480 *Macromolecules* **2010**, *43*, 480–487
DOI: 10.1021/ma9018312

Macromolecules
ARTICLE

Microcalorimetric Study of the Effects of a Chaotropic Salt, KSCN, on the Lower Critical Solution Temperature (LCST) of Aqueous Poly(*N*-isopropylacrylamide) (PNIPA) Solutions

Inna Shechter,[†,§] Ory Ramon,[‡,§] Irina Portnaya,[‡] Yaron Paz,[†] and Yoav D. Livney*[,‡]

[†]*Department of Chemical Engineering and* [‡]*Laboratory of Biopolymers and Food-Nanotechnology, Department of Biotechnology & Food Engineering, The Technion, Israel Institute of Technology, Haifa 32000, Israel.* [§] *Equally contributing authors*

Received August 17, 2009; Revised Manuscript Received November 17, 2009

ABSTRACT: The LCST phase-transition of aqueous PNIPA solutions in rising concentrations of the strong chaotropic salt KSCN was studied microcalorimetrically by DSC and apparently for the first time by ITC. An endothermic (entropy driven) binding of KSCN onto PNIPA was observed, explained by electrostatic perturbation of hydrophobic hydration by adsorbed ions. A good fit was found for the one-type-of-sites binding model, and the binding affinity increased with rising temperature from 15 to 20 °C but decreased at 25 °C. DSC measurements emphasized the lowering and broadening of the endothermic peak of PNIPA phase-transition with rising KSCN concentration, explained by reduced cooperativity of coil-to-globule collapse with increased heterogeneity along the polymer chain, caused by salt adsorption. A hysteresis was observed between heating and cooling DSC peaks, which decreased asymptotically with rising KSCN concentration, further supporting that binding occurs. This work provides new insights into the mechanisms of chaotropic salt effects on polymers and biopolymers in aqueous solutions.

Introduction

Many aspects of the physical interactions of macromolecules such as proteins, polysaccharides and synthetic polymers, with aqueous salt solutions are still unclear, after numerous studies. The classical Hofmeister series[1] ranks various salts according to their relative ability to promote the unfolded, solubilized state of proteins (a salting-in effect), versus stabilizing of the native state and promoting protein precipitation (a salting-out effect).[2−4] Beyond protein stability,[5] there are important implications for these ion-specific effects with regards to enzyme activity,[6−9] self-assembly,[10] and other protein interactions.[11]

A typical Hofmeister series of anions is:

$$SO_4^{-2} > HPO_4^{-2} > Cl^- > Br^- > I^- > SCN^-$$

This order (left to right) generally shows decreasing salting out (except for net positively charged proteins, i.e., below their isoelectric point, for which this order reverses at low salt concentrations).[12−14] The ions left of the chloride are called kosmotropes and are strongly hydrated, while those on the right are called chaotropes, and are weakly hydrated. Several mechanisms have been suggested to explain the origin of ion specific effects on macromolecules. The preferential interactions theory,[15−17] which focuses on the relative strength of polymer−salt and polymer−water interactions, and on excluded volume effects,[18,19] is widely accepted as the foundation, but many questions remain, particularly regarding the impact of salt-water interactions. A complementary mechanism focusing on water−ion interactions, suggests that the origin of ion specificity is the differences in charge density of the ions, which affects their strength of hydration.[20,21] Ions of high charge density, whose interaction with water is more favorable than water−water interactions, are kosmotropes, and they reduce the entropy of adjacent water molecules compared to bulk water entropy. On the other hand, ions of low charge density interact more weakly with water than water−water bonds, hence their hydration is less favorable, and they disrupt adjacent water molecule hydrogen bonding, and increase their entropy compared to bulk water.[20,21] While the above mechanism focuses on electrostatic forces, another mechanism proposed for explaining ion specificity, and particularly chaotropic ion interactions, is based on electrodynamic dispersion forces. These forces intensify with the increasing ion polarizability, which, for a given charge, tends to increase with ion size, but depends also on its shape,[22] and its specific electronic configuration.[12] Ion−water affinity plays a major role in Hofmeister, or lyotropic phenomena,[21] and thus various properties related to this affinity were used in attempt to explain the mechanisms leading to the order observed, e.g., partial molar volume,[23] molar surface tension increment,[22,24] and free energy of hydration.[12,22,23] It is commonly assumed that the contributions from different ions are additive and that approximately the same sequence holds for various systems and properties.[21,25] A similar series is observed for cations but their effect on stabilizing macromolecules is generally opposite and smaller than that of anions[4] as they interact with the oxygen of the water, whose charge density is lower than that of a water hydrogen,[20] and there is much less polarizability variation in cations than in anions.[12]

A similar ranking was found to hold when salts were compared in their ability to shrink or swell macromolecules such as synthetic polymers and gels.[15,24,26] Many aspects of salt effects on nonionic polymer solutions and gels were investigated, particularly regarding *n*-substituted acrylamides,[27−29] predominantly poly(*N*-isopropylacrylamide) (PNIPA).[30−35] PNIPA is a polyvinyl polymer, containing both a hydrophilic amide and a hydrophobic

*Corresponding author. E-mail: Livney@technion.ac.il. Telephone: 972-4-8294225. Fax: 972-4-8293399.

 Published on Web 12/07/2009

isopropyl in its side groups.[36] The great research attention drawn by PNIPA in linear and gel forms, is related both to its thermosensitivity[36−38] which is important in a plethora of biomedical and other applications, and to its unique structure containing both hydrophilic (amide) and hydrophobic (isopropyl and backbone) groups, making PNIPA an important model system for studying cold denaturation/renaturation of proteins.[39,40] It is noteworthy that PNIPA is an isomer of polyleucine and poly isoleucine where the amide group is rather in the backbone. The temperature response of PNIPA is characterized by a lower critical solution temperature (LCST) behavior; i.e., the polymer is water-soluble below its LCST phase transition temperature (~32 °C) while at higher temperatures the solution becomes cloudy as the polymer collapses and aggregates. Cross-linked PNIPA hydrogels display a significant reversible volume-phase transition at this LCST.[41−50] PNIPA phase transition was investigated by many experimental techniques such as: UV-turbidometry,[51,52] static light scattering,[48,49,53] NMR spectroscopy,[54,55] FTIR,[45,56,57] and calorimetry.[58−62]

Cosolutes such as salts, sugars, and surfactants affect PNIPA phase transition. For salts, this effect follows Hofmeister series.[56,57,63,64] Analogous effects with hydrophilic polymers like polyacrylamide[65] and dextran[66] have been studied by Livney and co-workers. A relationship was quantified between the anion radius and its effect on the osmotic pressure of the polymer solution, as well as on the swelling of its gel.

The main mechanisms proposed thus far for chaotropic and kosmotropic anion effects on macromolecules may be summarized as follows:

(1) Kosmotropic anions strongly attract,[20] coordinate,[39,67] and polarize[22] water molecules. Thus, enthalpically, they are worse cosolvents for amide-rich polymers, as the polarization decreases the hydrogen bond donation capacity of the hydration water. Because an amide group can accept three H-bonds and donate only one, kosmotropic anions decrease H-bonding of their hydration water with such polymers.[68] Entropically, kosmotropic anions are worse cosolvents for the polymer due to the large size of their hydration cluster, decreasing the entropy of mixing with the polymer.[65]

(2) Both kosmotropes and chaotropes may interfere with hydrophobic hydration of PNIPA by increasing the surface tension[22,69−71] of the "polymer−water interface", though to different extents.

