Effect of Comonomer Hydrophilicity and Ionization on the Lower Critical Solution Temperature of *N*-Isopropylacrylamide Copolymers

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Received October 19, 1992; Revised Manuscript Received February 10, 1993

ABSTRACT: Differential scanning calorimetry (DSC) was performed on aqueous solutions of poly(*N*-isopropylacrylamide-*co*-butyl methacrylate-*co*-X), with X being hydrophilic, hydrophobic, cationic, or anionic comonomers, to elucidate the mechanism of temperature-induced phase separation and the effect of comonomer content, hydrophilicity, and charge on the lower critical solution temperature (LCST). The endothermic heat of phase separation, which is related to the breaking of hydrogen bonds between water molecules surrounding hydrophobic moieties on the polymer, was a linear, decreasing function of the LCST. This suggests that the hydrophobic interactions between polymer side groups, which are the major driving force for phase separation, are enhanced at elevated temperatures due to a decrease in the structuring of water around hydrophobic side groups. It is concluded that the changes in LCST caused by the incorporation of comonomers are due to changes in overall hydrophilicity of the polymer and are not due to a direct influence of comonomer hydrophilicity or charge on the structuring of water around hydrophobic groups.

Introduction

Polymers which demonstrate good solubility in aqueous solutions at low temperatures but separate from solution when the temperature is raised above the lower critical solution temperature (LCST) have received increased attention in recent years. Cross-linked polymers exhibiting LCST behavior have been investigated for applications such as controlled drug delivery[1-3] and solute separation.[4,5] An important and useful feature of thermosensitive polymers is the possibility of controlling their LCST by various means, in particular by varying the monomer composition.[6-9] However, the mechanisms of the temperature-induced phase separation and the influence of comonomers on the LCST are not fully understood.

In general, the incorporation of hydrophobic comonomers leads to a lower LCST and hydrophilic comonomers to a higher LCST. It has been found that copolymerizing *N*-isopropylacrylamide (NIPAAm, poly(NIPAAm) exhibits an LCST in water at 31 °C) with acrylamide (hydrophilic) leads to a higher LCST and a lower endothermic heat of phase separation, but no detailed reasons for these effets have been given.[6] The presence of charge on the polymer also has a major effect on the LCST.[10-13] For certain polypeptide solutions which demonstrate temperature-induced phase separation, it was found that increasing the ionization of carboxylic acid side groups decreased the heat and increased the temperature of phase separation.[12] However, these changes in phase transition temperature have not been interpreted in thermodynamic terms.

In order to elucidate the LCST phenomenon and the role of comonomer hydrophilicity and charge, DSC was performed on NIPAAm copolymer solutions to obtain the temperature and heat of phase separation and the corresponding entropy changes ($\Delta H = T\delta S$, since $\Delta G = 0$ for the phase transitions[14-16]). The polymers studied were poly(NIPAAm-*co*-butyl methacrylate-*co*-X) with X = butyl methacrylate (BMA, hydrophobic), acrylamide (AAm, hydrophilic), acrylic acid (AAc), and (diethylamino)ethyl methacrylate (DEAEMA). Based on the results, a new mechanism is proposed for the effects of comonomer hydrophilicity and charge on the LCST.

Experimental Section

Materials. *N*-Isopropylacrylamide (NIPAAm, Eastman Kodak), was recrystallized in hexane. Butyl methacrylate (BMA), ethylene glycol dimethacrylate (EGDMA), and (diethylamino)-ethyl methacrylate (DEAEMA), all from Polysciences Inc., were purified by distillation at 58 °C/18 mmHg, 60 °C/115 μmHg, and 40 °C/190 μmHg, respectively. Acrylamide (AAm, Eastman Kodak), acrylic acid (AAc, Aldrich), and all other materials were used as received.

