Effects of Ligand Binding on Relative Stability of Subchain Conformations of Weakly Charged *N*-Isopropylacrylamide Gels in Swollen and Shrunken States

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ABSTRACT: The thermotropic collapse of the hydrogel of copolymer *N*-isopropylacrylamide with acrylic acid (NIPA−AAc) upon heating has been studied by high-sensitivity differential scanning calorimetry. Curves of the polymer partial heat capacity have been measured at different pH (from pH 2 to 11) and different concentrations of cationic ligands: $CaCl_2$, dodecyltrimethylammonium bromide (DTAB), and cetyltrimethylammonium bromide (CTAB). Dependencies of the main thermodynamic parameters of the gel collapse—temperature, enthalpy, and width of the transition on pH and ligand concentration—have been obtained. It has been found that the collapse is characterized by a negative value of the heat capacity increment $\Delta_t c_p$. Its average value determined over all variables studied is $\Delta_t c_p = -0.62 \pm 0.02$ J/(g K). This result is in accordance with the observed correlation between the transition enthalpy and temperature of the NIPA−AAc gel. The free energy of the collapse has been calculated as a function of pH and concentration of $CaCl_2$, DTAB, and CTAB and analyzed using the model of macromolecular binding on the independent equivalent sites. Parameters characterizing affinity of the gel in swollen and collapsed state with respect to the ionic ligands have been determined. A preferential electrostatic binding of protons, Ca^{2+}, and surfactant ions by subchains in collapsed gel has been evaluated. The results also reveal a weak affinity of the subchains in swollen gel to amphiphilic ligands.

Introduction

Recently, an intense interest in both experimental[1−8] and theoretical[9−12] respects appeared in the design of polymer systems capable of the simplest protein functions. One of these functions consists of a radical change in affinity of a protein molecule to definite low-molecular-weight substances (ligands) upon minor change of external conditions.[13,14] The sensitivity of a protein to external conditions is realized via corresponding changes in its conformation, so that the affinity to a ligand is ultimately determined by the conformational state of the protein. In this view a question emerges, is manifestation of similar conformation-dependent properties possible for simpler polymer systems? Among such systems may be, in particular, thermosensitive polymer networks (for example, *N*-isopropylacrylamide gels) with inclusion of a small amount of ionogenic groups.[15−23]

In this work we study the effects of some low-molecular-weight ligands (H^+, Ca^{2+}, and cationic surfactants) on the relative stability of swollen and collapsed states of the weakly charged hydrogel of copolymer of *N*-isopropylacrylamide with acrylic acid (NIPA−AAc) with low cross-linking density. The investigation has been carried out by high-sensitivity differential scanning calorimetry (HS-DSC). The main thermodynamic parameters (temperature, enthalpy, heat capacity increment, and width of the transition) of the gel collapse upon heating have been determined as a function of ligand concentration. Dependencies of the transition free energy on the ligand concentration have been calculated. The analysis of these dependencies has been carried out in terms of the model of macromolecular ligand binding on independent identical sites. It has been shown that the ligands under investigation are preferentially bound by the subchains in a conformation that corresponds to the collapsed state of the gel.

Experimental Section

Gels NIPA−AAc were prepared by free-radical copolymerization of *N*-isopropylacrylamide (6000 mM) with acrylic acid (100 mM) in DMSO using *N*,*N*-methylenebisacrylamide (40 mM) as a cross-linking agent.[18] After adding the initiator 2,2′-azobis(isobutyronitrile) (10 mM), the monomer solution was placed into a glass ampule and degassed under vacuum. The polymerization reaction was performed at 60 °C for 24 h. After reaction was completed gels were taken from ampules and washed consecutively with solutions of HCl (100 mM), NaOH (100 mM), and deionized water to remove nonreacted monomers. The equilibrium concentration of the NIPA−AAc hydrogel at 20 °C was determined to be ∼10%.

Stock suspensions of gel in water were obtained as reported earlier.[24] Polymer concentration in the stock suspension was determined by the dry residue method. Gel suspensions for calorimetric measurements at different pH and at different concentration of ligands were prepared by adding stock buffer solution of a ligand to the stock gel suspension in water. The following buffer solutions were used for buffering systems at different pH: 5 mM phosphate (pH 5.9−7.7); 5 mM glycine (pH 2−3.5 and pH 7.9−11); 5 mM citrate (pH 4.1−5.2), and in some cases 5 mM Tris-HCl (pH 6.7−7.0). Gel suspensions in the presence of ligands were incubated at 4 °C for 40−42 h before calorimetric measurements for attainment of ionic equilibrium.