(3) A third effect that involves only chaotropic anions is related to their ability to bind directly to amides.[72] The ion specificity has been shown to relate to the number and position of vicinal methyl groups attached to the amide.[73] By applying ^{127}I NMR competitive binding technique Oh et al.[74] and Song et al.,[75] measured the binding affinity of various anions to poly(vinylpyrrolidone) (PVP) in aqueous solutions, and showed that the intrinsic binding constant of SCN^- (at 298 K) on PVP is of the largest magnitude followed by other anions according to the Hofmeister series: $SCN^- > I^- > Br^- > NO_3^- > Cl^-$, while kosmotropic anions, F^- and SO_4^{-2}, resulted in apparent negative intrinsic binding constants. They also demonstrated that the binding of the chaotropic anions was not significantly influenced by the countercation.

The following model has been proposed by Cremer and coworkers[63,68,71] to describe ion effects on PNIPA phase transition. The model comprises a constant (The cloud point temperature at zero salt concentration), a linear term (suggested to correlate with the surface tension increment of chaotropic salts), and a Langmuir isotherm expression, to capitulate the chaotropic anion adsorption effect:

$$T = T_0 + c[\mathrm{M}] + (B_{\max} K_\mathrm{A}[\mathrm{M}])/(1 + K_\mathrm{A}[\mathrm{M}]) \quad (1)$$

Here, T_0 is the LCST of PNIPA in water; [M] is the molar concentration of salt; c (°C/M) is a constant, which is negatively proportional to the surface tension increment σ ($\partial\Delta\gamma/\partial$[M] of chaotropes, where $\Delta\gamma$ is the surface tension difference between the salt solution and pure water); K_A (M^{-1}) is the binding constant of the anion to polymer and $B_{\max}$ (°C) the asymptotic increase in LCST due the anion binding at saturation.

A Langmuir binding isotherm approach has been previously proposed by our group to describe the binding of chaotropic anions to nonionic polymers, e.g., of SCN^- to Dextran,[66] and of I^- on PAAM[65] based on salt concentration differences evaluated during osmotic pressure equilibrium measurements. Yet, the mechanisms leading to salting-in of macromolecules at low concentrations of chaotropic anions, and salting-out effects at high concentrations remain largely obscure.

The main objective of the present work was to study the concentration dependent salting-in and salting-out effects of a strong chaotropic salt on the phase transition of PNIPA. Particularly we were interested in obtaining evidence for the adsorption of the anion onto the polymer, and in studying its mechanism. For these purposes, we investigated the interactions between KSCN and PNIPA in aqueous solutions, using high sensitivity microcalorimetric techniques, ITC and DSC. This is apparently the first report of an ITC study of the interaction between a chaotropic salt and PNIPA.

Experimental Section

Materials. PNIPA (M_w = 98 000 Da, $M_\mathrm{w}/M_\mathrm{n}$ = 2.5, atactic) was purchased from Polymer Source, Inc. Analytical grade KSCN was purchased from Riedel-de Haen and used as received. All solutions were prepared with purified deionized water with resistivity of at least 18 MΩ.

Methods. *Isothermal Titration Microcalorimetry* (*ITC*). ITC is the most direct method to measure the enthalpy change during a binding reaction at constant temperature.[76] For the following binding interaction

$$\mathrm{L} + \mathrm{M} \overset{K_\mathrm{A}}{\rightleftharpoons} \mathrm{ML}$$

where L is the ligand, M is the macromolecule, and K_A is the association constant of each binding site (assuming one affinity class of independent sites):

$$K_\mathrm{A} = \frac{[\mathrm{ML}]}{[\mathrm{M}][\mathrm{L}]} \quad (2)$$

(The square brackets indicate molar (M) concentrations; thus K_A has units of M^{-1}.)

During a typical ITC titration, the ligand solution is placed in the syringe, and injected stepwise into the cell containing the macromolecule solution while stirring.[76] The measured heat absorbed or released following injection i is the area under the ith peak in the raw plot of heat flow vs time, e.g., in Figure 1a (top). This heat is the measured enthalpy change, $\Delta h_{i,\mathrm{m}}$, between consecutive injections $i - 1$ and i. In the first injections, as the molar ratio [L]/[M] is low (and if the affinity (or K_A) is high), practically all added ligand is bound to the polymer, and peak areas are large and similar.

As titration progresses, $\Delta h_{i,\mathrm{m}}$ gradually decreases until ultimately all binding sites are saturated. Further injections show small peaks due to heat of ligand dilution, $\Delta h_{i,\mathrm{dil}}$ and other nonspecific effects, $\Delta h_{i,\mathrm{ns}}$.[76] (Therefore, an ITC titration of a macromolecule with a ligand usually leads to a typical sigmoidal

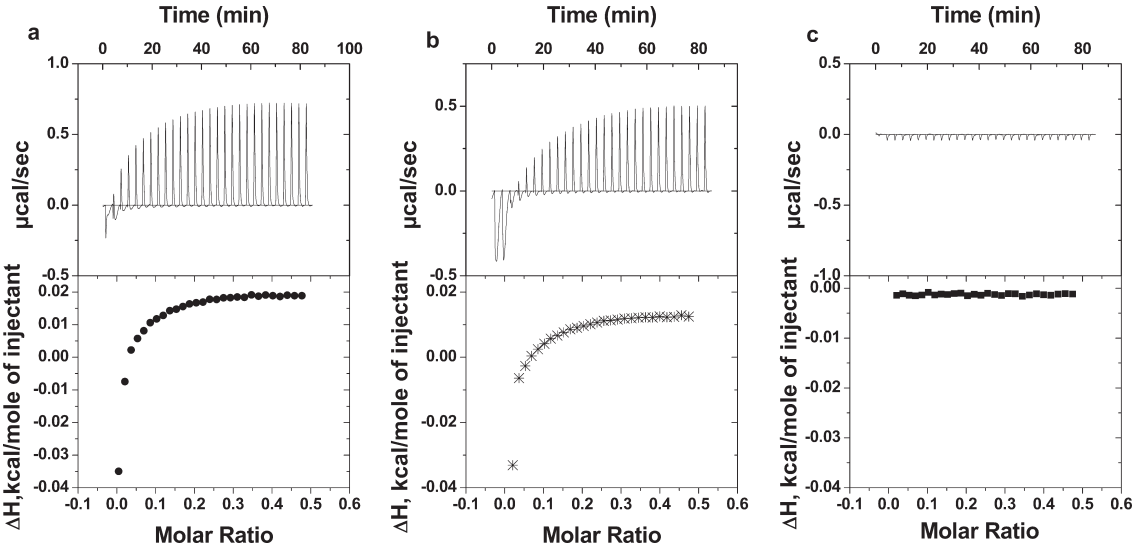

Figure 1. Raw ITC plots obtained at 15 °C: (a) Titration of 0.1 M KSCN solution into 0.5% (0.044 M NIPA) PNIPA solution; (b) titration of 0.1 M KSCN solution into water (blank I); (c) titration of water into 0.5% PNIPA solution (blank II).