Polymer Synthesis. Synthesis of linear poly(NIPAAm-*co*-BMA-*co*-X), with a NIPAAm:BMA ratio of 95:5 mol/mol, containing various amounts (0, 2, 5, 10, or 20 mol %) of X, with X = AAm, BMA, DEAEMA, or AAC, was carried out in dioxane (50 w/v %) with BPO as an initiator (1.3×10^{-3} mol of BPO/mol of monomer). Dried nitrogen was bubbled through the solution for 10 min prior to polymerization. After polymerization at 80 °C for 16 h, the mixtures were dissolved in acetone and purified in diethyl ether. Cross-linked poly(NIPAAm-*co*-BMA-*co*-X), having the same monomer compositions as the linear polymers, cross-linked with 1 mol % ethylene glycol dimethacrylate, were synthesized by a similar method.[5]

^{1}H NMR analysis showed a NIPAAm:BMA molar ratio of 95.5:4.5 in the the no X polymer (200-MHz IBM NR/200 FT-NMR spectrometer, $n = 3$, standard deviation 0.5 mol %). Acid–base titrations indicated that the relative differences between the DEAEMA and AAc contents in the X = DEAEMA or AAc polymers and the feed compositions were less than 20%. Combined with the extended synthesis reaction time (16 h), these results suggest that the comonomer feed ratios reflect the relative comonomer incorporation levels in the polymers (within a margin of (relative) error of 20%).

Differential Scanning Calorimetry. DSC experiments were performed from 0 to 75 °C (scanning rate 10 °C/min) on both linear polymer solutions and swollen cross-linked polymers using a DSC-7 (Perkin-Elmer) calorimeter. Linear poly(NIPAAm-*co*-BMA-*co*-X) samples were prepared in phosphate buffered saline (PBS), pH = 7.4 (1 and 3 w/v %, with 50 μL of solution used for each experiment). Cross-linked p(NIPAAm-*co*-BMA-*co*-X) samples, with X = DEAEMA or AAc, were prepared by immersing approximately 4 mg of dried polymer in PBS, pH = 7.4, while cross-linked samples of poly(NIPAAm-*co*-DEAEMA), containing 10 mol % DEAEMA, were prepared by immersing approximately

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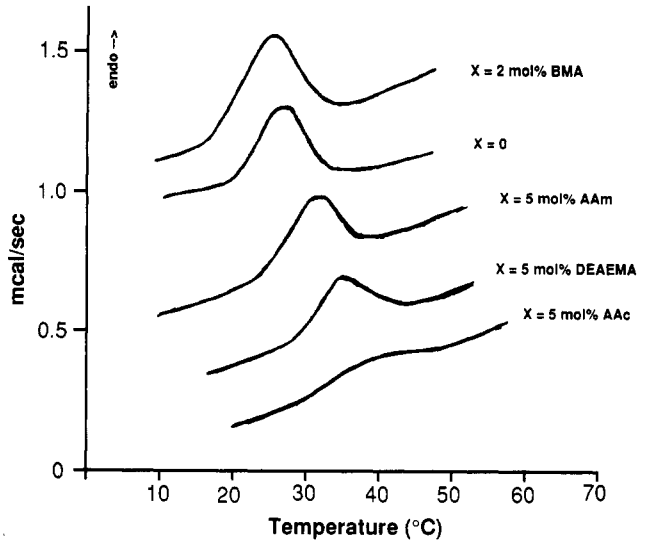

Figure 1. Typical DSC thermograms of p(NIPAAm-*co*-BMA-*co*-X) solutions in PBS (3 w/v %), pH = 7.4. Scanning rate: 10 °C/min.

4 mg of dried polymer in PBS of different pH. All samples were equilibrated at 4 °C for 1 day prior to the experiments.

Cloud Point Determination. Solutions of poly(NIPAAm-*co*-BMA-*co*-DEAEMA), containing 0, 2, 5, 10, or 20 mol % DEAEMA, were prepared in PBS, pH = 7.4. For each polymer, three solutions (0.01, 0.1, and 1 w/v %) were prepared. The temperature of the solutions was raised from 15 to 53 °C in 2-deg increments every 10 min, and the absorbance at 450 nm was measured using a Perkin-Elmer Lambda 19 UV/Vis/near-IR spectrometer. The cloud point was defined as the temperature at the inflection point in the absorbance versus temperature curve.

Titrations. Acid–base titrations using poly(NIPAAm-*co*-BMA-*co*-X), with X = 10 mol % DEAEMA or X = 5 mol % AAc, were performed at various temperatures. A 100-mg amount of polymer was dissolved in 15 mL of 0.15 M NaCl and titrated with 0.15 N NaOH (for X = DEAEMA) or 0.15 N HCl (for X = AAc) after all groups were ionized by adding small amounts of 0.15 N HCl or 0.15 N NaOH. The temperature was kept constant (±0.2 °C) for 30 min before and during each titration using a water bath.