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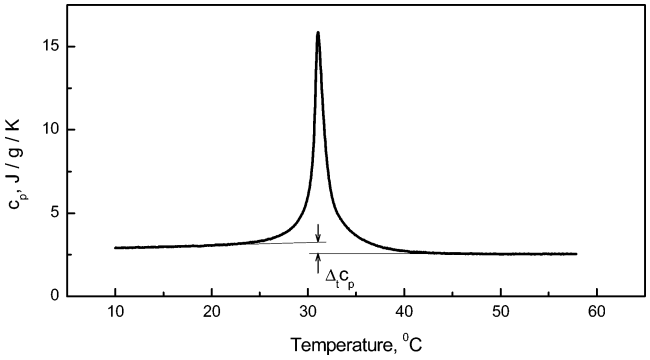

Figure 1. Thermogram of the NIPA−AAc gel at pH 2.25. $\Delta_t c_p$ is the transition heat capacity increment.

Calorimetric measurements were carried out with gel suspensions at concentration ∼ 2 mg/mL. The amount of polymer in the calorimetric cell was controlled by weight. To check the reliability of this procedure, 15 independent measurements of the collapse parameters were done for NIPA−AAc gel at pH 5.8. According to these data, the following values of the transition parameters were obtained: the transition temperature $T_t = 35.2 \pm 0.4$ °C; the transition enthalpy $\Delta_t h = 31 \pm 3$ J/g; the transition heat capacity increment $\Delta_t c_p = -0.74 \pm 0.09$ J/(g K), and the transition width $\Delta_t T = 3.9 \pm 0.4$ °C. These results indicated that the procedure of sample preparation is reliable and that calorimetric measurements are quite accurate.

Calorimetric measurements were carried out with the differential adiabatic scanning microcalorimeters DASM-4 (NPO "Biopribor", Russia) within the temperature range 10−80 °C and under excess pressure of 2 atm. The heating rate in all experiments was 1 K/min. At least three subsequent scans were done with each sample. The parameters of the second scan were used for analysis. Primary data processing was performed using the WSCAL software (Institute of Protein Research, Pushchino, Russia). Partial heat capacity of polymer network was calculated using the partial specific volume of linear NIPA polymer at 20 °C (0.870 cm^3/g).[25] Transformation of the temperature dependencies of the partial heat capacity to the excess heat capacity function was carried out using the NAIRTA software (Institute of Biochemical Physics, Moscow). The temperature of the maximum of the excess heat capacity function was considered as a transition temperature, T_t. The transition enthalpy, $\Delta_t h$, was determined by integration of the excess heat capacity function. The transition heat capacity increment, $\Delta_t c_p$, was calculated as a difference in partial heat capacities of the gel in the shrunken and swollen state, respectively, at the transition temperature. The transition width, $\Delta_t T$, was determined as a ratio of area of the excess heat capacity peak to its height.

Results

Figure 1 shows a curve of the partial heat capacity for the NIPA−AAc gel at pH 2.25. The thermotropic collapse of the gel upon heating manifests itself as a single endothermic peak of heat capacity. The distinctive feature of the peak is a negative heat capacity increment, $\Delta_t c_p$, i.e., difference in partial heat capacities of the gel in shrunken and swollen state. Thermograms of the first and second scanning revealed some minor differences in the transition profile related probably to the effect of thermal prehistory of the gel samples. Thermograms of the second and third scanning coincided completely, thus indicating reversibility of the transition.

Thermograms of the collapse of the NIPA−AAc gel at different pH are presented in Figure 2. It can be seen that with increasing pH the collapse endotherm alters

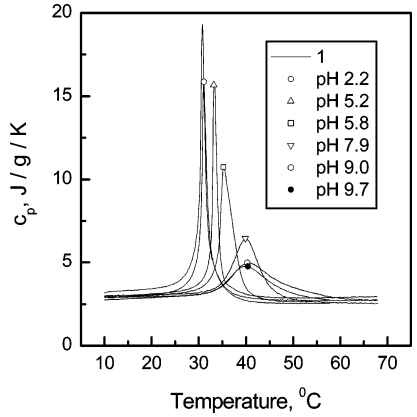

Figure 2. Thermograms of the NIPA−AAc gel at different pH. Curve 1 is the thermogram of a neutral NIPA gel with the same cross-linking density.

