3. It has been understood that a surfactant like SDS at low concentrations does little affect T_t but only hinders the successive aggregations of collapsed chains. Actually we and other researchers have shown that, as far as turbidimetric measurements are

Table I. Parameters obtained by DSC measurement of PNVIBA and PNIPAM solutions^a

Polymer	M_n kDa	conc. (w/v)%	T_t °C	ΔH kJ mol ⁻¹	ΔH^{VH} kJ mol ⁻¹	ΔS kJ K ⁻¹ mol ⁻¹	ΔC_p kJ K ⁻¹ mol ⁻¹	n^b
PNVIBA	11	0.001	45.0	616.8	245	1.939	-2.6	2.5
		0.005	43.1	682.5	1496	2.158	-6.6	0.46
		0.01	42.5	714.4	1351	2.263	-9.9	0.53
		0.02	42.0	764.4	1112	2.426	-11	0.69
		0.04	41.7	766.3	1415	2.434	-16	0.54
	66	0.001	41.7	4412	5468	14.01	-211	0.81
		0.005	41.3	4650	3734	14.79	-22	1.2
		0.01	41.1	4792	3548	15.25	-71	1.4
		0.02	40.9	4807	3634	15.31	-73	1.3
		0.04	40.8	4859	3633	15.48	-74	1.3
	460	0.001	40.2	27252	5754	86.97	-413	4.7
		0.005	39.9	27991	2840	89.41	-448	9.9
		0.01	39.7	28144	2677	89.96	-500	10.5
		0.02	39.6	28274	2874	90.40	-513	9.8
		0.04	39.4	28348	3169	90.70	-515	8.9
PNIPAM	49	0.001	37.0	1873	1671	6.040	-8.9	1.1
		0.005	36.4	2339	1926	7.555	-11.9	1.2
		0.01	36.4	2426	2010	7.838	-12.4	1.2
		0.02	36.3	2499	2019	8.074	-13.5	1.2
		0.04	36.2	2736	1916	8.846	-19.3	1.4

^aThermodynamic parameters were calculated based on polymer mole using M_n values. ^b $n = \Delta H/\Delta H^{VH}$.

