

Specific Ion Effects on the Water Solubility of Macromolecules: PNIPAM and the Hofmeister Series

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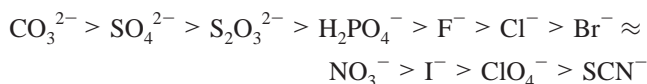
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Abstract: Aqueous processes ranging from protein folding and enzyme turnover to colloidal ordering and macromolecular precipitation are sensitive to the nature and concentration of the ions present in solution. Herein, the effect of a series of sodium salts on the lower critical solution temperature (LCST) of poly(*N*-isopropylacrylamide), PNIPAM, was investigated with a temperature gradient microfluidic device under a dark-field microscope. While the ability of a particular anion to lower the LCST generally followed the Hofmeister series, analysis of solvent isotope effects and of the changes in LCST with ion concentration and identity showed multiple mechanisms were at work. In solutions containing sufficient concentrations of strongly hydrated anions, the phase transition of PNIPAM was directly correlated with the hydration entropy of the anion. On the other hand, weakly hydrated anions were salted-out through surface tension effects and displayed improved hydration by direct ion binding.

Introduction

It was noted in 1888^{1,2} that the ability of salts to precipitate certain proteins from an aqueous solution follows a recurring trend now known as the Hofmeister series.^{3–13} This behavior is more pronounced for anions than cations and is a general phenomenon. The typical order of the anion series is:



The species on the left are referred to as kosmotropes, while those on the right are called chaotropes. These terms were originally used to describe the capacity of a particular ion to “make” or “break” water structure, respectively, as this was thought to be central to the mechanism of the effect. However, recent experimental data have cast doubt on the notion that changes in bulk water structure can explain the Hofmeister

series.^{14–16} Newer theories involving the relative polarizabilities of the ions and specific ion binding have been proposed, but have only received limited testing.^{10,13} Here we show that the Hofmeister series can be explained for a thermal responsive polymer purely on the basis of direct interactions of the anions with the macromolecule and its immediately adjacent hydration shell. Significantly, the phase transition of the polymer proceeds through separate mechanisms for chaotropes and kosmotropes.

It has been known for over 40 years that PNIPAM becomes insoluble above its lower critical solution temperature causing the aqueous system to turn cloudy and, hence, scatter light.^{17,18} PNIPAM bears both hydrophilic (amide) and hydrophobic (isopropyl) groups and has been described as a model system for the cold denaturation of peptides and proteins.¹⁹ Salt effects on the LCST of PNIPAM have been reported and related to the Hofmeister series.^{20–23} Unfortunately, however, the measurements are generally not of sufficient precision to allow for a detailed analysis to be made on the effects of salts. In this contribution, we use our recently developed temperature gradient microfluidic apparatus^{24–27} together with dark-field microscopy

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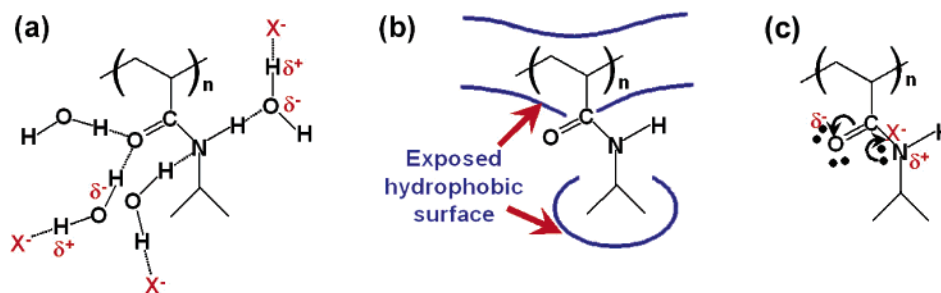


Figure 1. Interactions among anions, PNIPAM, and hydration waters. (a) Hydrogen bonding of the amide and its destabilization through polarization by the anion, X^- . (b) The hydrophobic hydration of the molecule is associated with surface tension and can be modulated by salt. (c) Direct binding of the anion to the amide group of PNIPAM.