curve, when the integrated enthalpy change per mole of ligand is plotted vs the molar ratio [L]/[M]). The apparent enthalpy change following the *i*th injection due to the studied association (corrected for ligand dilution and nonspecific effects) $\Delta h_{i,a}$, is obtained by:

$$\Delta h_{i,\,a} = \Delta h_{i,\,m} - \Delta h_{i,\,dil} - \Delta h_{i,\,ns} = \Delta[L_i] V_{cell} \Delta H_a \qquad (3)$$

The sum ($\Delta h_{i,dil} + \Delta h_{i,ns}$) is obtained by blank titration of the ligand into buffer (and if significant, another blank may be measured—that of injecting buffer into the macromolecule solution). $\Delta[L_i]$ is the change in bound ligand concentration in the *i*th injection, V_{cell} is the cell volume, and ΔH_a is the apparent molar association enthalpy change. To calculate ΔH_a, as well as *n*, the number of binding sites on M, the following equations are used:[76]

$$\Delta h_{i,\,a} = n[M]_{tot} V_{cell} \Delta H_a Y \qquad (4)$$

$[M]_{tot}$ is the total concentration of M in the sample cell, and *Y* is the root of the quadratic equation:

$$y_i^2 - y_i(1 + 1/(nK_A[M]_{tot}) + [L_i]_{tot}/(n[M]_{tot}) + n[L_i]_{tot}[M]_{tot} = 0 \qquad (5)$$

In which y_i is the degree of saturation, defined as

$$y_i = \Delta[L_i]_{bound}/[M]_{tot} \qquad (6)$$

$[L_i]_{tot}$ is the total concentration of ligand added until injection *i*. A nonlinear model-fitting using the MicroCal Origin analysis software, according to eqs 4−6 ("one-type-of-sites") yields *n*, K_A and ΔH_a based on a titration experiment.

By normalizing the molar concentration to the standard state concentration of 1 mol/L, K_A turns dimensionless, and the ΔG of binding can be obtained from the normalized K_A according to the expression

$$\Delta G = -RT \ln K_A \qquad (7)$$

where *R* is the gas constant (8.314 J/(K mol)) and *T* is in kelvin.[76]

The entropy change may then be calculated from the fundamental Gibbs equation:

$$\Delta G = \Delta H - T\Delta S \qquad (8)$$

Characterization of Binding of SCN⁻ and PNIPA. VP-ITC (MicroCal) was used to study the binding interactions between the chaotropic salt KSCN and PNIPA. We measured the enthalpy change during the addition of KSCN to PNIPA solution, as a function of KSCN concentration at different temperatures. The reaction cell (V_{cell} = 1.43 mL) was filled with degassed PNIPA aqueous solution ([NIPA] = 44.2 mM, 0.5 wt %/wt) or with water (blank). To obtain the stoichiometric binding ratio between the salt and the amide groups, we expressed PNIPA concentration in terms of monomer units (M_w = 113 Da). 0.05 M salt (KSCN) solutions were injected (1 step of 3 μL and 27 more steps of 10 μL each) into the polymer solution. The duration of each injection was 20 s. The injector stirred the solution at 310 rpm to ensure complete mixing within a few seconds. The system was re-equilibrated for 180 s before the following injection. Such an interval was found to be sufficient to equilibrate the system. Each experiment was performed in duplicate. Calorimetric data analysis was carried out using Origin 5.0 software (MicroCal). The system temperature was set to 15, 20, or 25 °C, i.e., below the phase transition of PNIPA. ITC titration was performed as follows: (1) titration of a PNIPA solution with KSCN solution, (2) titration of water with the same KSCN solution (blank I), (3) titration of the PNIPA solution with water (blank II). Appropriate subtractions of these two blanks were preformed in order to explore the possible direct binding interaction between the salt and the polymer.

Differential Scanning Microcalorimetry. The LCST phase transition of aqueous PNIPA solutions was studied using a VP-DSC microcalorimeter (Micro-Cal Inc., Northampton, MA). KSCN concentrations were varied in the range of 0−2 M. Solutions of 0.5−1 wt % PNIPA were prepared by dissolving the polymer in pure water or in salt solutions at a low temperature (4 °C). Degassed solutions were transferred to a sample cell (0.5 mL) with a calibrated syringe. The thermograms were measured at heating and cooling rates of 0.5 °C/min. Polymer-free solutions (water or salt solutions at same salt concentrations) were used in the reference cell. The LCST was determined from the onset temperature (T_{onset}) of the endothermic peak, determined as the intersection of the tangent of the endotherm's maximal rising slope, and the baseline[45] (Figure 2).

Debye Length Calculation. Debye length (denoted κ^{-1}) was calculated by the following expression[77] (eq 9):

$$\kappa^{-1} = \sqrt{\frac{\varepsilon_0 \varepsilon_r KT}{2N_A e^2 I}} \qquad (9)$$

where *I* is the ionic strength of the electrolyte. As KSCN is a monomonovalent salt, *I* is simply the molar concentration, converted to mol/m^3.

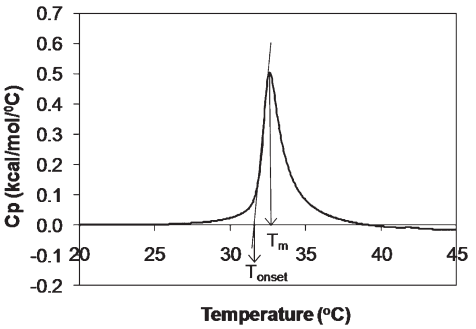

Figure 2. Typical Micro-DSC curve of a PNIPA LCST phase transition endotherm. T_{onset} was obtained from the intersection of the maximal slope tangent and the baseline. T_m is the temperature at the maximum point.

ε_0 is the permittivity of free space: $\varepsilon_0 \approx 8.85 \times 10^{-12}$ Amp^2 s^4 kg^{-1} m^{-3}.

ε_r is the dielectric constant, which in water at 20 °C is 80.1.

k is the Boltzmann's constant, 1.38×10^{-23} J/K.

T is the absolute temperature in kelvin.

N_A is Avogadro′s number: 6.022×10^{23} mol^{-1}.

e is the elementary charge: 1.602×10^{-19} C.

Statistical analysis was performed using JMP statistical analysis software (SAS Institute Inc.).

Results and Discussion

Isothermal Titration Calorimetry (ITC) Experiments. To test the conception that a strong chaotrope exerts its salting-in effect by binding to the polymer and, if so, to gain better understanding of the binding mechanism of KSCN to PNIPA, we employed isothermal titration calorimetry, which apparently has not yet been applied for studying this interaction.

Herein, we employed the commonly used "one-type-of-sites" model (eqs 4−6), which assumes all binding sites have similar affinity to the ligand, a reasonable assumption in the case of KSCN and the relatively simple homopolymer PNIPA.

Binding takes place only if the total free energy decreases, regardless of the actual enthalpy change due to direct molecular contact between the associating chaotropic anions[20,78] and the PNIPA amide nitrogen groups. The free energy of binding can be very small as a result of a fragile balance between favorable and unfavorable contributions and in this case, solvent effects may play a dominant role in the energy balance.

Figures 3 presents an ITC plot of a titration of PNIPA with KSCN obtained at 20 °C, after subtraction of two blank titrations (of KSCN into water, and of water into PNIPA solution, as shown in Figure 1 for 15 °C, and detailed in the methods section).