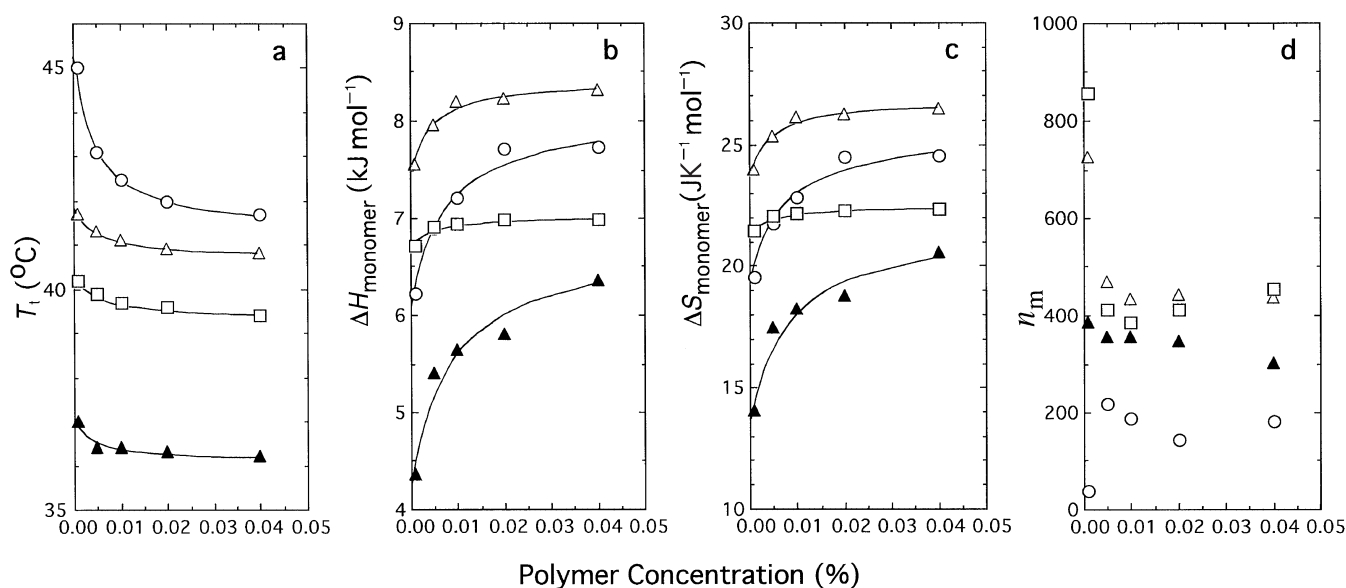


Figure 2. Polymer concentration dependence of T_t (a), $\Delta H_{\text{monomer}}$ (b), $\Delta S_{\text{monomer}}$ (c), and n_m (d) for PNVIBA and PNIPAM solutions. PNVIBA: \circ , $M_n = 11$ kDa; \triangle , $M_n = 66$ kDa; \square , $M_n = 460$ kDa. PNIPAM: \blacktriangle , $M_n = 49$ kDa. Curves were drawn on an assumption of a simple saturation (hyperbolic) function.

concerned, the apparent T_t did not show distinct change with addition of SDS up to *ca.* 0.03 (w/v)%. Therefore, this technique was applied for the dynamic light scattering measurements, in order to prevent turbidity, for the measurement of the collapsed (single) chain size.^{10, 12, 14, 20} Actually, SDS addition up to 0.02% concentration showed very small shift in T_t or even slightly lowered it.

The thermodynamic parameters calculated from these and other thermograms for PNVIBA and PNIPAM are compiled in Table II. The critical micelle concentration of SDS around room temperature is known to be about 8 mM (*ca.* 0.23 (w/v)%). Even well below this value, however, addition of, *e.g.*, 0.05 or 1 (w/v)% SDS drastically shifted T_t . No distinct C_p peaks were observed for simple aqueous solutions of SDS at this

Table II. Effects of SDS addition on the DSC parameters of PNVIBA and PNIPAM solutions^a

Polymer	M_n kDa	SDS conc. (w/v)%	T_t °C	ΔH kJ mol ⁻¹	ΔS kJ K ⁻¹ mol ⁻¹	ΔH^{VH} kJ mol ⁻¹	ΔC_p kJ K ⁻¹ mol ⁻¹	n
PNVIBA	11	0	42.5	714.4	2.34	1351	-9.9	0.53
		0.005	42.1	634.6	2.01	1384	-3.6	0.45
		0.02	43.5	524.1	1.66	1222	-7.2	0.43
		0.05	50.7	409.6	1.27	1602	-7.4	0.26
		0.1	60.5	382.6	1.15	1497	-13.1	0.26
	66	0	41.1	4792	15.3	3548	-70.9	1.35
		0.005	40.8	4503	14.3	2795	-72.0	1.61
		0.02	41.9	3661	11.6	1206	-61.7	3.04
		0.05	44.1	2555	8.05	1124	-30.3	2.27
		0.1	50.8	2490	7.69	891	-19.6	2.79
	460	0	39.7	27678	88.5	2722	-500	10.2
		0.005	38.6	24541	78.7	1963	-195	12.5
		0.02	38.5	22365	71.8	1187	-477	18.8
		0.05	40.2	17816	56.9	1219	-456	14.6
		0.1	45.6	15924	50.0	1316	-449	12.1
PNIPAM	49	0	36.4	2426	7.84	2010	-12.4	1.21
		0.005	36.5	2405	7.77	1785	-10.3	1.35
		0.02	36.5	2364	7.64	1525	-19.2	1.55
		0.05	40.7	2310	7.36	2166	-17.4	1.07
		0.1	51.3	2284	7.04	1880	-16.0	1.22

^aThermodynamic parameters were calculated based on polymer mole using M_n values. [Polymer] = 0.01 (w/v)%.