Swelling. The swelling of cross-linked polymer samples of poly(NIPAAm-*co*-BMA-*co*-X) was determined gravimetrically as a function of temperature in PBS, pH = 7.4 (n = 3). The equilibrium weight of swollen samples at each temperature, which was reached within 3 days, was determined after less than 0.3% change in weight over a period of 12 h was detected. The swelling was defined as the ratio of swollen to dry sample weight.

Results

Effect of Comonomers on LCST. To determine the LCST's of poly(NIPAAm-*co*-BMA-*co*-X) in PBS, pH = 7.4, DSC and cloud point experiments were performed. In Figure 1, typical examples of DSC thermograms are shown. The relatively large breadth of the DSC peaks, which is also observed for cross-linked, BMA-containing NIPAAm copolymers during their collapse, is probably related to the specific thermosensitive properties of these polymers. In general, cross-linked polymers (for which thermosensitive properties are reflected in their swelling characteristics) exhibiting gradual deswelling with increasing temperature show broad endothermic peaks, while cross-linked polymers exhibiting sharp deswelling at their LCST show narrow endothermic peaks.[9,17] It was found that the temperatures at the maxima of the DSC endotherms of the X = DEAEMA polymers corresponded most closely to their cloud point temperatures, while the onset temperatures of the DSC endotherms were 5–10-deg lower, as shown in Table I. Hence, the temperatures at the maxima of the DSC endotherms were referred to as the LCST's of the polymers.

Table I. Cloud Point Temperatures and DSC Endothermic Peaks of Solutions of p(NIPAAm-*co*-BMA-*co*-DEAEMA) in PBS, pH = 7.4[a]

DEAEMA content (mol %)	cloud point temperature (°C)	maximum of endothermic peak (°C)	onset of endothermic peak (°C)
0	25	28.3(0.3)	22.3(0.6)
2	26	29.4(0.6)	20.4(0.6)
5	33	34.4(0.6)	30.4(0.8)
10	44	41.8(0.3)	36.2(0.2)
20	48		

[a] Numbers in parentheses represent standard deviations (n = 3).

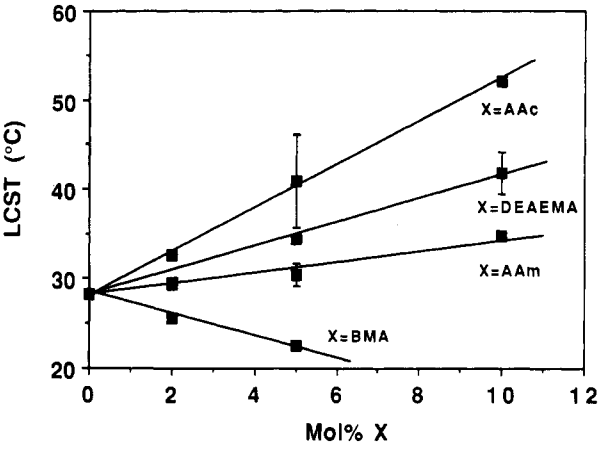

Figure 2. LCST of p(NIPAAm-*co*-BMA-*co*-X) solutions in PBS, pH = 7.4, as a function of comonomer X content (LCST's obtained from DSC experiments, n = 3).

In Figure 2 the LCST of poly(NIPAAm-*co*-BMA-*co*-X) is shown as a function of comonomer (X) content for X = BMA, AAm, DEAEMA, or AAc. The change in LCST was found to be proportional to the comonomer content. The increase in LCST was the largest for the anionic comonomer, followed by the cationic and the hydrophilic comonomers, while the hydrophobic comonomer caused a decrease in the LCST.

Effect of Charge on LCST. Besides the effect of comonomer content on the LCST, the specific role of charge on phase separation was examined. Therefore, the amount of charge on X = AAc and DEAEMA polymers in PBS, pH = 7.4, was determined at their respective LCST. By determining the pK's of AAc and DEAEMA in the polymers as a function of temperature (shown in Figure 3), the amount of charge on the polymers in PBS, pH = 7.4, as a function of temperature could be calculated, as shown in Figure 4. A decrease in charge on the polymers with increasing temperature was observed due to the decreased acidity and basicity of AAc and DEAEMA with increasing temperature. This can be explained by the increased hydrophobicity of NIPAAm. Several studies have shown that the incorporation of hydrophobic comonomers into polyelectrolytes leads to a decrease in acidity or basicity.[12,18–22] This phenomenon may be due to the decreased dielectric constant in the polymer environment.[18,19] For all the polymers examined, the total amount of charged monomers at their LCST's was approximately 4 mol %. This indicates that there is no phase separation until the amount of charged groups on the polymer is reduced to a critical limit.

