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Order−Disorder Conformational Transitions of *N*-Isopropylacrylamide−Sodium Styrene Sulfonate Copolymers in Aqueous Solutions

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ABSTRACT: Copolymers of *N*-isopropylacrylamide (NIPA) with sodium styrene sulfonate (SSS) synthesized in aqueous medium at 70 °C undergo reversible cooperative conformational transitions without phase separation of their aqueous solution. Data obtained by high-sensitivity differential scanning calorimetry and velocity sedimentation for the NIPA-SSS copolymers have shown that depending on the SSS content either intermolecular association (micellization) or intramolecular chain folding take place upon heating.

Conformation-dependent design of thermosensitive functional copolymers is a field of both theoretical and experimental interest. Theoretical studies predict that flexible copolymer chains composed of two monomer types, hydrophobic (H) and hydrophilic (P), can reversibly form folded water-soluble particles: either individual globules[1,2] or finite aggregates.[3] To be soluble in aqueous medium the associated copolymer chains must adopt a core−shell structure, i.e., a hydrophobic globule-like interior surrounded by a hydrophilic corona.[3–5] It has been shown that the core−shell copolymer structure dictates a particular nonrandom distribution of the H- and P-blocks along the chain.[3–7] Such copolymers of nonrandom H−P sequences were named "protein-like" copolymers since their sequences roughly resemble the distribution of hydrophobic and hydrophilic residues in primary structure of globular proteins.[4] The term "protein-like copolymers" has another sense related to a potential technological significance of smart functional copolymers as protein analogs.[8] The core−shell conformation provides solubility in water and reproducibility of a parent conformation thus allowing to perform operating cycles, while the globular core provides stability of the macromolecular machine to global chain fluctuations.

Practical realization of the core−shell copolymer concept is still behind the theoretical developments.[9] The main problem is how to attain a nonrandom distribution of H- and P-comonomers in a copolymer chain during its synthesis. One of possible strategies implies that heterogeneous extended sequences can be synthesized under conditions of segregation of H- and P-comonomers.[7,10] Such a strategy can be employed upon synthesis of a thermosensitive HP-copolymer at temperatures above LCST of the corresponding H-homopolymer. These conditions promote segregation of H- and P-sequences thus resulting in formation of the chain segments enriched in comonomers of the same type. This approach was first realized in synthesis of copolymers of *N*-vinylcaprolactame with *N*-vinylimidazole (NVCL-NVI).[11] As it has been proved by high sensitivity differential scanning calorimetry (HS-DSC), some fractions of the copolymers, synthesized at 65 °C, undergo cooperative thermotropic transitions in aqueous solution without loss of solubility at high temperatures.[12] Later a difference in thermal behavior was shown for copolymers of *N*-isopropylacrylamide with vinylpyrrolidone (NIPA-VP) synthesized at 60 °C (so-called, segmented copolymers) and 30 °C (random copolymers).[13] Light scattering (LS) detected a "coil-globule" transition of individual chains of the random and segmented NIPA-VP copolymers, and HS-DSC apparently confirmed the LS data. However, the HS-DSC experiments were carried out with the copolymer solutions of three order more concentrated than those used in the LS measurements. It was not definitely demonstrated whether a phase separation occurred in the HS-DSC experiments or not. Strictly speaking, the NVCL-NVI copolymer has represented until now the only example of thermosensitive copolymers with the cooperative conformational transitions in a single-phase system evidently demonstrated by direct methods.[11,12]

Table 1. Characteristics of the NIPA-SSS Copolymers: the Molar Fraction of the SSS Units (x_{SSS}) and the Weight-Average Molar Mass (M_w)

NIPA-SSS sample	x_{SSS}, mol %	M_w, kDa
1	1.6	986
2	2.8	140
3	5.7	139
4	6.8	169
5	7.3	182
6	16.8	245
7	28.6	293
8	64.7	145

In this communication we report data on the thermotropic cooperative transitions in aqueous solutions of copolymers of *N*-isopropylacrylamide and sodium styrene sulfonate (NIPA-SSS) synthesized at 70 °C that is above LCST of the NIPA homopolymer. A particular feature of these transitions is that they are observed in solution without phase separation of the system.