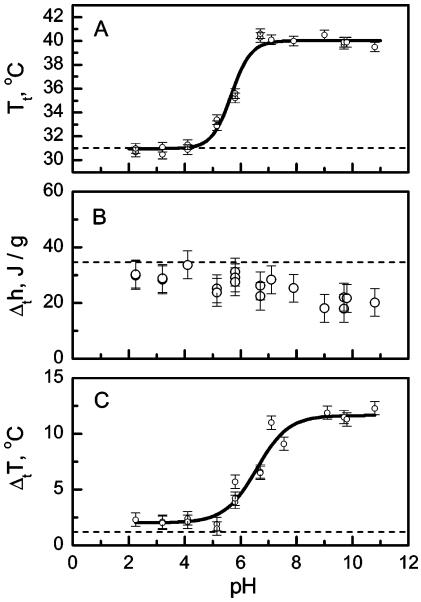

Figure 3. pH dependencies of thermodynamic parameters of collapse of the NIPA−AAc gel: transition temperature (A), enthalpy (B), and width (C). The background buffer: 5 mM glycine (pH 2.25−3.2 and pH 7.9−10.8); 5 mM citrate (pH 4.1 and pH 5.15); 5 mM phosphate (pH 5.7−7.1). Dashed lines show values of the transition parameters for a neutral NIPA gel with the same cross-linking density.

notably: it shifts to higher temperatures, decreases in height, and becomes wider.

Figure 3 displays pH dependencies of the collapse parameters of the NIPA−AAc gel: transition temperature, T_t; enthalpy, $\Delta_t h$, and width of the transition, $\Delta_t T$. The corresponding parameters of collapse of the neutral NIPA gel with equal cross-linking density are indicated in Figure 3 for comparison.

At pH values from 2 to 4 the transition temperature of the NIPA−AAc gel is constant and coincides with that of the NIPA gel (Figure 3A). In the pH range from 4 to 7 an increase in the transition temperature of the NIPA−AAc gel from 31 to 40 °C is observed. At pH > pH 7 the temperature of collapse of the NIPA−AAc gel does not practically change.

In the most acid medium (pH 2−4) the enthalpy of the collapse of the NIPA−AAc gel is close to that of the NIPA gel (Figure 3B). With increasing pH a tendency to some decrease in the enthalpy is observed.

Figure 3C displays the change in the transition width of the NIPA−AAc gel. In acid medium (pH 2−5) the

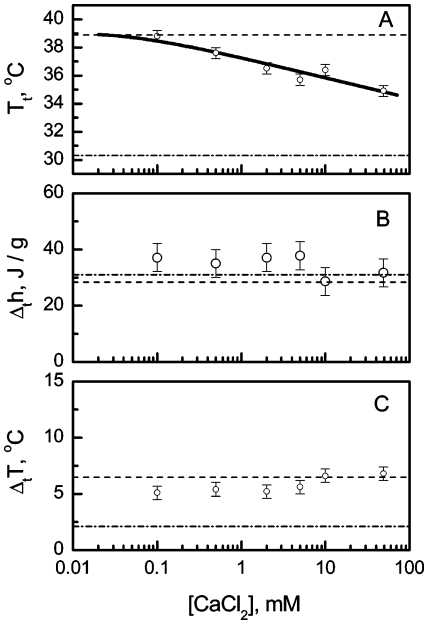

Figure 4. Dependencies of the transition temperature (A), enthalpy (B), and width (C) for the collapse of the NIPA−AAc gel on the concentration of $CaCl_2$. Dashed and dot−dashed lines show the transition parameters for the NIPA−AAc gel and for a neutral NIPA gel with the same cross-linking density in the absence of $CaCl_2$, respectively. Buffer: 5 mM Tris-HCl, pH 7.0.

transition width of the NIPA−AAc gel is close to that of the NIPA gel. With increasing pH the transition width of the NIPA−AAc gel increases more than 1 order of magnitude. The width of a transition is considered to be a measure of cooperativity of the system: the wider the transition, the lower the cooperativity. Introduction of charged groups into the polymer network results evidently in a dramatic decrease of the cooperativity of the gel structure. It is noteworthy that profiles of the pH dependencies of the transition width and of the transition temperature are similar, in general.

Figure 4 shows dependencies of the transition parameters of the NIPA−AAc gel on $CaCl_2$ concentration. The corresponding transition parameters for the NIPA gel as well as for the NIPA−AAc gel in the absence of $CaCl_2$ are given for comparison. With increasing concentration of Ca^{2+} ions the transition temperature of the NIPA−AAc gel decreases (Figure 4A). The enthalpy and the transition width of collapse of the NIPA−AAc gel do not reveal notable dependencies on the concentration of $CaCl_2$ (Figure 4B,C) being close to the values of corresponding parameters for the neutral NIPA gel.