When the charge of a polymer (X = 10 mol % DEAEMA polymer) was varied by varying the pH, large changes in LCST were observed, as shown in Figure 5. Decreasing the pH led to an increased LCST. However, due to the

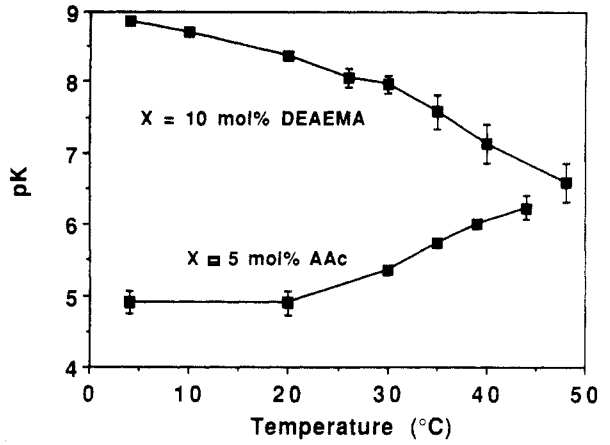

Figure 3. pK of p(NIPAAm-*co*-BMA-*co*-X), with X = 10 mol % DEAEMA or 5 mol % AAc, as a function of temperature in 0.15 M NaCl. (pK values are averaged at each temperature from 10% to 90% ionization, with error bars respresenting standard deviations.)

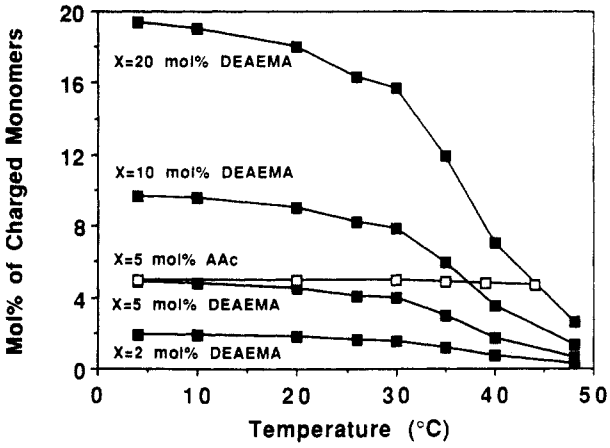

Figure 4. Total amount of charged monomers in p(NIPAAm-*co*-BMA-*co*-X), with X = various amounts of DEAEMA or AAc, in PBS, pH = 7.4, as a function of temperature.

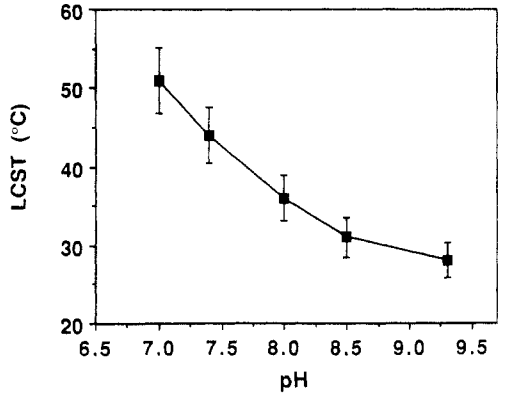

Figure 5. LCST of solutions of p(NIPAAm-*co*-BMA-*co*-DEAEMA), containing 10 mol % DEAEMA, in PBS as a function of pH (LCST's obtained from cloud point experiments, n = 3).

increased neutralization of DEAEMA at higher temperatures, the same amount of monomer units (about 4 mol %) was charged at each LCST. Thus, charge density plays a critical role in determining the LCST of these polymers. Increasing charge leads to a large increase in LCST, with no phase separation taking place if more than approximately 4 mol % of the monomer units are charged.