The titrations were done in duplicate at 15, 20, and 25 °C. The results obtained at 15 and 20 °C are described in Table 1. The titrations performed at 25 °C resulted in highly scattered data, which did not resemble a typical binding sigmoidal plot, and the model fit was not achievable, presumably due to too low a calorimetric signal.

It is evident from the plot in Figure 3, and from the data in Table 1 that the binding enthalpy is endothermic, suggesting an entropically driven process. Endothermic ion binding processes have been observed with ITC,[79,80] though in these cases the ligands were kosmotropic cations, e.g., Ca^{2+} and Cd^{2+}, and the entropy gain was explained by the release of their (highly ordered) hydration-sphere water as well as, hydration water of the binding-partner, and possibly by

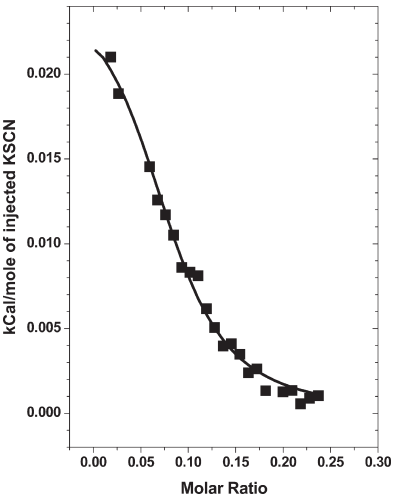

Figure 3. ITC titration curve showing the binding of KSCN to PNIPA at 20 °C. Line is a one-type of sites model fit (MicroCal Origin). Curve fit parameters are in Table 1.

disruption of ordered hydration shell surrounding the nonpolar residues of the peptide.[80] The low χ^2 values support a good fit of the one-type-of-sites model in Figure 3. The negative free energy change resulting from the model fit suggests a spontaneous binding in this KSCN concentration range (0−9 mM) at a 0−0.25 KSCN/NIPA molar ratio range. Even though SCN^- is small enough to distinguish the different local functional groups of PNIPA, the good fit obtained for the one-type of sites model was in line with our hypothesis, which was based on the homopolymeric structure of PNIPA, and on the notion that the amide nitrogen is likely to be the only favorable binding site for the SCN^- anion, as had been suggested by Von Hippel et al.[72] The amide group can be described in terms of two resonant states:[15]

$$>N-\underset{|}{C}=O \rightleftarrows >N^+=\underset{|}{C}-O^-$$

This resonance provides the amide nitrogen with a partial positive charge and the carbonyl with a partial negative charge enabling the chaotropic anion to bind at or near the nitrogen. Collins[20,78] suggested that weakly hydrated ions (chaotropes) are "pushed" onto weakly hydrated surfaces or nonionic polymers as water−water interactions are more favorable than ion and polymer hydration. This binding process is further explained by Collins based on the assumption that the amide nitrogen acts as a chaotropic cation, which forms "inner sphere" (or nearest neighbor) ion pairs with chaotropic anions, driven by the rejection of the water favoring water−water bonds.[20]

We propose the following mechanism to explain the entropically driven binding interaction we observed by ITC:

On the basis of the ion-pairing mechanism,[20,78] it is reasonable to assume that the chaotropic SCN anion is "pushed" by the water onto the slightly positive nitrogen of the PNIPA amide so as to free the hydration water molecules of both the ion and the amide. However, this mechanism alone cannot fully account for the endothermic binding we observed with the ITC, as the release of hydration water from the vicinity of chaotropic ions to the bulk should decrease entropy. Moreover, the binding of the SCN^- anion to the amide should be somewhat exothermic, and so are the formed hydrogen bonds of the released water molecules. Therefore, we propose that an additional mechanism, whose

Table 1. Values of the Binding Parameters Obtained by ITC

T (°C)	n	K_A (M^{-1})	ΔH (cal/mol K SCN)	ΔG (cal/mol K SCN)	ΔS (cal/(K mol KSCN))	χ^2 of model fit
15 av	**0.09**	**615**	**34.8**	**−3660**	**12.8**	0.50
15 std error	0.02	137	23.9	129	0.5	
20 av	0.11	**1486**	**16.4**	**−4251**	**14.6**	0.25
20 std error	0.02	98	9.2	37	0.1	

entropic effect is more dominant, takes place immediately following the ion binding to the amide. At these temperatures, below the cloud point of PNIPA, the nonpolar backbone and isopropyl groups are hydrophobically hydrated, and it is widely accepted that this hydration is highly ordered (Frank and Evans icebergs[81] or water cages). At the salt concentrations used in the ITC experiment (~4 mM), the Debye length in the solution is about 5 nm, which is ~20 times larger than the distance between the amide nitrogen and the isopropyl or the polymer backbone (each ~2.5 Å away). Therefore, when an SCN^- anion adsorbs onto the amide nitrogen, its electrical field can most probably disturb the hydrophobic hydration water near these nonpolar groups, resulting in increased entropy. The potassium counterion has a very slightly chaotropic nature as its charge density is not as low as that of KSCN, so it might be also binding to the amide oxygen, but to a much lesser extent. It is more likely to move (approximately within the Debye length radius) around the bound SCN^-, thereby intensifying the disordering effect on the hydration of the hydrophobic groups in this vicinity. As KSCN concentration increases, the Debye length decreases and this effect vanishes. Thus, approaching saturation is gradual (as seen below in Figure 6) as it does not necessarily reflect a complete coverage of the accessible sites but rather a diminished entropic driving force for binding. Therefore, in this case, we propose that both mechanisms (ion pairing and disturbance of the hydrophobic hydration by bound ions) prevail, leading to an increase in the overall entropy, thus facilitating a spontaneous endothermic binding process. When ITC titrations were performed at higher KSCN concentrations (0.1, 0.15, 0.3 M in the syringe), above 0.15 M no sigmoidal binding curves were obtainable (not shown). That is possibly because at the higher chaotropic salt concentrations, the change in the endothermic signal due mainly to breaking of hydrogen bonds of disturbed cages became too weak to be detected.

The slight increase in the binding constant from 15 to 20 °C, and then the decrease toward 25 °C (as no binding was observed) is an intriguing observation. We hypothesize that the increasing temperature initially decreases ΔG, but as the temperature continues to rise, the balance between the two mechanisms described-above is shifted, and the cages become weaker and less ordered due to thermal motion, thereby decreasing the relative importance of this ion disturbance effect, thus reducing the entropic driving force. Complete understanding of this observation may require further study.