The partial heat capacity curves for the NIPA−AAc gel obtained at different concentrations of cationic surfactants are shown in Figure 5. Two surfactants were studied: dodecyltrimethylammonium bromide, DTAB (Figure 5A), and cetyltrimethylammonium bromide, CTAB (Figure 5B), which differ by the length of the hydrophobic tail. Within the whole range of the surfactant concentrations studied the collapse of the NIPA−AAc gel was characterized by a single endothermic peak of heat capacity. It can be seen that in both cases with increasing concentration of the surfactant the collapse endotherm changes markedly its position and profile: it shifts first to lower temperatures, increasing in height and decreasing in width, but then a reverse shift to higher temperatures is observed (Figure 5).

Dependencies of the transition parameters of the NIPA−AAc gel on the concentration of DTAB and CTAB

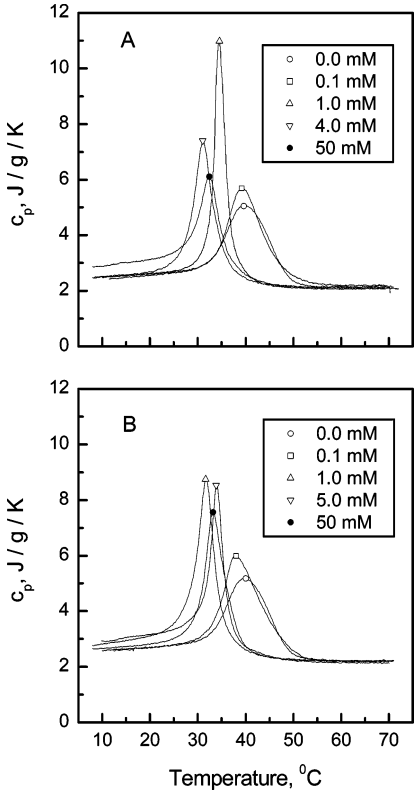

Figure 5. Thermograms of the NIPA−AAc gel at different concentrations of DTAB (A) and CTAB (B). Buffer: 5 mM phosphate, pH 7.3.

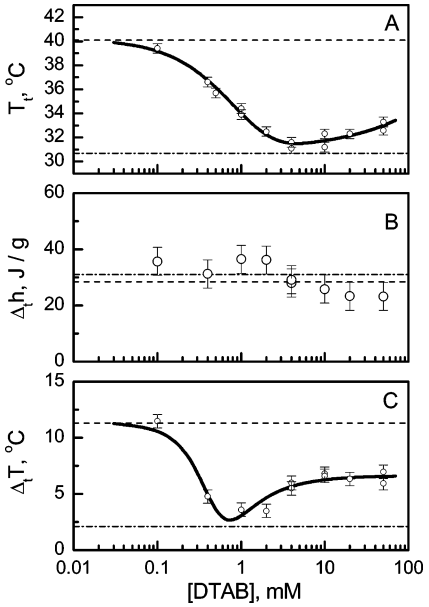

Figure 6. Dependencies of the transition temperature (A), enthalpy (B), and width (C) for the NIPA−AAc gel on DTAB concentration. DTAB is the cationic surfactant with C_{12} hydrocarbon tail. Dashed and dot−dashed lines show the transition parameters for the NIPA−AAc gel and for the neutral NIPA gel with the same cross-linking density in the absence of DTAB, respectively. Buffer: 5 mM phosphate, pH 7.3.

are given in Figures 6 and 7, respectively. For comparison, the graphs indicate also values of the corresponding transition parameters for the neutral gel NIPA and for the charged gel in the absence of surfactant. When the concentration of both surfactants increases, the transition temperature decreases almost down to the level of