Heat of Phase Separation. In Figure 6, the temperature and ΔH of phase separation of poly(NIPAAm-*co*-BMA-*co*-X) solutions in PBS, pH = 7.4, are shown. The ΔH, which decreased with increasing AAm, AAc, and

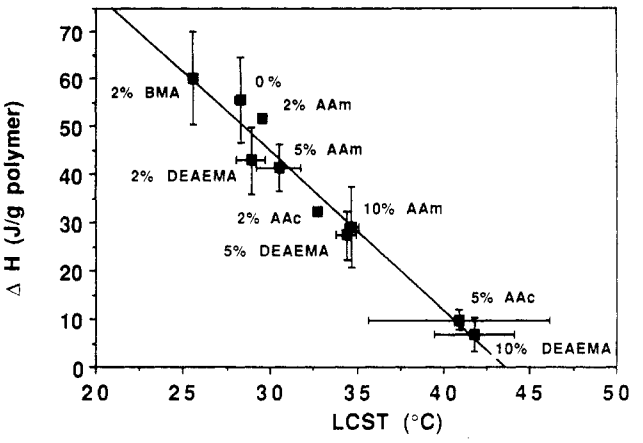

Figure 6. Heat (ΔH) and temperature (LCST) of the phase separation of p(NIPAAm-*co*-BMA-*co*-X) solutions in PBS, pH = 7.4 (n = 3).

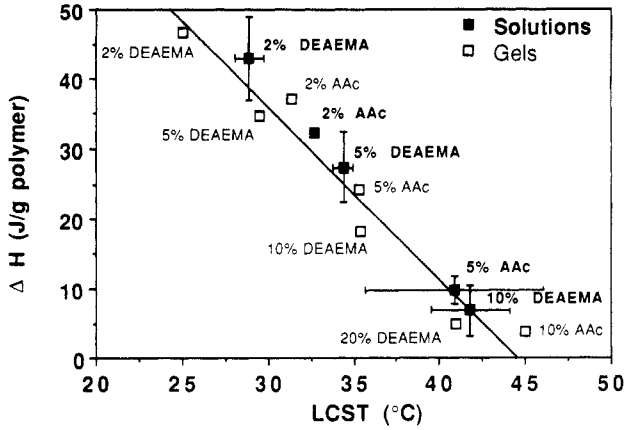

Figure 7. Comparison between the heat (ΔH) and temperature (LCST) of the phase separation of p(NIPAAm-*co*-BMA-*co*-X) solutions (n = 3) and gels (n = 1) in PBS, pH = 7.4, with X = various amounts of DEAEMA and AAc, respectively.

DEAEMA content, was found to be a linear function of the LCST (correlation coefficient = 0.961, as determined by the least squares method). The same relationship was observed for all three comonomers, even though the change in LCST was very different for each of the three comonomers. In contrast with the other comonomers, BMA caused a decrease in LCST and an increase in ΔH.

In Figure 7, the temperature and ΔH of phase separation are shown for poly(NIPAAm-*co*-BMA-*co*-X) solutions and their corresponding gels. The LCST of the gels was also defined as the temperature at the maximum of their DSC endotherms. At this temperature the gels, which shrink with increasing temperature, showed collapse and became opaque, indicating collapse of the polymer chains and phase separation in the gels. The gels showed a similar increase in LCST and decrease in ΔH with increasing DEAEMA or AAc content as the linear polymers. Even though the relative increase in LCST with increasing DEAEMA and AAc content appeared to be somewhat larger for the linear polymer solutions, roughly the same linear relationship between ΔH and the LCST was found for both polymer solutions and gels.

In Figure 8, the temperature and heat of phase separation are shown for cross-linked poly(NIPAAm-*co*-BMA-*co*-DEAEMA), containing 10 mol % DEAEMA, at different pH values. Similar to what was found for its linear polymer analog, an increase in phase separation temperature was observed with the increasing charge of DEAEMA (decreasing pH). The increase in phase separation temperature was accompanied by a decrease in ΔH. Approx-

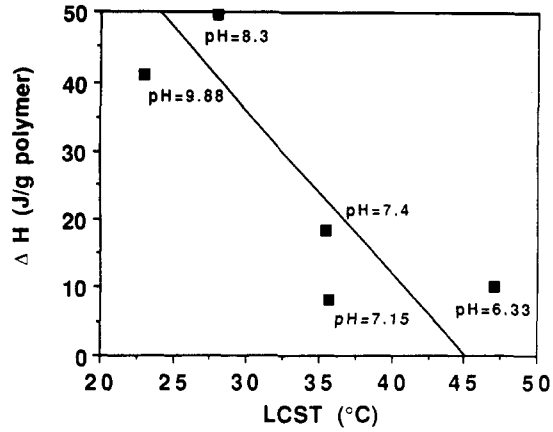

Figure 8. Heat (ΔH) and temperature (LCST) of the phase separation of p(NIPAAm-*co*-BMA-*co*-DEAEMA) gels, containing 10 mol % DEAEMA, in PBS at various pH.

imately the same relationship between ΔH and phase separation temperature was found as in the cases where the LCST was varied by changing the comonomer chemical structure or by changing the comonomer content. (N.B. Due to the relatively large error margins, the same line was drawn in Figure 8 as in Figure 7.)