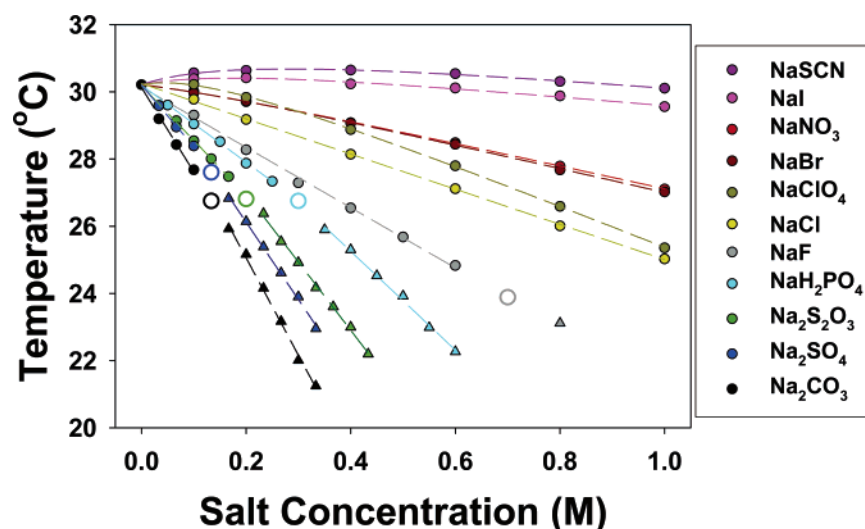


Figure 2. LCST values of PNIPAM measured for sodium salts at concentrations from 0 to 1.0 M. The dashed lines are curve fits to the data (see eq 1). The upward pointing filled triangles (▲) for the kosmotropes are for the lower-temperature transition of the two-step phase transition. The higher-temperature phase transition for kosmotropes (see Figure 4) is not shown. The open circles, ○, denote the point where the LCST crosses over from a single- to a two-step transition. All error bars are within the data points drawn. The order of the curves (top-to-bottom) corresponds to the color legend at the right of the figure.

to determine the mechanism of aggregation and precipitation of PNIPAM, resulting from the polymer's interactions with various anions. The device allows rapid measurements of the LCST to be made with extremely high temperature precision while only requiring a few microliters of solution.^{25–27} These measurements showed for the first time that the LCST proceeds in two separate steps in the presence of a sufficiently high concentration of kosmotropic anions. Also, Langmuir isotherms and, hence, anion binding data can actually be extracted from LCST measurements in the presence of chaotropes.

Our studies have revealed that all the effects of Hofmeister anions on PNIPAM solvation can be explained by three interactions of the ions with the polymer and its hydration waters. First, the anions can polarize an adjacent water molecule that is in turn involved in hydrogen bonding with the amide (Figure 1a). Second, these species can interfere with the hydrophobic hydration of the macromolecule by increasing the surface tension of the cavity surrounding the backbone and the isopropyl side chains (Figure 1b). Third, the anions may bind directly to the polyamide (Figure 1c). The first and second of these effects should lead to the salting-out of the polymer—

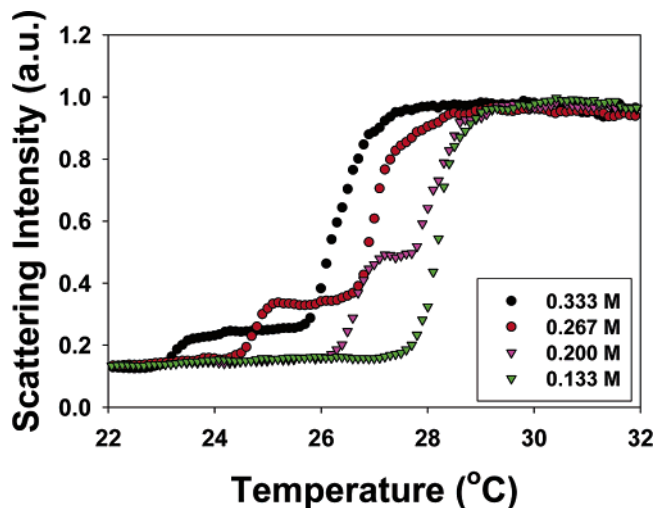


Figure 3. Light-scattering curves for PNIPAM in Na₂SO₄ solutions at various salt concentrations. The two-step phase transition can be visualized at 0.200 M and above.

thereby lowering the LCST. The third effect should lead to the salting-in of the polymer.