N-Isopropylacrylamide (NIPA) was purified by recrystallization from a mixture of benzene and *n*-hexane (v/v, 1/3). Sodium styrene sulfonate (SSS) and potassium persulfate (KPS) were recrystallized three times from methanol and deionized water, respectively. The NIPA-SSS copolymers of various SSS content were synthesized by free radical polymerization in deionized water under N_2 atmosphere at 70 °C using KPS as an initiator. Typically, initial concentrations of NIPA, SSS, and KPS were 124−185 mM, 20−53 mM, and 0.2 mM, respectively. A remarkable feature of the NIPA-SSS copolymers of various comonomer contents is their full solubility during polymerization at high temperatures. The crude product was dialyzed against water using semipermeable membrane with a cutoff molar mass of 13 kDa. The content of SSS was determined by ^{1}H NMR spectra in D_2O measured on a Brucker 300 MHz spectrometer with tetramethylsilane (TMS) as an internal standard. The weight-average molar mass (M_w) was

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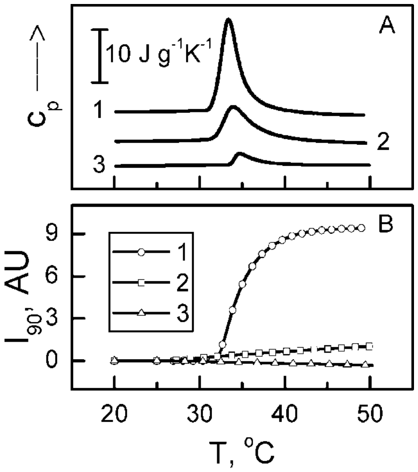

Figure 1. Temperature dependences of the excess heat capacity (A) and intensity of light scattering (B) of the NIPA homopolymer (1) and of the NIPA-SSS copolymers with SSS contents of 1.6 (2) and 16.8 (3) mol % in aqueous solutions. Polymer concentration 1−2 mg mL^{-1}; heating rate 1 K min^{-1}. The heat capacity curves are shifted arbitrarily along the ordinate axis.

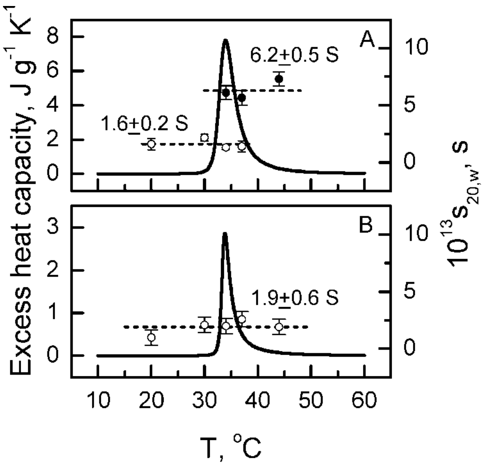

Figure 2. Temperature dependences of the excess heat capacity (left axis) and sedimentation coefficients (right axis) of the NIPA-SSS copolymers with the SSS content of 1.6 (A) and 16.8 (B) mol %. Open and closed symbols refer to the slowly and fast sedimenting components, respectively. The values of sedimentation coefficients are reduced to 20 °C.

measured by static light scattering in 1.0 M NaCl at 25.0 °C. Molecular characteristics of the NIPA-SSS copolymers are given in Table 1.