Discussion

It has been suggested that LCST behavior is caused by a critical hydrophilic/hydrophobic balance of polymer side groups.[6,9,12,23-25] For p(NIPAAm), the –CONH– groups are hydrophilic and the $-CH(CH_3)_2$ groups hydrophobic. It has been proposed that the mechanism for LCST behavior is as follows.[6,9,12,16,23,26-29] At low temperatures, the strong H-bonding between the hydrophilic groups and water outweighs the unfavorable free energy related to the exposure of hydrophobic groups to water, leading to good solubility of the polymer in water. At increasing temperatures, H-bonding weakens, while hydrophobic interactions between hydrophobic side groups increase. Above the LCST, interactions between hydrophobic groups become dominant, leading to an entropy-driven polymer collapse and phase separation. The reduced motional freedom of the polymer chain is compensated by a gain in entropy due to the release of structured water around the hydrophobic groups on the polymer. Supporting evidence for this model has been given by DSC experiments on solutions of LCST polymers which show an endothermic phase separation.[9,23,28,29]

Several questions about the LCST, however, remain to be solved. First, what is the effect of temperature on the structured water around the hydrophobic moieties? It has been suggested by Bae et al.[9] that the increased hydrophobic interactions which are responsible for phase separation are caused by an increased structuring of water around the hydrophobic moieties with increasing temperature (increased unfavorable entropy of the dissolved polymer system), while other authors[23,30-32] point to a phase separation driven by a reduced amount of structured water around hydrophobic groups with increasing temperature. (In this view, the structuring of water around hydrophobic groups is seen as stabilizing the hydrophobic groups in water. The unfavorable free energy related to the exposure of hydrophobic groups to water is partially compensated for by the increased H-bonding between surrounding water molecules.[33-45] Hence, a reduction in the structuring of water around hydrophobic groups results in increased hydrophobic interactions between the polymers.) Secondly, what is the mechanism of the effects of comonomer hydrophilicity and charge on the LCST? The observed increase in LCST of certain polypeptides (and the decrease in heat of the phase transition), caused by an increase in charge, has been interpreted by assuming a disruption of the structured water around hydrophobic moieties of the polymer by the charges, leading to a reduced driving force for a phase transition (reduced gain in entropy from release of structured water) and a reduced endothermic transition process[12] (less structured water to disrupt). However, due to the limited amount of data it is not clear if this interpretation of the effect of charge is correct.

The DSC experiments showed endothermic phase separations for all poly(NIPAAm-*co*-BMA-*co*-X) solutions. The heat of phase separation of aqueous poly(NIPAAm) solutions is mainly related to the destructuring of water around the hydrophobic *N*-isopropyl groups, based on calculations of the heat associated with hydrophobic interactions between *N*-isopropyl groups in water.[23] This is probably related to the fact that after phase separation of poly(NIPAAm) solutions, the polymer rich phase contains more than 50 w % water, indicating that the polymers, presumably the –CONH– groups, are still highly hydrated.[15] Studies on the temperature-induced phase transitions of polypeptides also related the heat of phase separation to the destructuring of water around apolar moieties, on the basis of the unchanged hydration state of the backbone amide groups and on calculations of the heat associated with hydrophobic interactions between the hydrophobic moieties.[16,29]

The DSC experiments showed that the enthalpies of phase separation of the poly(NIPAAm-*co*-BMA-*co*-X) solutions in PBS, pH = 7.4, are a linear function of the phase separation temperature. A single ΔH–LCST relationship was found, whether the LCST was varied by AAm, BMA, DEAEMA, or AAc content. Moreover, the same ΔH–LCST relationship was found when the LCST of one polymer (X = 10 mol % DEAEMA) was varied by pH (while having the same degree of ionization at each LCST). From this it can be concluded that ΔH is directly related to the phase separation temperature and is not determined directly by the hydrophilicity of the comonomer or by charge. Since ΔH decreases with increasing temperature, it follows that an increase in temperature leads to a reduction in the structuring of water around the *N*-isopropyl groups, rather than an increase. This is in accord with the general observation of the decreased structuring of water around hydrophobic groups in water with increasing temperature.[30-32,40,41]