Results and Discussion

LCST vs Salt Concentration. Figure 2 shows the initial onset values of the LCST for PNIPAM solutions as a function of anion

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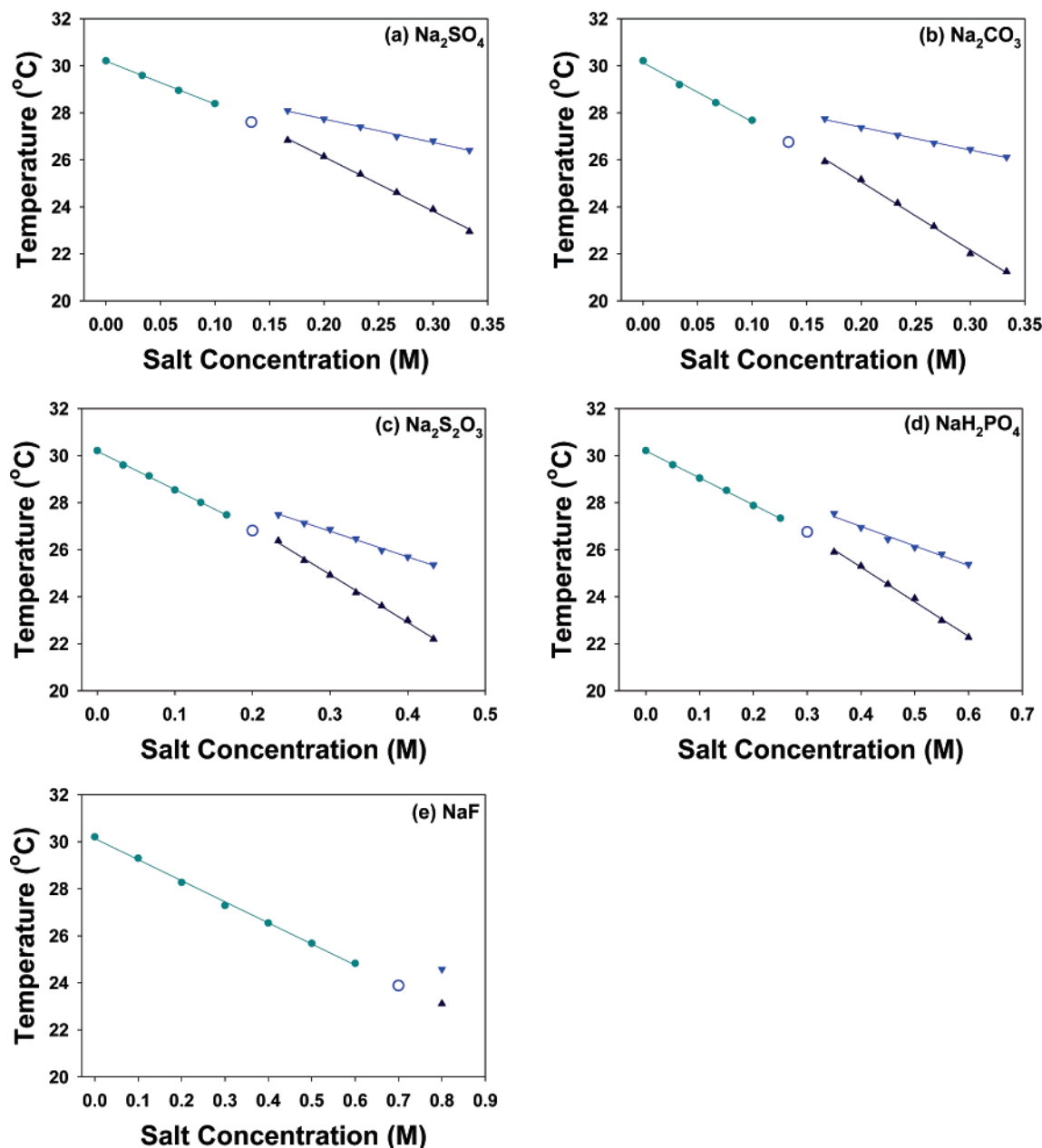


Figure 4. Plots of LCSTs vs salt concentration for kosmotropes: (a) Na_2SO_4 ; (b) Na_2CO_3 ; (c) $\text{Na}_2\text{S}_2\text{O}_3$; (d) NaH_2PO_4 ; (e) NaF . Initial slope (gray-green \bullet). Lower-temperature step in the two-step phase transition (blue \blacktriangle). Higher-temperature transition (blue \blacktriangledown). (\circ) represent the point where the phase transition widens without explicitly splitting into two separate steps. This designates the transition from a single- to a two-step process.