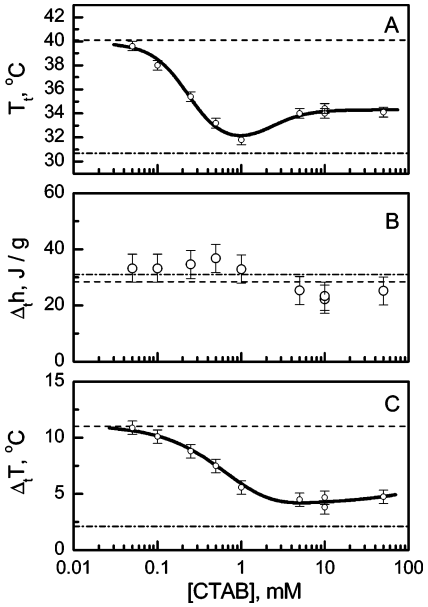

Figure 7. Dependencies of the transition temperature (A), enthalpy (B), and width (C) for the NIPA−AAc gel on CTAB concentration. CTAB is the cationic surfactant with C_{16} hydrocarbon tail. Dashed and dot−dashed lines show respectively the transition parameters for the NIPA−AAc gel and for the neutral NIPA gel with the same cross-linking density in the absence of CTAB. Buffer: 5 mM phosphate, pH 7.3.

the transition temperature of the neutral NIPA gel (Figures 6A and 7A). This effect is more pronounced for CTAB than for DTAB; i.e., in the presence of CTAB the temperature of collapse of the NIPA−AAc gel approaches that of the NIPA gel at lower surfactant concentration than in the presence of DTAB. Consequently, one can note that the surfactant of longer hydrophobic tail has a more pronounced effect on the collapse of the NIPA−AAc gel.

The enthalpy of collapse of the NIPA−AAc gel changes slightly with the surfactant concentration showing a tendency to decrease at high concentrations of both surfactants (Figures 6B and 7B).

Changes in the transition width for the NIPA−AAc gel, $\Delta_t T$, induced by the surfactants are shown in Figures 6C and 7C. With increasing surfactant concentration, the transition width decreases significantly, tending to that one for the neutral NIPA gel. At high surfactant concentrations a slight reverse effect of broadening of the NIPA−AAc transition is observed.

The heat capacity increment of the collapse of the NIPA−AAc gel, $\Delta_t c_p$, was found to be negative for the all variables studied (pH, [$CaCl_2$], [DTAB], [CTAB]). This result points to the dehydration of the hydrophobic groups of the network chains upon gel collapse. No significant effect of the agents studied on the $\Delta_t c_p$ was observed. Its average value is of -0.62 ± 0.02 J/(g K). This estimate is close to that one for the neutral NIPA gel with the same cross-linking density ($\Delta_t c_p = -0.63 \pm 0.04$ J/(g K)).[25] Thus, it can be concluded that the investigated ligands do not affect the heat capacity increment of collapse of the NIPA−AAc gel.

Discussion

Depending on the temperature the NIPA−AAc gels exist in either the swollen or shrunken state. The gel collapse transition is frequently considered as a macroscopic analogy of the coil−globule transition in a single polymer chain (see, for instance, ref 26). This analogy, however useful, should not be brought too far. Indeed, in both gel collapse and single chain collapse the local number density of monomers dramatically increases, while the amount of water (or, in general, other solvent) inside the gel or the coil drastically decreases. This creates conditions for the decreased hydration because the local environment around each monomer gets enriched with other monomers. So far runs the analogy between the gel situation and the single molecule situation. This analogy proves fruitful, as it allows one to formulate the mean-field theory of the gel collapse along the lines of the single molecule theory:[26] in both cases the average density serves as an order parameter, and in both cases the equilibrium value of the average density is determined by the balance of attractive and repulsive parts of volume interactions between monomers.

What does this analogy tell us about individual subchain conformations in the gel? With regards to the swollen gel, the subchains of such gel are coils, either Gaussian or swollen (excluded volume) coils. But what is the subchain conformation in the collapsed gel is much less clear. Indeed, increased density and overall gel collapse can be achieved by either collapse of all (or many) of subchain coils or by increasing overlap and interpenetration of such coils. Of course, these two scenarios do not exclude each other, and any combination of them is possible. In this sense, chain conformations in collapsed gel are understood[27]only in the framework of the model in which subchains are phantom (pass freely through one another). Topological constraints are expected to modify the situation quite significantly.[28]

Whatever the general resolution of this important puzzle, for our purposes here it is sufficient to realize that subchains in thermosensitive gel undergo the transition between coil type state when the gel is swollen and some other state, which we call "condensed", when gel is collapsed. It is worth emphasizing once again that we shall make no restrictive assumption about conformations, spatial line shapes, or fold of the subchains in the condensed state; we shall only rely on the fact of the elevated density in this state.