Copolymer solutions in deionized water (1−2 mg mL^{-1}) were used in calorimetric experiments. Measurements were carried out with a differential adiabatic scanning microcalorimeter DASM-4 (BIOPRIBOR, Russia) within temperature range 10−100 °C at the heating rate of 1 K·min^{-1} and excess pressure of 0.25 MPa. Primary data processing was performed using the WSCAL software (Institute of Protein Research, Pushchino, Russia). The partial heat capacity curves were converted into the excess heat capacity functions using the NAIRTA 2.0 software (Institute of Organoelement Compounds, Moscow, Russia). For this purpose the transition baseline was constructed by a spline interpolation. The transition temperature, T_t, was determined as the temperature of maximum of the excess heat capacity peak. The transition enthalpy, $\Delta_t h$, was determined by integration of the excess heat capacity peak. The width of the transition, $\Delta_t T$, was calculated as a ratio of the transition enthalpy to the maximal ordinate of the excess heat capacity curve.

Velocity sedimentation experiments were carried out using an analytical ultracentrifuge 3170B (MOM, Hungary) at 40 000 rpm (120 000 g) at different temperatures from 20 to 45 °C. Polymer concentration was 5−7 mg mL^{-1}.

Light scattering measurements were performed at an angle of 90° with a spectrocolorimeter Specol (Carl Zeiss, Jena), equipped with a cell thermoholder allowing heating of the sample with a constant rate of 1 K min^{-1} from 20 to 70 °C.

Data of HS-DSC were obtained for the NIPA-SSS copolymers of various SSS content (from 1.6 to 64.7 mol %) as well as for the NIPA homopolymer. The examples of thermograms are shown in Figure 1A. The copolymers with the SSS content from 1.6 to 16.8 mol % demonstrate a cooperative transition as a narrow asymmetric heat capacity peak. The copolymers with the SSS content from 28.6 to 64.7 mol % do not reveal cooperative transitions upon heating. In Figure 1B the temperature dependences of the light scattering intensity of solutions of the NIPA homopolymer as well as NIPA-SSS copolymers of different SSS content are presented. In contrast to the NIPA homopolymer, the solutions of the copolymers do not show increase in light scattering up to temperature 50 °C that is well above the transition area registered by HS-DSC (Figure 1A). Consequently, the cooperative thermotropic copolymer transitions revealed by HS-DSC are not the phase separation transitions.

In order to elucidate conformational mechanism of the copolymer transitions, the velocity sedimentation experiments were carried out with the solutions of the NIPA-SSS copolymers of various SSS content at different temperatures. In Figure 2 the sedimentation coefficients and the excess heat capacity functions of the NIPA-SSS copolymers with the SSS content of 1.6 and 16.8 mol% are shown as a function of temperature. For the weakly charged copolymer (Figure 2A) a single slowly sedimenting component with the sedimentation coefficient of 1.6 S is observed at temperatures below the transition. Within the transition region, two sedimentation components—slow and fast ones (1.3 and 6.2 S, respectively) are observed. Above the transition, only the fast component is present. This result implies an increase in particle size during the transition most likely due to chain association. For the highly charged copolymer (Figure 2B), a single slowly sedimenting component is detected at all temperatures. Its sedimentation coefficient (of about 2 S) does not change significantly with temperature within experimental error limits indicating that no chain association is apparently related to the transition.

The sedimentation behavior of the NIPA-SSS copolymers above the transition temperature differs principally from that of the NIPA homopolymer mesoglobule[14] or the NIPA copolymer microgels.[15] In fact, suspensions of these particles possess a high gravitation stability at rest. However, such particles of size ~50 nm should have sedimentation coefficients of order of 150 S, and consequently they will precipitate completely in the ultracentrifuge for a few minutes. Besides, the ultracentrifugation of such particles will give a continuous polymer phase (liquid or gel-like) incapable of spontaneous redispersion. In case of the NIPA-SSS copolymers the sedimentation components (clusters) at a centrifugation force of about 120 000 g do not reach the bottom of the ultracentrifuge cell for a typical time of the experiment (about of 1 h). Moreover, concentration of the clusters in the cell after stop of the rotation is spontaneously equalized over the cell volume due to diffusion. It implies that the NIPA-SSS copolymer solutions represent equilibrium