type and concentration for all 11 anions studied. The five kosmotropic species show linear dependence on salt concentration at low salt concentration. Beyond a certain concentration, a two-step transition is observed for these anions, and both a low and high temperature change in the light scattering data is observed. Only the low-temperature phase transition point, \blacktriangle , is plotted in Figure 2. The other anions behave quite differently. For these chaotropic species, the effect of anion concentration on LCST is nonlinear. This is most obvious for ClO_4^- and SCN^- .

Increasing concentrations of kosmotropic anions such as sodium carbonate, sodium sulfate, sodium thiosulfate, sodium dihydrogen phosphate, and sodium fluoride affect PNIPAM solubility in a complex fashion. The solubility of PNIPAM in an aqueous solution with these species shows a linear dependence on salt concentration that abruptly changes slope as the

concentration of the anion is increased. This latter behavior occurs concomitantly with the resolution of the phase transition into a low- and a high-temperature transition as illustrated by the two-step transition seen for Na_2SO_4 solutions in Figure 3. At Na_2SO_4 concentrations ≤ 0.133 M, a single LCST is seen. Higher Na_2SO_4 concentrations cause a separation of the LCST into two distinct transitions. A plot of the temperatures for the onset of the lower- and higher-temperature phase transitions vs Na_2SO_4 concentration in Figure 4a shows that the individual steps are associated with distinct slopes. The lower-temperature phase transition has steeper salt concentration dependence than the single-step transition seen at low Na_2SO_4 concentrations. The higher-temperature phase transition in the two-step region has a markedly shallower slope. The other kosmotropic anions (CO_3^{2-} , $\text{S}_2\text{O}_3^{2-}$, H_2PO_4^- , and F^-) behave similarly (Figure 4b–e).

Table 1. Fitted Values for K_A , B_{\max} , and c from LCST Measurements as Well as Literature Values of Surface Tension Increments (σ) and Entropy of Hydrations (ΔS_{hydr}) for the Anions

anion	σ^a (mN L/m mol)	ΔS_{hydr}^b (J/K mol)	B_{\max} (°C)	K_A (M ⁻¹)	c (°C/mol)		
					initial	first step	second step
CO ₃ ²⁻	2.6	-245	—	—	-25.1	-29.0	-9.7
SO ₄ ²⁻	2.7	-200	—	—	-18.3	-23.1	-10.0
S ₂ O ₃ ²⁻	2.9	-180	—	—	-16.3	-20.3	-10.9
H ₂ PO ₄ ⁻	2.3	-166	—	—	-11.5	-14.7	-8.3
F ⁻	2.0	-137	—	—	-9.0	—	—
Cl ⁻	1.6	-75	—	—	-5.3	—	—
Br ⁻	1.3	-59	0.7	2.7	-3.7	—	—
NO ₃ ⁻	1.1	-76	0.6	2.9	-3.5	—	—
I ⁻	1.0	-36	1.1	4.3	-1.5	—	—
ClO ₄ ⁻	1.4	-57	1.8	5.1	-6.4	—	—
SCN ⁻	0.5	-66	1.6	4.3	-1.4	—	—

^a From ref 29 except for H₂PO₄⁻ from ref 3, and CO₃²⁻ and S₂O₃²⁻ from ref 30. ^b From ref 31.

Modeling Changes in the LCST with Added Salt. It is known that the surface tension of water at the hydrophobic/aqueous interface varies linearly with salt concentration for simple inorganic salts up to moderate concentrations.²⁸ The polarization of water molecules in the first hydration shell of a macromolecular solute should also depend linearly on salt concentration. We propose that each of these effects can dominate the precipitation of PNIPAM depending on whether the anion is a chaotrope or a kosmotrope. The third effect—a salting-in effect due to enthalpically favorable anion-polymer interactions—is also observed for the most weakly hydrated anions. Such direct ion binding to the polymer is a saturation phenomenon and can be modeled with a binding isotherm. The data in Figure 2 for the perturbation of PNIPAM's LCST in water by added salts can be modeled by eq 1 which includes a constant, a linear term, and a Langmuir isotherm:

$$T = T_0 + c[M] + \frac{B_{\max} K_A [M]}{1 + K_A [M]} \quad (1)$$

T_0 is the LCST of PNIPAM in the absence of salt, and $[M]$ is the molar concentration of salt. The constant, c , has units of temperature/molarity. We will show that this term is directly proportional to the hydration entropy of the anions, ΔS_{hydr} , if changes in polymer solvation with salt concentration dominate the mechanism of the phase transition as is the case for the kosmotropic anions. Alternatively, for chaotropic anions (and the second step of the two-step transition for kosmotropes), we will demonstrate that changes in the surface tension with salt concentration dominate the mechanism and c is proportional to the surface tension increment, σ , of each anion. Also, weak binding of the chaotropic anions to the polymer affects macromolecule solubility. Therefore, the third term in eq 1 is required. K_A is the binding constant of the anion to the polymer. B_{\max} is the increase in the phase transition temperature due to direct ion binding at saturation. The dashed lines in Figure 2 are fits to the data with eq 1. The abstracted K_A , B_{\max} , and c parameters, which are shown in Table 1, are employed below for all correlations with the well-known σ and ΔS_{hydr} values of the anions.

Influence of Hydration Entropy. The slope for the lower transition in the split phase transition of the kosmotropes, ▲, is

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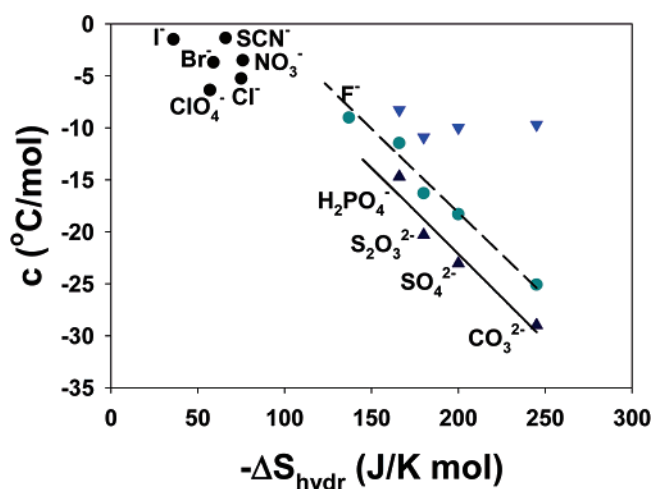


Figure 5. Plot of hydration entropy of the anions vs c . The two lines shown represent a least-squares fit to the data for the first slope of the split transition (—) and for the slope of the initial transition for the kosmotropes (---). Hydration entropy values for the anions are obtained from ref 31.

correlated to the hydration entropy of the anions, ΔS_{hydr} (Figure 5). No such trend exists for chaotropic anions, ●. This suggests that the lower-temperature phase transition is related to the dehydration of the amide group through a polarization effect (Figure 1a). In this case, the most strongly hydrated species are capable of polarizing the water sufficiently to weaken its interactions with the lone pairs on the carbonyl oxygen and amide nitrogen. This effect should be partly offset by the strengthening of the hydrogen bonding between the oxygen on water and the NH of the amide; however, three hydrogen bonds are weakened, while only one is strengthened, leading to the more facile dehydration of the amide.

If the salt concentration is sufficient, the hydrogen bonds to the amide become weak enough that the dehydration of this moiety occurs separately from the rest of the molecule. A substantial influence is also observed at lower salt concentration where the initial slope of the LCST vs kosmotropic salt concentration curve, gray-green points in Figure 5, also correlates well with ΔS_{hydr} . This is even the case for F⁻, for which only the initial concentration vs LCST slope could be obtained, because the phase transition ultimately decouples into two separate transitions very close to the saturation point for NaF (Figure 4e).