The presence of ionogenic groups provides to the network an ability to bind oppositely charged ligands. The shift of collapse of the NIPA−AAc gel induced by ligands points to their preferential interaction with one of the states of the gel subchains. As a thermodynamic measure of this interaction, we can take the excess free energy of transition as a function of ligand concentration, $\Delta_t g_L^E$, determined at a reference temperature.[29] This is a difference between the free transition energy at given ligand concentration (L) and that in the absence of the ligand, that is, $\Delta_t g_L^E = \Delta_t g(L) - \Delta_t g(0)$. It is convenient to choose the transition temperature at $L = 0$, $T_{t,0}$, as the reference temperature. Since in our case $\Delta_t c_p$ is a constant, one gets (see Appendix)

$$\Delta_t g_L^E = \Delta_t h_L(1 - T_{t,0}/T_{t,L}) + \Delta_t c_p(T_{t,0} - T_{t,L}) - \Delta_t c_p T_{t,0} \ln(T_{t,0}/T_{t,L}) \quad (1)$$

where $\Delta_t h_L$ is the transition enthalpy at $T_{t,L}$, $\Delta_t c_p$ is the transition heat capacity increment, and $T_{t,L}$ is the transition temperature at a given concentration of ligand, L.

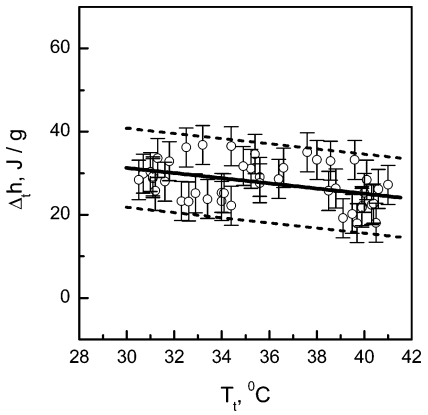

Figure 8. Correlation of the transition enthalpy and transition temperature for the NIPA−AAc gel plotted using data of Figures 3, 4, 6, and 7. Solid line shows approximation of the experimental points according to Kirchhoff's law with $\Delta_t c_p = -0.62$ J/(g K). A confidence interval of the approximation is given by dashed lines.

Values of $\Delta_t g_L^E$ at different concentrations of the ligands were estimated using the experimental values of the transition temperature, $T_{t,L}$, and values of the calculated transition enthalpy, $\Delta_t h_L$, at the temperature $T_{t,L}$. The calculations of $\Delta_t h_L$ were carried out according to Kirchhoff's law using the experimental transition heat capacity increment $\Delta_t c_p = -0.62$ J/(g K). A validity of this approximation for the NIPA−AAc gel collapse is illustrated in Figure 8.

The obtained dependencies of $\Delta_t g_L^E$ against ligand concentration were analyzed in terms of the model of macromolecular ligand binding to the independent equivalent sites assuming that in the extended and condensed states the network subchains carry n_1^{ext} and n_1^{cnd} of strong binding sites with binding constants K_1^{ext} and K_1^{cnd}, respectively. In this case, the excess transition free energy can be expressed in the form[29]

$$\Delta_t g_L^E = -n_1^{cnd} RT \ln(1 + K_1^{cnd} L) + n_1^{ext} RT \ln(1 + K_1^{ext} L) \quad (2)$$

It is reasonable to assume that in the NIPA−AAc gels the carboxylic groups provide sites for the strong binding of the ligands. In the absence of the imprinting effects[18] $n_1^{cnd} \approx n_1^{ext} \sim 2$ per subchain, as estimated from the copolymer composition and cross-linking density of the NIPA−AAc gels under study.[30]

The experimental pH dependence of the reduced excess free energy of the NIPA−AAc gel collapse, $\Delta_t g_L^E/RT_{t,0}$, is given in Figure 9. Upon decreasing pH, i.e., upon increasing the proton concentration, the transition free energy decreases indicating stabilization of the condensed conformation of the gel subchains. Hence, it can be concluded that protons bind preferentially to the subchains in the condensed state.

The experimental dependence of $\Delta_t g_L^E/RT_{t,0}$ on pH was approximated by eq 2. The proton binding constants for the subchain in the extended and the condensed states served as adjustable parameters of the approximation. Values of these constants obtained in result of fitting of eq 2 to the experimental data are given in Table 1. It was determined that $pK_1^{ext} \sim 5$ and $pK_1^{cnd} \sim 6$. These values agree with the results of direct determination of the equilibrium constants of proton binding by the NIPA−AAc gel with similar charge density and cross-linking density (pK_a 4.9 and pK_a 5.9 for the swollen and shrunken states of the gel, respectively[31]).