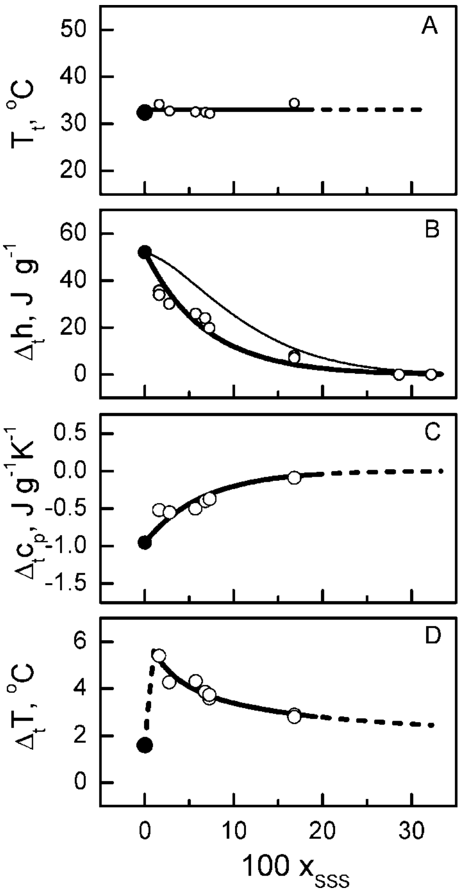

Figure 3. Transition parameters of the NIPA-SSS copolymers vs SSS content: A, transition temperature; B, transition enthalpy; C, transition heat capacity increment; D, transition width. Open and closed symbols indicate experimental data for the NIPA-SSS copolymers and NIPA homopolymer, respectively. The thin line in panel B is calculated by (eqs 1 and 2) (see text). The diameter of the open circles corresponds to the experimental errors.

true solutions, while the microgel and mesoglobule suspensions are just stable nonequilibrium colloid systems.

Thus, the thermotropic cooperative transitions of the NIPA-SSS copolymers take place in a single phase system. Consequently, they can be classified as conformational transitions resulting in formation of the folded copolymer conformation similar to that of the core−shell type.[1] Structure of the copolymer folded conformation is determined by its charge. The copolymer of low charge undergoes a cooperative interchain association (micellization). For the copolymer of high charge the interchain association is unlikely possible, so an intramolecular contraction (folding) becomes dominating. In such a case the conformational transition is most likely of the "coil−globule" type.

Figure 3 shows the thermodynamic transition parameters (temperature, T_t, enthalpy, $\Delta_t h$, heat capacity increment, $\Delta_t c_p$, and width, $\Delta_t T$) of the copolymers plotted against the molar fraction of the SSS units, x_{SSS}.

The transition temperature of the NIPA-SSS copolymers does not vary with increasing SSS content and is close to the transition temperature of the NIPA homopolymer (32.4 °C). This result is difficult to correlate with the presence of charges in the copolymer chain. Since electrostatic interactions must hinder the transition, one could expect an increase in the transition temperature with increasing charge content. This, for example, has been observed for the thermosensitive copolymers containing carboxyl and imidazole groups.[16–19] The constancy of the transition temperature of the NIPA-SSS copolymers seems to require special theoretical considerations.

The transition enthalpy $\Delta_t h$, normalized per gram of the copolymer, decreases with increasing SSS content, and reaches zero at $x_{SSS} = 28.6$ mol % (Figure 2B). A nonlinear character of the dependence $\Delta_t h(x_{SSS})$ indicates a nonadditive effect of the copolymer composition on the transition enthalpy.