An additional mechanism may become important at higher temperatures, approaching the cloud point of PNIPA. Wu and Wang (1998)[82] determined the temperature dependence of the average radius of gyration $\langle R_g \rangle$ of PNIPA in pure water, observing a decrease from $\langle R_g \rangle \sim 1.9 \times 10^2$ nm at 20 °C to $1.75*10^2$ nm at 25 °C. They interpreted this temperature-dependent change in the average $\langle R_g \rangle$ as a change from expanded coil conformation at 20 °C, to a "crumpled coil" conformation at 25° and eventually to a globule above the LCST. This change in the radius of gyration was accompanied by a decrease in A_2, the second virial coefficient of PNIPA-aqueous solution, representing a decrease in the solvent quality of the salt solution for the polymer. Therefore, one can expect that the number of sites accessible for SCN^- binding decreases as temperature increases approaching coil globule transition for this system. Krasovitski et al.,2004,[64] employed static light scattering (SLS) measurements of PNIPA aqueous solutions without and in the presence of SCN^- anions (247 mM NaSCN), showing that the radius of gyration, $\langle R_g \rangle$, of the polymer in the chaotrope solution (32.8 ± 0.3 nm) increased compared to its value in pure water (30.4 ± 0.3 nm) at the same temperature (18 °C). By using a Zimm plot they calculated R_g and A_2, the second virial coefficient, and concluded that the presence of SCN^- tended to reduce the free energy of mixing and significantly increase the second virial coefficient, A_2 (for PNIPA in the SCN^- solution, A_2 was 1.18×10^{-3}, and in water, A_2 was 6.67×10^{-4} cm^3mol/g^2). These measurements indicated that the presence of SCN^- turns the aqueous-chaotrope solution into a better solvent for PNIPA compared to pure water in the low concentration range studied, where the preferential binding is the dominant effect. This may also increase the osmotic pressure difference between a ternary (water−polymer−salt) solution and a respective polymer free binary solution, across a membrane impermeable to the polymer only. In this manner, Livney et al. showed an increase in the osmotic pressure of a Dextran solution with the addition of NaSCN compared to the osmotic pressure of the macromolecule in pure water.[66] They similarly showed an effect of iodide on polyacrylamide, and by measuring the equilibrium ion concentrations on both sides of the membrane, they derived a Langmuir binding isotherm.[65] A similar improvement in solvent quality due to preferential adsorption of the anion onto the polymer, may serve to explain the increase in the LCST of the polymer at low KSCN concentrations observed using Micro DSC, as described in the next section.

Effect of Increasing KSCN Concentration on the LCST Phase Transition of Aqueous PNIPA Solution, As Measured by Micro DSC. To study the effect of rising KSCN concentrations on the LCST of PNIPA, we employed a micro differential scanning calorimeter (Micro DSC). Figure 2 shows an endothermic peak obtained during heating a pure PNIPA solution in water. Figure 4 describes the effect of KSCN concentration on the measured heating endotherm of 0.5% PNIPA solutions during heating. When PNIPA is heated in pure water, the phase transition occurs as a sharp transition due to the cooperative collapse of long segments, and probably the entire polymer chain.[58] This cooperativity is related to the cooperative hydration[83] of this homopolymer. As salt concentration increases, the DSC peaks became shorter and broader. A somewhat similar behavior was observed by Schild and Tirrell for NaSCN and PNIPA.[52] We propose that the bound ions interfere with the cooperativity, hence the higher the salt concentration is, the less cooperative is the collapse process. The length of unperturbed segments decreases, and their size distribution heterogeneity increases, resulting in decreasing peak height and increasing width.

Microcalorimetric measurements were performed at several PNIPA concentrations. The peak shape was found to be independent of polymer concentration (not shown). The results shown in Figure 5 describe the phase transition onset temperatures obtained at 0.5, 0.75, and 1 wt % PNIPA with

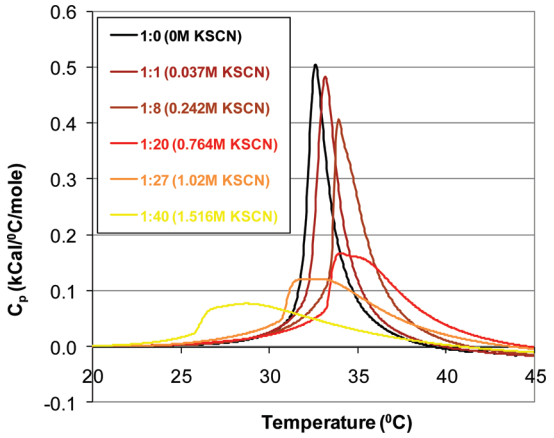

Figure 4. Microcalorimetric DSC endotherms for aqueous PNIPA solutions (0.5%wt) in presence of different KSCN concentrations. (The key shows the NIPA:KSCN molar ratio and KSCN molar concentration).

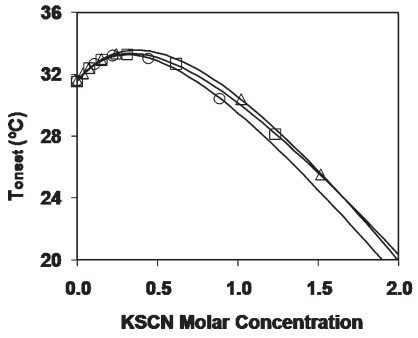

Figure 5. Effect of KSCN molar concentration on LCST onset temperature of PNIPA solutions (triangles, 0.5 wt % PNIPA; circles, 0.75 wt % PNIPA; squares, 1 wt % PNIPA). The lines are a model fit based on Zhang et al.[68]

rising KSCN concentration. At all PNIPA concentrations adding KSCN first increased the LCST up to a maximum (~0.4 M KSCN), (showing a "salting-in" behavior). Above this concentration, the LCST decreased with increasing salt concentration. Further to our discussion of the possible effect of the Debye length on the binding, it is noteworthy that at the concentration of maximal salting-in, ~0.4 M, the Debye length is about 5 Å, and at ~0.8 M, where the onset temperature is back to around its value in pure water, Debye length is ~3.4 Å, i.e., only slightly more than the distance between the amide and the centers of the nearest hydrophobic groups (~2.5 Å). (In fact, the Debye length is even shorter, taken that the local ion concentration near the polymer is actually higher than the average). Therefore, the hydration cages are at the limit of the range of the bound ion influence, which could explain the diminished binding-induced salting-in effect beyond this concentration.

The lines in Figure 5 represent model fits based on eq 1.[68] By subtracting the linear contribution ($T_0 + C$[M]) and plotting the temperature increase of the LCST compared to LCST in pure water, ΔT, vs KSCN concentration, a Langmuir shape binding isotherm is obtained,[68] (see Figure 6 as an example, at 0.5 wt % PNIPA). This Langmuir-curve shape provides an additional evidence for the binding of KSCN onto PNIPA, thus corroborating the ITC results shown above. Table 2 presents the values of the model parameters obtained by fitting the model at different polymer concentrations.

Table 2. Values of C, B_{max} and K_A, (Eq 1) Obtained for KSCN, by Fitting the Model of Cremer and Co-Workers,[63,68,71] at Different PNIPA Concentrations

PNIPA concentration (% wt)	C (°C/M) (std. err)	B_{max} (°C) (std error)	K_A (M^{-1}) (std error)
0.5	−15.8 (0.4)	30.8 (1.7)	0.92 (0.04)
0.75	−14.2 (1.1)	21.9 (3.1)	1.2 (0.1)
1.0	−13.2 (0.7)	21.6 (2.1)	1.2 (0.2)

The lower binding constants obtained from this model fit, compared to the values obtained by ITC are partly due to the fact that the ITC results were obtained at constant low temperatures, where the binding is optimal. In contrast, the DSC-based Langmuir curves represent experiments done during heating to the phase transition temperature, where the polymer collapses, and the binding is much weaker, due to the reasons discussed above. Nevertheless, the binding constants we obtained here (0.92−1.2 M^{-1}) were within the same order of magnitude as those obtained by Zhang et al.[63] (2.8 M^{-1} for M_n = 30 070 Da, and 4.3 M^{-1} for 121 000 Da, while the polymer used here was 39 200 Da). This is a reasonably good agreement, given the different methods used, and the fact that the cations were different (potassium vs sodium). Likewise, differences in the values of B_{max} and C obtained in the two studies are reasonable given these experimental differences.