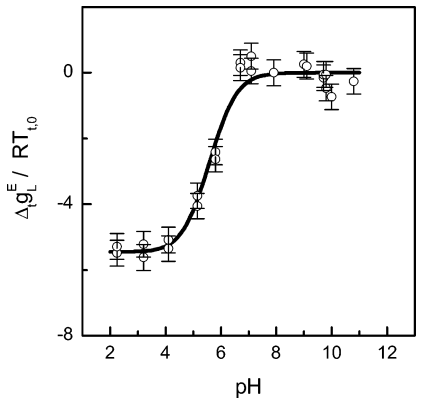

Figure 9. pH dependence of the reduced excess transition free energy of the NIPA−AAc gel. Points are experimental, and the solid line is the best fit curve by eq 2 according to the model of macromolecular binding on independent identical sites (see comments in the text). The best fit parameters are summarized in Table 1. $T_{t,0} = 40$ °C.

Table 1. Binding Parameters of the NIPA−AAc Gel with Respect to Various Cationic Ligands

ligand	n_1[a]	K_1^{cnd},[b] M^{-1}	K_1^{ext},[b] M^{-1}	$n_2^{ext}K_2^{ext}$,[c] M^{-1}
H^+	2	$10^{6.2}$	$10^{5.0}$	
Ca^{2+} [d]	1	16000	200	
DTAB[e]	2	6000	300	40
CTAB[e]	2	14000	600	300

[a] n_1 is the apparent number of strong ionic binding sites per subchain for the condensed and the extended conformations. [b] K_1^{cnd} and K_1^{ext} are the equilibrium constants of the strong ionic binding for the NIPA−AAc gel network subchains in the condensed and the extended conformations, respectively. [c] $n_2^{ext}K_2^{ext}$ is the total affinity of the weak (hydrophobic) binding for the NIPA−AAc gel network subchains in the extended conformations. [d] 5 mM Tris-HCl buffer, pH 7.0. [e] 5 mM phosphate buffer, pH 7.3.

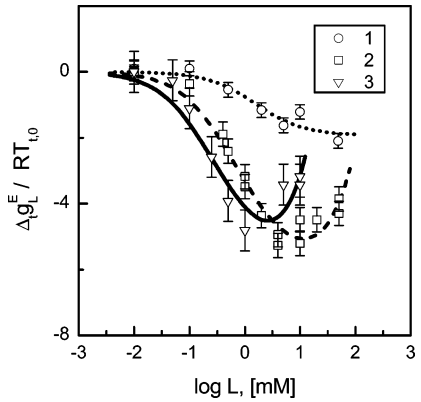

Figure 10. Dependencies of the reduced excess transition free energy of the NIPA−AAc gel on ligand concentration for $CaCl_2$ (1, $T_{t,0} = 38.6$ °C), DTAB (2, $T_{t,0} = 40.1$ °C), and CTAB (3, $T_{t,0} = 40.1$ °C). Lines show the best fit curves by eq 2 for 1 and by eq 4 for 2 and 3 according to the model of macromolecular binding on independent identical binding sites (see comments in the text). The best fit parameters are summarized in Table 1.

Dependencies of the reduced excess free energy of collapse of the NIPA−AAc gel on concentration of Ca^{2+}, DTAB, and CTAB are presented in Figure 10.

Analysis of the $\Delta_t g_L^E/RT_{t,0}$ as a function of Ca^{2+} concentration was performed in the same way as it was done for the $\Delta_t g_L^E/RT_{t,0}$ against pH, but the numbers of

binding sites for Ca^{+2} cations per subchain for both subchain conformations were used as adjusting parameters. This was necessary since several stoichiometries of the Ca^{2+} binding possible (for example, one or two cations per subchain as well as two cations per two subchains), statistical weights of which are unknown. The analysis revealed that the 1:1 stoichiometry ($n_1^{cnd} \approx n_1^{ext} \sim 1$) is prevailed and Ca^{2+} cations are preferentially bound by subchains in the condensed conformation, i.e., $K_1^{cnd} > K_1^{ext}$ (Table 1). This result is in agreement with the results of the direct determination of the equilibrium constants of Ca^{2+} binding for hydrogels of copolymers of NIPA with methacrylic acid (NIPA−MAAc) in swollen and shrunken states.[19] The effect of preferential binding of Ca^{2+} cations observed in our work ($K_1^{cnd}/K_1^{ext} \sim 8$) is comparable with that reported for the NIPA−MAAc gels ($K_1^{cnd}/K_1^{ext} \sim 10$).