One could explain a nonlinear decrease in the transition enthalpy of the copolymers with increasing SSS content assuming the existence of "non-active" NIPA sequences, i.e. rather short sequences incapable of cooperative transitions. In such an approximation, the specific transition enthalpy of the copolymer depends on its composition in a following way:

$$\Delta_t h(x_{SSS}, n^*_{NIPA}) = \frac{\Delta_t h_{NIPA} \delta^*_{NIPA}(x_{SSS}, n^*_{NIPA})(1 - x_{SSS})M^0_{NIPA}}{(1 - x_{SSS})M^0_{NIPA} + x_{SSS}M^0_{SSS}} \qquad (1)$$

where x_{SSS} is the molar fraction of SSS units; n^*_A is the critical length of the NIPA sequences; $\Delta_t h_{NPA}$ is the apparent transition enthalpy of the active NIPA sequences; $\delta^*_{NIPA}(x_{SSS},n^*_{NIPA})$ is the fraction of the active NIPA units; M^0_{NIPA} and M^0_{SSS} are the molecular mass of the NIPA and SSS units, respectively. The NIPA sequence undergoes a cooperative transition if its polymerization degree $n_{NIPA} \geq n^*_{NIPA}$. By definition:[20]

$$\delta^*_{NIPA}(x_{SSS}, n^*_{NIPA}) = \frac{\sum\limits_{n_{NIPA} - \delta^*_{NIPA}}^{\infty} n_{NIPA} \times f_{NIPA}(n_{NIPA})}{\sum\limits_{n_{NIPA} - 1}^{\infty} n_{NIPA} \times f_{NIPA}(n_{NIPA})} \qquad (2)$$

where $f_{NPA}(n_{NPA})$ is the length distribution function of the NIPA sequences.

Using eqs 1 and 2, we have calculated the dependence $\Delta_t h(x_{SSS})$ for a random copolymer, i.e., using the random distribution function $f_{NIPA}(n_{NIPA})$.[20] For calculations, we have taken the calorimetrically determined value of the NIPA transition enthalpy ($\Delta_t h_{NIPA}$=52.1 J g^{-1}) and the experimental value of the critical length $n^*_{NIPA} \sim 14$.[21] The calculated curve is shown by a thin line in Figure 3B. It is seen a notable deviation of the calculated dependence $\Delta_t h(x_{SSS})$ for a random copolymer from the experimental one. Apparently, there are two probable sources of such a disagreement. First, the distribution function $f_{NIPA}(n_{NIPA})$ for the NIPA-SSS copolymers does not seem to be random. Second, local electrostatic fields of the charged SSS units could disturb the hydration structure of the NIPA sequences that should both decrease the number of the active NIPA sequences and the enthalpy $\Delta_t h_{NIPA}$.

The transition heat capacity increment decreases by absolute value with increasing SSS content and reaches zero at 28.6 mol % SSS content (Figure 2C). In general, this tendency reflects an evident decrease in the dehydration degree of the copolymer upon the transition since the introduced charges reduce hydrophobic hydration of the copolymer in coil conformation.

The dependence of the transition width on the copolymer composition passes through a maximum (Figure 3D). A broadening of the transition, particularly at a rather small content of the SSS units, is unexpected. According to a widely spread theoretical point of view,[22] insertion of charges into polymer chain should cause a narrowing of the coil-globule transition due to the Donnan effect. This contradiction seems to be of importance for theoreticians working on the theory of the coil-globule transition in partly charged polymer chains.

Thus, we have presented experimental facts indicating that the NIPA-SSS copolymers, synthesized in water at 70 °C, undergo upon heating cooperative conformation changes in solution without phase separation. This is a nontrivial behavior that was never observed for random copolymers. In particular, the NIPA-SSS copolymers synthesized by a standard protocol of radical polymerization at ~20 °C[23] (that is known to result in random comonomer distribution) were shown to precipitate from their aqueous solutions upon heating (the cloud point is close to the LCST of the NIPA homopolymer).

Conclusion.A hypothesis can be put forward that the NIPA-SSS copolymers synthesized in water at 70 °C have a nonrandom distribution of the NIPA and SSS units along the chain. This hypothesis is consistent with the demonstrated fact that the copolymers undergo thermotropic cooperative conformational transitions in aqueous solutions without precipitation.

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