The idea that the lower-temperature transition seen with the kosmotropes is due to the perturbation of the solvation waters hydrogen-bonded to the amide rather than interactions with the hydrophobic portions of the molecule was tested by solvent isotope studies. It is well established that the increase in the LCST for PNIPAM in the presence of D₂O in the absence of salts is approximately 0.6 °C.^{27,32} The same increase is also observed, for example, in the presence of 1 M NaCl (Figure 6a). This modest solvent isotope effect is consistent with a process which is dominated by the dehydration of the hydrophobic portion of the molecule. On the other hand, the solvent isotope effect is different for each step of the two-step transition. An example of this difference can be seen with 0.333 M Na₂-

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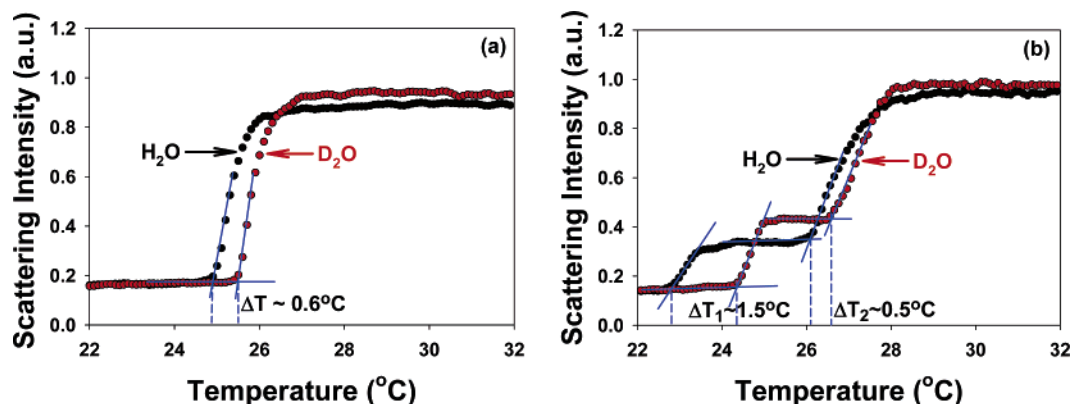


Figure 6. (a) Temperature vs light-scattering curve for 10 mg/mL PNIPAM and 1 M NaCl in H₂O and D₂O. (b) Temperature vs light-scattering curve for 10 mg/mL PNIPAM and 0.333 M Na₂SO₄ in H₂O and D₂O. The ionic strength of the solutions in (a) and (b) is identical. The data points for H₂O are black, while the data for D₂O are red.

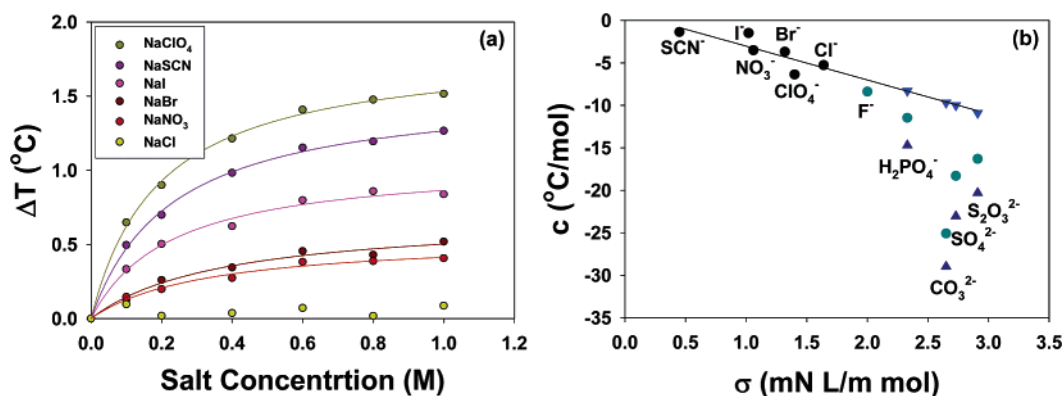


Figure 7. (a) The residual LCST data for the chaotropic anions from Figure 2 after removing the linear portion of the LCST vs concentration curves. The lines represent Langmuir isotherm fits to the data points. (b) Plot of the surface tension increment of the anions vs c : initial slopes are shown with filled gray-green circles; first slope of the split transition (\blacktriangle) and second slope of the split transition (\blacktriangledown) for kosmotropes; and (\bullet) for the chaotropes. The surface tension increments of the anions were obtained from ref 29, except for H₂PO₄[−] which was from ref 3, and CO₃^{2−} and S₂O₃^{2−} which were from ref 30.