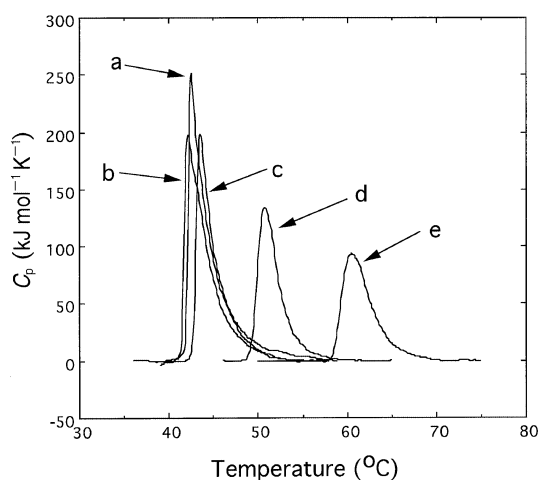


Figure 3. DSC thermograms for PNVIBA aqueous solutions in the presence of various concentrations of SDS. SDS concentrations ((w/v)%): a, 0; b, 0.005; c, 0.02; d, 0.05; e, 0.1. Scanning rate; 1 K min⁻¹.

concentration level. Both ΔH and ΔS of transition decreased with increasing SDS concentrations. The concentration dependence of $\Delta H_{\text{monomer}}$ and $\Delta S_{\text{monomer}}$ are shown in Figure 4, in order to compare the thermodynamic values obtained for polymers of different M_n , together with T_t and n_m . The concentration dependence of T_t is larger for shorter PNVIBA, and that of $\Delta H_{\text{monomer}}$ and $\Delta S_{\text{monomer}}$ were larger in PNVIBA than in PNIPAM. Changes in n_m are rather scattered, ranging between 200–400 monomer units, but the effects

seemed to be larger for shorter polymers.

DISCUSSION

Microcalorimetric studies^{13–15, 18, 21–23} and dynamic light scattering studies^{12, 24–27} on thermoresponsive vinyl polymers in dilute solutions have indicated that these polymers showed a coil-collapse change as a single molecular event at the same temperature as detected by the cloud point measurement. The changes are not driven by the aggregation of collapsed chains, but the visible changes occur as a successive result of the formation of the collapsed chains. For long polymers, changes occur at several cooperative domains in a single chain, and, for short polymers, association of some chains seems to be necessary in order to perform the observable change.

Even for the well-fractionated preparations of polymers as in the present study, the molecular weight distribution is still significant, when compared with those of proteins. However, the size of cooperative domain, as determined by the ratio of $\Delta H/\Delta H^{VH}$, has significant meaning when we compare the samples of significant differences in their molecular weight. The all-or-none coil-collapse transition²⁵ of these vinyl polymers occurs at about 400 monomer units in a polymer chain. Although the present shortest sample gave $n_m \sim 200$, it is still larger than the mean DP for this sample, and