When analyzing the experimental dependencies $\Delta_t g_L^E/RT_{t,0}$ on concentrations of the cationic surfactants (Figure 10), it was assumed that in addition to the strong ionic binding there is also a relatively weak hydrophobic binding of these ligands to the gel network. If it is the case, one can get

$$\Delta_t g_L^E = -n_1^{cnd} RT \ln(1 + K_1^{cnd} L) - n_2^{cnd} RT \ln(1 + K_2^{cnd} L) + n_1^{ext} RT \ln(1 + K_1^{ext} L) + n_2^{ext} RT \ln(1 + K_2^{ext} L) \quad (3)$$

where n_2^{cnd}, K_2^{cnd} and n_2^{extl}, K_2^{ext} are the number of hydrophobic binding sites and hydrophobic binding constants for the condensed and the extended states of the subchain, respectively. The relative weakness of the hydrophobic binding can be introduced presuming that $n_2K_2 \ll 1$ for both conformations. Then it can be assumed that the number of hydrophobic binding sites of the subchain in the extended state is much larger than that for the subchain in the condensed state, i.e., $n_2^{ext} \gg n_2^{cnd}$. Additionally, it is evident that $K_2^{cnd} \sim K_2^{ext}$ due to the nonspecificity of the hydrophobic binding. Therefore, eq 3 can be simplified as follows:

$$\Delta_t g_L^E = -n_1^{cnd} RT \ln(1 + K_1^{cnd} L) + n_1^{ext} RT \ln(1 + K_1^{ext} L) + n_2^{ext} RT K_2^{ext} L \quad (4)$$

This simplified equation was used to fit experimental dependencies of $\Delta_t g_L^E/RT_{t,0}$ on DTAB and CTAB concentrations (Figure 10). Results of the fitting (Table 1) illustrate that the cationic surfactants at low concentrations are preferentially bound by subchains in the condensed state ($K_1^{cnd}/K_1^{ext} \sim 20$). This effect is probably caused by a cooperation of the ionic binding with the hydrophobic interaction upon inclusion of the amphiphilic ligand into the interior of the condensed subchains. At higher concentrations of the surfactants, the contribution of the hydrophobic ligand binding to the extended subchains becomes apparent. The total affinity of this interaction, $n_2^{ext}K_2^{ext}$, increases from DTAB to CTAB by about 1 order of magnitude upon increasing the length of the aliphatic radical of the surfactant by four methylene groups.

The effect of ionic surfactants on the relative stability of the extended and condensed conformations of subchains in the NIPA−AAc gels reproduces qualitatively the effect of these amphiphilic ligands on the conformational stability of some globular proteins.[32−35]

Conclusion

The affinity of positively charged ligands to the NIPA−AAc gel network depends drastically on the states of the gel subchains. Condensation of the subchains upon the gel collapse results in a remarkable rise of affinity. At least in this modest way this copolymer network mimics binding properties of globular proteins.

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Appendix

Equation 1 was derived from a general form of temperature dependence of transition free energy, $\Delta_t g(T)$, and from the fact that the transition enthalpy is a unique function of temperature (see Figure 8).

According to general definitions[36]

$$\Delta_t g(T) = \Delta_t h(T) - T\Delta_t s(T) \quad (A1)$$

$$\Delta_t g(T_t) = 0 \quad (A2)$$

$$\Delta_t h(T) = \Delta_t h(T_t) + \int_{T_t}^{T} \Delta_t c_p \, dT \quad (A3)$$

$$\Delta_t s(T) = \Delta_t s(T_t) + \int_{T_t}^{T} \frac{\Delta_t c_p}{T} \, dT \quad (A4)$$

$$\Delta_t s(T_t) = \frac{\Delta_t h(T_t)}{T_t} \quad (A5)$$

Assuming that $\Delta_t c_p \neq f(T)$ as was experimentally observed, we get

$$\Delta_t g(T) = \Delta_t h(T_t)(1 - T/T_t) + \Delta_t c_p(T - T_t) - \Delta_t c_p T \ln(T/T_t) \quad (A6)$$

Then introducing a dependence of the transition temperature on ligand concentration, $T_{t,L} = T_t(L)$, the transition temperature in the absence of ligand, $T_{t,0} = T_t(0)$, as a reference temperature, and $\Delta_t h_L = \Delta_t h(T_{t,L})$, we come to eq 1, that is, $\Delta_t g_L^E$ at $T_{t,0}$.

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