Since ΔH is determined by temperature and is not directly affected by charge, it is unlikely that the effect of charge on the LCST is caused by a destructuring of water around the hydrophobic side groups by charge. Since the incorporation of either hydrophilic or charged comonomers led to the same decrease in ΔH, given the same increase in LCST, it seems likely that the effect of charge and the effect of hydrophilic comonomers on the LCST are based on the same mechanism, a mechanism not based on a destructuring of water around the hydrophobic side groups by the comonomers.

Based on these results, the following mechanism is proposed for the effects of charge and comonomer hydrophilicity on the LCST: Incorporation of hydrophilic or charged comonomers reduces the amount of hydrophobic groups and increases the polymer hydrophilicity due to the strong interactions between water and charged or hydrophilic groups on the polymer. This leads to an increased LCST, since the hydrophobic interactions, which increase with temperature, are compensated for up to a higher temperature by the increased polymer–water

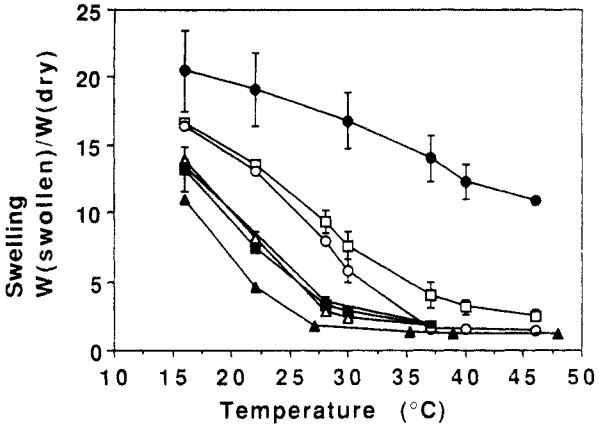

Figure 9. Swelling of p(NIPAAm-*co*-BMA-*co*-X) gels in PBS, pH = 7.4, as a function of temperature (n = 3): (○) X = 10 mol % DEAEMA; (△) X = 5 mol % DEAEMA; (▲) X = 2 mol % DEAEMA; (●) X = 10 mol % AAc; (□) X = 5 mol % AAc; (■) X = 2 mol % AAc; (▩) X = 10 mol % AAm.

interactions. A higher LCST leads to a reduced ΔH of phase separation due to a smaller amount of structured water at higher temperatures. The amount of structured water is only a function of temperature, while the phase separation temperature is determined by the relative hydrophilicity of the polymer, with charge merely increasing the polymer hydrophilicity.

In order to verify this hypothesis, swelling experiments were performed. According to the above hypothesis, the hydrophilicity of two polymers with the same LCST, one having charged and the other having hydrophilic comonomers (X), should be the same. In order to determine the hydrophilicity of linear polymers having the same LCST, the swelling ratios of their cross-linked analogs were measured at different temperatures. In Figure 9 the swelling of various cross-linked poly(NIPAAm-*co*-BMA-*co*-X), with X = various amounts of AAc, DEAEMA, or AAm, is shown. The swelling of polymers having the same LCST, such as the X = 2 mol % AAc, 5 mol % DEAEMA, and 10 mol % AAm polymers, as well as the X = 5 mol % AAc and 10 mol % DEAEMA polymers, was equal at various temperatures, indicating that the LCST is determined by the overall hydrophilicity of the polymer.

Conclusions

The experimental results indicate that temperature-induced phase separations of solutions of LCST polymers are mainly driven by increased interactions between hydrophobic moieties on the polymers, caused by a reduced structuring of water around hydrophobic polymer side groups (decreased stabilization of hydrophobic groups in water) with increasing temperature. Changes in LCST caused by the incorporation of comonomers are due to changes in overall hydrophilicity of the polymer. Both hydrophilic and charged comonomers, while having no direct effect on the structuring of water around hydrophobic moieties, increase the LCST due to the increase in hydrophilicity of the polymer. These results might have important implications for the understanding of cold denaturation processes of proteins, since these processes are similar to the phase transitions of solutions of LCST polymers.

Acknowledgment. This work was supported by NIH Grant HL 117623-18.

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