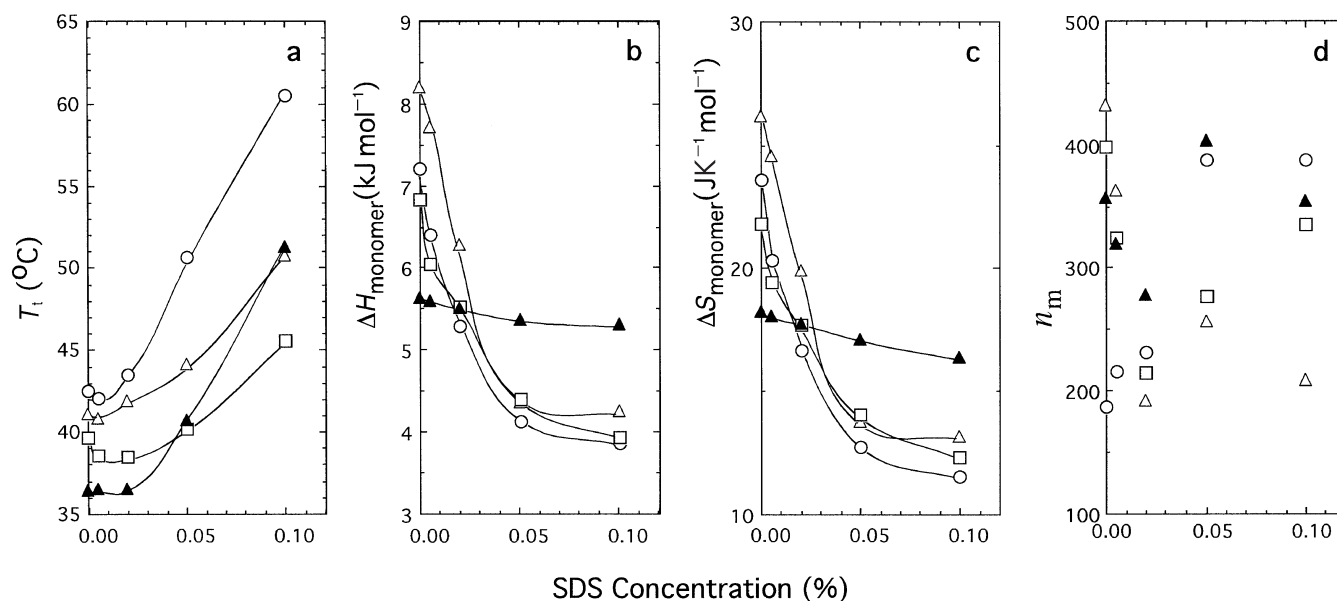


Figure 4. SDS concentration dependence of T_t (a), $\Delta H_{\text{monomer}}$ (b), $\Delta S_{\text{monomer}}$ (c), and η_m (d) for PNVIBA and PNIPAM solutions. PNVIBA: \circ , $M_n = 11$ kDa; \triangle , $M_n = 66$ kDa; \square , $M_n = 460$ kDa. PNIPAM: \blacktriangle , $M_n = 49$ kDa. Curves in (a)–(c) are arbitrary.

hence some intermolecular interaction will be necessary. This might be the reason of molecular weight and concentration dependence of T_t , especially for shorter polymers. For samples of several hundreds kDa and concentrations over 0.05 (w/v)%, these dependences are practically unobservable and will give almost flat phase diagrams.²⁶

Compared with PNIPAM, PNVIBA gives larger ΔH and ΔS , but the size of the cooperative domain is similar. The difference in T_t is only 15% in absolute temperature scale, but this larger ΔH value will result in the sharper transition in turbidimetric measurements, which is very important for practical applications. The origin of this ΔH is thought to be mainly the hydrogen bond cleavage at the side chain of the polymer. Thus, PNVIBA seems to be hydrated or intramolecularly hydrogen-bonded more tightly than PNIPAM. The sole difference in these two polymers is in the direction of the amide bond in the side chain, and the coordination in PNVIBA, NH being inside and CO being outside, closer to isopropyl group, seems to be favorable for the hydrogen bond formations with water.

With addition of SDS, both ΔH and ΔS of transition decreased. The decrements were much larger in PNVIBA and in the presence of 0.05 or 0.1 (w/v)% SDS both ΔH and ΔS became even smaller than those of PNIPAM. These changes were mostly compensated and as a result the effects of SDS addition on T_t at higher concentrations are rather larger in PNIPAM than in PNVIBA. Addition of SDS at low concentrations does little affect T_t and seems to hinder the successive aggregations of collapsed chains. The solutions PNIPAM or PNVIBA of sufficient molecular weight did not

strongly become turbid in the presence of 0.02%, and T_t did not show distinct change,¹² which afforded the dynamic light scattering measurements, by preventing higher aggregation of the collapsed (single) chains.

The presence of very small amount of SDS in the polymer solutions destabilizes the coil state of polymer, though weakly, and the increase of SDS concentration over a certain level in turn stabilizes the coil state. During these processes, the hydrogen bonding on the polymer side chains is weakened. The entropy of the coil state is also increased, or that of the collapse state will be decreased, in order to give smaller entropy change, which is insufficient to compensate the enthalpy change. Thus the transition temperature increased drastically at high concentrations of SDS. All these results will come from the direct interactions of SDS molecules with polymer (side chain and main chain). The differences in the changes of these thermodynamic parameters in two polymers will reflect the differences in the strength of hydrogen-bonding properties of polymer side chains, and in the strength of interactions with surfactant molecules.