The constant c(M) was shown[63] to correlate with the surface tension increment σ[22] for chaotropic anions, hence it has been proposed[63] to explain the salting-out, or decrease in PNIPA phase transition temperature at higher KSCN concentration (Figure 5). The increase in surface energy around hydrophobic groups would indeed favor polymer−polymer interactions. However, further study has to be performed to test the validity of the surface tension mechanism, because there are considerable uncertainties[22] regarding reliability of surface tension increment data in the literature. Moreover, any solution property, which is linearly dependent on salt concentration, would correlate with c. The salting-out effect of KSCN at high concentration may even be the result of mere competition over available hydration water. Table 1 shows that the negative values of c slightly decreased in absolute value with increasing polymer concentration, however this was not statistically significant. Zhang et al.[63] also observed small insignificant changes in the value of c with increasing PNIPA concentration in the presence of another chaotrope ($NaClO_4$).

Table 2 also shows that the magnitude of the Langmuir asymptote B_{max} decreased and that of the binding constant K_A slightly increased, when the polymer concentration increased from 0.5% to 0.75%, though no further significant changes were observed from 0.75% to 1%. Zhang et al.[63] also observed an increase in K_A and decrease in B_{max} with increasing PNIPA concentration in the presence $NaClO_4$.

Figure 7 shows a heating−cooling cycle of 0.5% PNIPA (A) in pure water and (B) in a 0.242 M KSCN solution. Cooling commenced immediately after heating to 50 °C (the holding time at the collapsed state may affect hysteresis, however this was not varied in this study). The transition midpoint temperature (T_M) during the cooling process was about 2 °C lower than that during the heating process, indicating hysteresis. Such a hysteresis phenomenon was previously observed in binary aqueous PNIPA solutions by static light scattering, SLS[82] and also by microcalorimetric DSC experiments in K_2SO_4 solution made by Paz et al.[57] As can be seen in Figure 8 the hysteresis (ΔT_M) decreased sharply with increasing KSCN concentration in all PNIPA

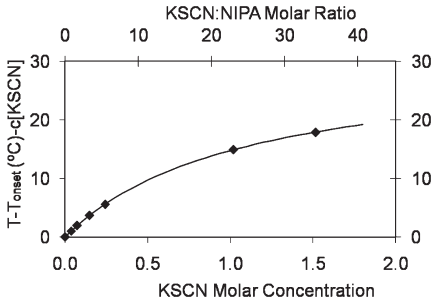

Figure 6. Langmuir-plot of the effect of KSCN molar concentration (bottom axis) and KSCN:NIPA molar ratio (top axis) on (T_{onset} (°C) − T^0 − c[KSCN]) in 0.5% PNIPA solutions. Diamonds are based on the experimental points, and the line is the Langmuir component ($B_{max}K_A$[KSCN]/(1 + K_A[KSCN])) of the Zhang et al.[68] model fit.

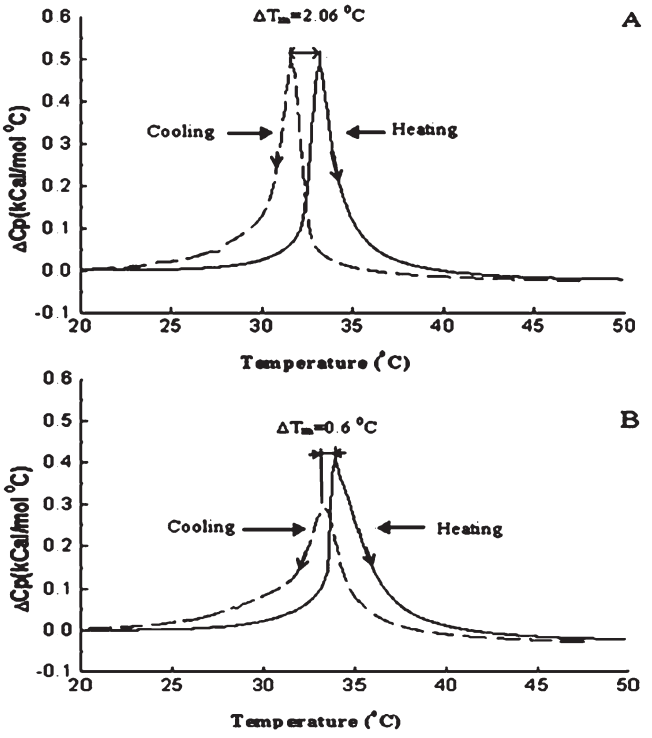

Figure 7. MicroCal DSC thermograms of (A) 0.5% PNIPA in water and (B) ternary solution containing 0.242 M KSCN (solid lines refer to heating, and dashed lines refer to cooling).

concentrations studied and reached a magnitude very close to zero at about 0.6 M KSCN.

The effect of KSCN on hysteresis was not significantly affected by changes in polymer concentrations. Moreover, the curves exhibited a saturation effect, by decreasing toward an asymptote with increasing salt concentration. This is most likely another expression of the binding saturation process. Interestingly, in the above-mentioned study[57] the hysteresis was found to decrease by the addition of a strong kosmotropic salt, Na_2SO_4. The effect was explained there based on the notion that the sulfate ions cause a relative stabilization of the so-called crumpled coil stage (partial intrachain collapse[82,84]) due to the salting-out effect of the salt. It was further suggested that this stabilization causes a smaller extent of interchain penetration above LCST, resulting in a smaller extent of hysteresis.[57]

Zeng et al.,[85] showed rheological evidence that during PNIPA phase separation a reversible network is created, apparently due to physical association of the hydrophobic

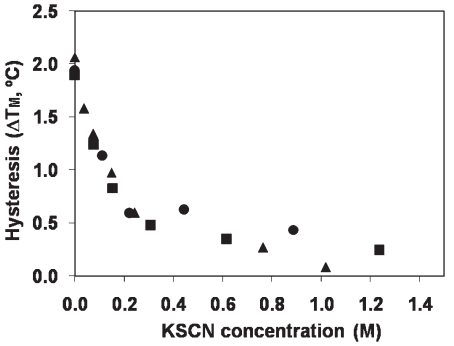

Figure 8. LCST hysteresis dependence on KSCN concentration (triangles, 0.5 wt % PNIPA solutions; circles, 0.75 wt % PNIPA solutions; squares, 1 wt % PNIPA solutions).

side groups of the collapsed polymer chains. We propose that in the case of a chaotropic salt, such as KSCN, the presence of bound ions diminishes segment−segment attraction both on the intrachain and on the interchain level, thereby assisting the globule-to-coil transition and the dissociation of aggregates and making the rehydration during cooling easier than in pure water, thereby decreasing the hysteresis (ΔT_m) with salt addition. Thus, both a kosmotropic and a chaotropic salt decrease hysteresis, but they do so via two very different mechanisms.

Conclusions

The LCST phase transition of aqueous PNIPA solutions in the presence of the strong chaotropic salt KSCN at rising salt and polymer concentrations were studied by two complementary microcalorimetric methods, ITC and DSC.