SO₄ (Figure 6b). Both phase transitions shift to higher temperature in D₂O, but the magnitude of the shift for the lower-temperature transition is approximately 3 times greater (~ 1.5 °C) than for the second step (~ 0.5 °C). This is a strong indication that specific hydrogen bonds are being broken in the first step of the process. In fact, the deuterium bond in D₂O is about 5% stronger than the hydrogen bond in H₂O.^{33–35} This leads to an increase in the LCST on enthalpic grounds.^{32,36}

The uncoupling of the amide dehydration from the rest of the phase transition manifests itself in the formation of partially collapsed structures which scatter less light than fully collapsed PNIPAM. In fact, Wu and co-workers^{37,38} performed dynamic light-scattering studies in the absence of salt, which demonstrated that several dynamic populations of different sized PNIPAM particles are present upon cooling the solution through the LCST. Such intermediates are associated with crumpled coil and molten globule structures. In the present experiments these intermediates should merely be stabilized by the presence of a sufficient concentration of strongly hydrated ions.

Influence of Direct Ion Binding and Surface Tension. For the chaotropes, subtracting the linear contribution ($T_0 + c[M]$) to the concentration vs LCST curves in Figure 2 and replotting

the residual data reveals Langmuir-shaped binding isotherms (Figure 7a). The species with the strongest binding yielded the largest values of B_{\max} within experimental error. The order of B_{\max} values (ClO₄[−] > SCN[−] > Br[−] > NO₃[−] > Cl[−]) and the magnitude of the binding constants is consistent with prior reports describing the binding of these anions to polyacrylamide gels,³⁹ poly(*N*-vinylpyrrolidone),⁴⁰ and uncharged *N*-capped peptide helices.⁴¹ Furthermore, species such as perchlorate are even known to salt-in small organic molecules.⁴² It should also be noted that all species beyond Br[−] and NO₃[−] bound weakly enough to the amide moiety that deviation from linear LCST vs concentration behavior was not observed.

The c value for each anion is plotted against σ in Figure 7b. The correlation is remarkably good for the chaotropes. For these anions, the increased surface tension of the water/hydrophobic interface should cause the polymer to undergo its phase transition at lower temperatures as salt is added. For anions beyond fluoride, however, there are three different values of c to consider: the initial LCST vs concentration slope, the first step of the split phase transition, and the second step. The c values for both the single-step phase transition at low salt concentration and the lower-temperature step in the split

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transition are uncorrelated with the surface tension of the anion. However, the change in LCST with salt concentration for the higher-temperature step in the two-step process correlates well with the anions' surface tension increment. This suggests that the latter step corresponds to the removal of the hydrophobic hydration waters from the backbone and isopropyl side chain during polymer folding.

Under conditions where the surface tension mechanism is dominant, added salt lowers the LCST by destabilizing the hydrophobic hydration of the polymer. This is analogous to the water solubility of small alkanes, which become increasingly less soluble as salt is added to the solution.⁴³ The solubility of alkyl groups in water becomes unfavorable at higher temperatures due to the entropic cost of organizing water around these hydrophobic groups.

Conclusions

It has been well over 100 years since Hofmeister noted that the behavior of colloidal systems in aqueous solutions follow a systematic order based upon ion type. Since it is now known that bulk water structure is not greatly influenced by the nature of the salt present in solution outside the first hydration shell, the Hofmeister effects of salts on macromolecule solubility need

to be explained by interactions of the ions with the macromolecule and its first hydration shell, as noted above. Species which are strongly hydrated do not readily shed their innermost hydration shell and, hence, have the weakest binding constants for the polyamide groups.^{39,40} On the other hand, chaotropes act through surface tension and direct ion binding. The change in mechanism from a polarization effect to a surface tension and direct ion binding effect is the hallmark of the division between kosmotropic and chaotropic behavior at least for the LCST of PNIPAM.

Acknowledgment. This work was supported by the National Science Foundation (CHE-0094332, P.S.C., DMR 0348477, D.E.B.) and the Robert A. Welch Foundation (Grant A-1421, P.S.C., and A-0639, D.E.B.). P.S.C. acknowledges additional fellowship support from the Beckman Foundation, the Sloan Foundation, and the Dreyfus Foundation.

Supporting Information Available: Experimental protocol for PNIPAM preparation, microfluidic design, the effect of pH on the LCST of PNIPAM, as well as ion identity information for the multivalent ions. This information is available free of charge via the Internet at <http://pubs.acs.org>.

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