REFERENCES

1. M. Heskins and J. E. Guillet., *J. Macromol. Sci. A-2*, 1441 (1968).
2. H. G. Schild, *Prog. Polym. Sci.*, **17**, 163 (1992).
3. M. Shibayama and T. Tanaka, *Adv. Polym. Sci.*, **109**, 1 (1993).
4. Y. H. Bae, T. Okano, R. Hsu, and S. W. Kim. *Makromol. Chem. Rapid Commun.*, **8**, 481 (1987).
5. A. S. Hoffman, *J. Control. Release*, **6**, 297 (1987).
6. A. Gustafsson, H. Wennerstrom, and F. Tjerneld, *Fluid Phase Equilib.*, **29**, 365 (1986).

7. M. Akashi, S. Nakano, and A. Kishida, *J. Polym. Sci., Part A: Polym. Chem.*, **34**, 301 (1996).
8. K. Suwa, Y. Wada, Y. Kikunaga, K. Morishita, A. Kishida, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **35**, 1763 (1997).
9. K. Suwa, K. Morishita, A. Kishida, and M. Akashi, *J. Polym. Sci., Part A: Polym. Chem.*, **35**, 3377 (1997).
10. S. Kunugi, K. Takano, N. Tanaka, K. Suwa, and M. Akashi, *Macromolecules*, **30**, 4499 (1997).
11. K. Suwa, K. Yamamoto, M. Akashi, K. Takano, N. Tanaka, and S. Kunugi, *Colloid Polym. Sci.*, **276**, 529 (1998).
12. S. Kunugi, Y. Yamazaki, K. Takano, N. Tanaka, and M. Akashi, *Langmuir*, **15**, 4056 (1999).
13. S. Kunugi, T. Tada, Y. Yamazaki, K. Yamamoto, and M. Akashi, *Langmuir*, **16**, 2040 (2000).
14. E. I. Tiktopulo, V. E. Bychkova, J. Ricka, and O. B. Ptitsyn, *Macromolecules*, **27**, 2879 (1994).
15. E. I. Tiktopulo, V. N. Uversky, B. B. Lushchik, S. I. Klenin, V. E. Bychkova, and O. B. Ptitsyn, *Macromolecules*, **28**, 7518 (1995).
16. O. B. Ptitsyn, "Protein Folding", T. E. Creighton, Ed., W. H. Freeman, New York, N.Y., 1992, p 243.
17. S. Kunugi and N. Tanaka, *Biochim. Biophys. Acta*, (in press).
18. H. G. Schild and D. A. Tirrell, *J. Phys. Chem.*, **94**, 4352 (1990).
19. P. L. Privalov, "Protein Folding", T. E. Creighton, Ed., W. H. Freeman, New York, N.Y., 1992, p 83.
20. M. Meewes, M. Ricka, M. de Silva, R. Nyffenegger, and T. Binkert, *Macromolecules*, **24**, 5811 (1991).
21. Y. Yamazaki, T. Tada, and S. Kunugi, *Colloid Polym. Sci.*, **278**, 80 (2000).
22. W. S. Cai, L. H. Gan, and K. C. Tam, *Colloid Polym. Sci.*, **279**, 793 (2001).
23. Y. Maeda, T. Higuchi, and I. Ikeda, *Langmuir*, **17**, 7535 (2001).
24. S. Fujishige, K. Kubota, and I. Ando, *J. Phys. Chem.*, **93**, 3311 (1989).
25. S. Fujishige, K. Kubota, and I. Ando, *J. Phys. Chem.*, **94**, 5154 (1990).
26. K. Kubota, S. Fujishige, and I. Ando, *Polym. J.*, **22**, 15 (1990).
27. H. G. Schild and D. A. Tirrell, *Langmuir*, **7**, 665 (1991).