Our main findings in the current work were as follows:

(1) The ITC observation of the endothermic binding of KSCN onto PNIPA at constant temperatures provides strong support to the notion of binding of KSCN (more likely, the SCN^- anion) to PNIPA amide based on the good fit found for the one-type-of-sites binding model. We suggest that this weak endothermic binding is driven by the increase in entropy due to disturbance of the hydrophobic hydration ("cages") of the isopropyl and the backbone effected by the electrostatic field of the bound anion (and its more freely moving vicinal counterion) at low salt concentration (long Debye length). The observed increase in binding affinity from15 to 20 °C and then the decrease at 25 °C are suggested to be mainly due to the opposite effects of T and ΔS, because as temperature rises, the entropy of the "cages" increases and becomes less different from that of bulk water, hence diminishing the entropic gain of disrupting the cages.

(2) Our DSC results emphasize the gradual change in shape of the endothermic peak of PNIPA phase transition, which becomes lower and broader with rising KSCN concentration, in addition to the fact the peak first shifts to higher temperatures (salting-in) and then to lower temperatures (salting-out). We propose that the broadening of the peak is due to increased heterogeneity of local domains due to inhomogeneous adsorption of the salt on the polymer, and consequently reduced cooperativity of the chain collapse.

(3) Another unique observation is the hysteresis of the DSC endotherms upon heating and cooling PNIPA solutions, which was found to decrease in its extent

upon rising KSCN concentration, approaching an asymptote. This result further supports the conclusion that binding of KSCN to PNIPA occurs.

We conclude that an entropically driven adsorption is a major mechanism in the salting-in effect of chaotropic salts, of which KSCN is one of the strongest, on phase transition of PNIPA, which in some respects may serve as a model for protein behavior in aqueous systems.

Acknowledgment. The authors thank Prof. S. Mizrahi for critically reviewing the manuscript.

References and Notes

(1) Hofmeister, F. *Naunyn-Schmiedeberg's Arch. Pharmacol* **1888**, *25* (1), 1–30.
(2) von Hippel, P. H.; Schleich, T. *Acc. Chem. Res.* **1969**, *2*, 257–265.
(3) von Hippel, P. H.; Wong, K. Y. *Science* **1964**, *145*, 577–580.
(4) von Hippel, P. H.; Schleich, T.; Timasheff, S. N.; Fasman, G. D. The Effects of Neutral Salts on the Structure and Conformational Stability of Macromolecules in Solution. In *Structure and Stability of Biological Macromolecules*; Marcel Dekker Inc.: New York, 1969; pp 417−574.
(5) Broering, J. M.; Bommarius, A. S. *J. Phys. Chem. B* **2005**, *109*, 20612–20619.
(6) Pinna, M. C.; Bauduin, P.; Touraud, D.; Monduzzi, M.; Ninham, B. W.; Kunz, W. *J. Phys. Chem. B* **2005**, *109*, 16511–16514.
(7) Pinna, M. C.; Salis, A.; Monduzzi, M.; Ninham, B. W. *J. Phys. Chem. B* **2005**, *109*, 5406–5408.
(8) Bauduin, P.; Nohmie, F.; Touraud, D.; Neueder, R.; Kunz, W.; Ninham, B. W. *J. Mol. Liq.* **2006**, *123*, 14–19.
(9) Vrbka, L.; Jungwirth, P.; Bauduin, P.; Touraud, D.; Kunz, W. *J. Phys. Chem. B* **2006**, *110*, 7036–7043.
(10) Banerjee, R.; Frilley, B.; Guissani, A. *Ind. J. Biochem. Biophys.* **1999**, *36*, 107–117.
(11) Perez-Jimenez, R.; Godoy-Ruiz, R.; Ibarra-Molero, B.; Sanchez-Ruiz, J. M. *Biophys. J.* **2004**, *86*, 2414–2429.
(12) Lagi, M.; Lo Nostro, P.; Fratini, E.; Ninham, B. W.; Baglioni, P. *J. Phys. Chem. B* **2007**, *111*, 589–597.
(13) Collins, K. D. *Biophys. Chem.* **2006**, *119*, 271–281.
(14) Zhang, Y. J.; Cremer, P. S. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 15249–15253.
(15) Timasheff, S. N.; Fasman, G. D., Eds.; *Structure and Stability of Biological Macromolecules* (*Biological Macromolecules, Vol. 2*); 1969; p 694.
(16) Arakawa, T.; Timasheff, S. N. *Biochemistry* **1982**, *21*, 6545–6552.
(17) Timasheff, S. N. *Biochemistry* **2002**, *41*, 13473–13482.
(18) Schellman, J. A. *Biophys.Chem.* **1990**, *37*, 140.
(19) Schellman, J. A. *Biophys. J.* **2003**, *85* (1), 108–125.
(20) Collins, K. D. *Biophys. J.* **1997**, *72* (Jan. 97), 65−72.
(21) Collins, K. D.; Washabaugh, M. W. *Q. Rev. Biophys.* **1985**, *18*, 323–422.
(22) Kunz, W.; Lo Nostro, P.; Ninham, B. W. *Curr. Opin. Colloid Interface Sci.* **2004**, *9* (1−2), 1–18.
(23) Marcus, Y. *Ion Properties*; Marcel Dekker Inc.: New York, NY, 1997.
(24) Melander, W.; Horvath, C. *Arch. Biochem. Biophys.* **1977**, *183*, 200–215.
(25) Kiriukhin, M. Y.; Collins, K. D. *Biophys. Chem.* **2002**, *99* (2), 155–168.
(26) Piculell, L.; Nilsson, S. *Prog. Colloid Polym. Sci.* **1990**, *82*, 198–210.
(27) Baltes, T.; Garret-Flaudy, F.; Freitag, R. *J.Polym.Sci.: Part A: Polym.Chem.* **1999**, *37*, 2977–2989.
(28) Hong, J. S.; Nakahara, T.; Maeda, H.; Kikunaga, Y.; Kishida, A.; Akashi, M. *Colloid Polym. Sci.* **1996**, *274*, 1013–1019.
(29) Suwa, K.; Yamamoto, K.; Akashi, M.; Takano, K.; Tanaka, N.; Kunugi, S. *Colloid Polym. Sci.* **1998**, *276*, 529–533.
(30) Inomata, H.; Goto, S.; Otake, K.; Saito, S. *Langmuir* **1992**, *8*, 687–690.
(31) Park, T. G.; Hoffman, A. S. *Macromolecules* **1993**, *26*, 5045–5048.
(32) Dhara, D.; Chatterji, P. R. *J. Macromol. Sci.—Rev. Macromol. Chem. Phys.* **2000**, *C40* (1), 51–68.
(33) Annaka, M.; Motokawa, K.; Sasaki, S.; Nakahira, T.; Kawasaki, H.; Maeda, H.; Amo, Y.; Tominaga, Y. *J. Chem. Phys.* **2000**, *113*, 5980–5985.
(34) Kawasaki, H.; Mitou, T.; Sasaki, S.; Maeda, H. *Langmuir* **2000**, *16*, 1444–1446.
(35) Suzuki, A. *Adv. Polym. Sci.* **1993**, *110*, 201–240.
(36) Schild, H. G. *Prog. Polym. Sci.* **1992**, *17*, 163–249.
(37) Idziak, I.; Avoce, D.; Lessard, D.; Gravel, D.; Zhu, X. X. *Macromolecules* **1999**, *32*, 1260–1263.
(38) Liu, H. Y.; Zhu, X. X. *Polymer* **1999**, *40*, 6985–6990.
(39) Takano, M.; Ogata, K.; Kawauchi, S.; Satoh, M.; Komiyama, J. *Polym. Gels Networks* **1998**, *6*, 217–232.
(40) Graziano, G. *Int. J. Biol. Macromol.* **2000**, *27* (1), 89–97.
(41) Hirotsu, S.; Hirokawa, Y.; Tanaka, T. *J. Chem. Phys.* **1987**, *87*, 1392–1395.
(42) Winnik, F. M. *Macromolecules* **1989**, *22*, 734–742.
(43) Winnik, F. M. *Macromolecules* **1990**, *23*, 233–242.
(44) Otake, K.; Inomata, H.; Konno, M.; Saito, S. *Macromolecules* **1990**, *23*, 283–289.
(45) Ramon, O.; Kesselman, E.; Berkovici, R.; Cohen, Y.; Paz, Y. *J. Polym. Sci., Part B: Polym. Phys.* **2001**, *39*, 1665–1677.
(46) Hirotsu, S. *J. Chem. Phys.* **1988**, *88*, 427–431.
(47) Kratz, K.; Hellweg, T.; Eimer, W. *Polymer* **2001**, *42*, 6631–6639.
(48) Wu, C.; Zhou, S. Q. *Macromolecules* **1995**, *28*, 8381–8387.
(49) Wu, C.; Zhou, S. Q. *Macromolecules* **1995**, *28*, 5388–5390.
(50) Li, Y.; Tanaka, T. *J. Chem. Phys.* **1989**, *90*, 5161–5166.
(51) Heskins, M.; Guillet, J. E. *J. Macromol. Sci., Pure Appl. Chem.* **1968**, *2*, 1441–1455.
(52) Schild, H. G.; Tirrell, D. A. *J. Phys. Chem.* **1990**, *94*, 4352–4356.
(53) Meewes, M.; Ricka, J.; Desilva, M.; Nyffenegger, R.; Binkert, T. *Macromolecules* **1991**, *24*, 5811–5816.
(54) Tokuhiro, T.; Amiya, T.; Mamada, A.; Tanaka, T. *Macromolecules* **1991**, *24*, 2936–2943.
(55) Zeng, F.; Tong, Z.; Feng, H. *Polymer* **1997**, *38*, 5539–5544.
(56) Kesselman, E.; Ramon, O.; Berkovici, R.; Paz, Y. *Polym. Adv. Technol.* **2002**, *13*, 982–991.
(57) Paz, Y.; Kesselman, E.; Fahoum, L.; Portnaya, I.; Ramon, O. *J. Polym. Sci., Part B: Polym. Phys.* **2004**, *42*, 33–46.
(58) Schild, H. G.; Tirrell, D. A. *J. Phys. Chem.* **1990**, *94*, 4352–4356.
(59) Tiktopulo, E. I.; Bychkova, V. E.; Ricka, J.; Ptitsyn, O. B. *Macromolecules* **1994**, *27*, 2879–2882.
(60) Boutris, C.; Chatzi, E. G.; Kiparissides, C. *Polymer* **1997**, *38*, 2567–2570.
(61) Yamazaki, Y.; Tada, T.; Kunugi, S. *Colloid Polym. Sci.* **2000**, *278* (1), 80–83.
(62) Cho, E. C.; Lee, J.; Cho, K. *Macromolecules* **2003**, *36*, 9929–9934.
(63) Zhang, Y.; Furyk, S.; Sagle, L. B.; Cho, Y.; Bergbreiter, D. E.; Cremer, P. S. *J Phys Chem C Nanomater Interfaces* **2007**, *111*, 8916–8924.
(64) Krasovitski, E.; Cohen, Y.; Bianco-Peled, H. *J. Polym. Sci., Part B: Polym. Phys.* **2004**, *42*, 3713–3720.
(65) Livney, Y. D.; Portnaya, I.; Faupin, B.; Ramon, O.; Cohen, Y.; Cogan, U.; Mizrahi, S. *J. Polym. Sci., Part B: Polym. Phys.* **2003**, *41*, 508–519.
(66) Livney, Y. D.; Ramon, O.; Kesselman, E.; Cogan, U.; Mizrahi, S.; Cohen, Y. *J. Polym. Sci., Part B: Polym. Phys.* **2001**, *39*, 2740–2750.
(67) Muta, H.; Kojima, R.; Kawauchi, S.; Tachibana, A.; Satoh, M. *J. Mol. Struct.* (*THEOCHEM*) **2001**, *536*, 210–226.
(68) Zhang, Y.; Furyk, S.; Bergbreiter, D. E.; Cremer, P. S. *J. Am. Chem. Soc.* **2005**, *127*, 14505–14510.
(69) Collins, K. D. *Methods* **2004**, *34*, 300–311.
(70) Gurau, M. C.; Lim, S. M.; Castellana, E. T.; Albertorio, F.; Kataoka, S.; Cremer, P. S. *J. Am. Chem. Soc.* **2004**, *126*, 10522–10523.
(71) Zhang, Y.; Cremer, P. S. *Curr. Opin. Chem. Biol.* **2006**, *10*, 658–663.
(72) von Hippel, P. H.; Peticolas, V.; Schack, L.; Karlson, L. *Biochem. J.* **1973**, *7*, 1256–1264.
(73) Hamabata, A.; von Hippel, P. H. *Biochem. J.* **1973**, *7* (1264), 1271.
(74) Oh, S. H.; Ryoo, R.; Jhon, M. S. *Macromolecules* **1990**, *23*, 1671–1675.
(75) Song, J. D.; Ryoo, R.; John, M. S. *Macromolecules* **1991**, *24*, 1730.
(76) Jelesarov, I.; Bosshard, H. R. *J. Mol. Recognition* **1999**, *12* (1), 3–18.
(77) Russel, W. B.; Saville, D. A.; Schowalter, W. R. *Colloidal Dispersions*; Cambridge University Press: Cambridge, U.K., 1989.
(78) Collins, K. D. *Proc. Natl. Acad. Sci. U.S.A.* **1995**, *92*, 5553–5557.
(79) Sinn, C. G.; Dimora, R.; Huin, C.; Sel, O.; Antonietti, M. *Macromolecules* **2006**, *39* (18), 6310–6312.
(80) Mukherjee, M.; Zhu, X.; Ogawa, M. Y. *Inorg. Chem.* **2008**, *47*, 4430–4432.
(81) Frank, H. S.; Evans, M. W. *J. Chem. Phys.* **1945**, *13*, 507–532.
(82) Wu, C.; Wang, X. H. *Phys. Rev. Lett.* **1998**, *80*, 4092–4094.
(83) Okada, Y.; Tanaka, F. *Macromolecules* **2005**, *38*, 4465–4471.
(84) Wang, X. H.; Qiu, X. P.; Wu, C. *Macromolecules* **1998**, *31*, 2972–2976.
(85) Zeng, F.; Zheng, X.; Tong, Z. *Polymer* **1998**, *39*, 1249–1251.