Effect of NaCl on the Exothermic and Endothermic Components of the Inverse Temperature Transition of a Model Elastin-like Polymer

Javier Reguera,[†] Dan W. Urry,[‡] Timothy M. Parker,[‡] David T. McPherson,[‡] and J. Carlos Rodríguez-Cabello*[,†]

Departamento de Física de la Materia Condensada, E.T.S.I. Industriales, Universidad de Valladolid, Paseo del Cauce SN, 47011 Valladolid, Spain, and Bioelastics Research Ltd., Birmingham, Alabama 35205

Abstract:
TMDSC data have been employed to observe the effect of NaCl on the inverse temperature transition of the model elastin-like polymer $(GVGVP)_{251}$. NaCl causes a decrease in T_t and an increase in ΔH. The increase in enthalpy appears both in the enthalpy related with the folding of the polymer and in the contribution associated with disruption of the structured water of hydrophobic hydration. It has been suggested that the presence of NaCl may cause a better formation of water structures surrounding the apolar polymer chains.

Introduction

The understanding of the "inverse temperature transition" (ITT)[1] in elastin-like polymers (ELPs) has become a key issue not only in this area but in more general research due to the interest in understanding the hydrophobic association[2] and its relation to protein folding.[3] Furthermore, the use of this phenomenon in new nano(bio)technological applications that involve smart molecules makes the study of the ITT much more relevant.[4−6]

ELPs are made from the recurrence of short peptide sequences, or some modification of them, that appear repeated in the natural elastin. The most common ELP is based on the monomer (GXGVP) where G stands for glycine, V for L-valine, P for L-proline, and X is any amino acid except proline.[1,7] The polymer $(GVGVP)_n$ is considered as a model for the ELPs.

The extraordinary properties of ELPs and their potential to be exploited in advanced applications have triggered their interest in hot areas such as biomedicine, biomimicry, or nanotechnology. Some of the relevant properties displayed by the ELPs include mechanical properties ranging from almost ideal elastomers[8] to plastics,[9] outstanding biocompatibility,[10] and acute smart[11] and self-assembly[11−13] behavior. Furthermore, they can be synthesized by recombinant DNA technology[14] in genetically modified organisms, which makes possible the development of polymers with an unmatched degree of complexity and control in their composition.[6] The biosynthesis provides an absolute control of the sequence and polymer architecture, with the inexistence of randomness in amino acid stereochemistry, comonomer arrangement, and molecular weight (MW).[6,15]

The most striking feature of the ELPs is their ITT. In aqueous solution, and below a certain critical temperature (T_t), the free polymer chains remain disordered, random coils in solution[16] that are fully hydrated, mainly by hydrophobic hydration.[11,17] On the contrary, above T_t, the chain hydrophobically folds and assembles to form a phase-separated state. The actual state of this aggregated phase is a matter of certain controversy. The most established model points to a folded state in which the polymer chains adopt a dynamic, regular, nonrandom structure, called a β-spiral, involving one type II β-turn per pentamer, and stabilized by intraspiral interturn and interspiral hydrophobic contacts.[1,11] During the initial stages of polymer dehydration, hydrophobic association of β-spirals takes on fibrillar form that grows to a several hundred nanometer particle before settling into the visible phase-separated state.[11,18] That regular repeating dynamic pentameric structure seems to account for the frequency localized relaxations that grow to remarkable intensity as the associations of the inverse temperature transition develop, say in the vicinity of 5 MHz and 3 kHz dielectric relaxations,[19−21] and in the NMR relaxation studies.[22,23] Additionally, that model structure also has been used to explain the entropic elasticity, perfect storage, and return of energy expended in elastomeric force development shown by these polymers.[8,24]

On the other hand, some works, such as those by Daggett's group, making use of molecular dynamics simulations[25,26] or the works from Gross et al.[27] point against the existence of these regular structures in the folded state of the polymer.

Shifts in T_t as a consequence of different stimuli are the base of the so-called "ΔT_t mechanism"[11,15,28,29] and the "amplified ΔT_t mechanism".[30] These two mechanisms make possible the design of smart polymers with sensitiveness to different stimuli such as pH, light, and pressure.

The ITT is a complex and multistep transition.[31] Some of the processes are endothermic, likely associated with the loss of hydrophobic hydration, while other are oppositely signed exothermic. Those are assigned to the physical association of chains (van der Waals cohesive interactions). The magnitude of the exothermic component is less than one-third of the endothermic component.[32] Temperature modulated differential scanning calorimetry (TMDSC) has been the only technique up to now able to separate both components.[32] In general terms, TMDSC is able to separate thermally overlapping phenomena with different time dependences by using a heating program containing an alternating function of the temperature, such as a sine wave, superimposed on the constant heating rate r.[33,34] In principle, two overlapping processes can split by TMDSC under

* Corresponding author. E-mail: roca@bioforge.uva.es.
[†] Universidad de Valladolid.
[‡] Bioelastics Research Ltd.

particular dynamical conditions by finding a frequency for the periodic component low enough for the faster process to follow the oscillating temperature changes ("reversing") but high enough to impede alternating behavior of the slower ("non-reversing") one. In the case of the ITT, it has been proven in a previous work[32] that the physical association of chains (exothermic) appears in the reversing component whereas the loss of hydrophobic hydration (endothermic) appears mainly in the non-reversing component, and that behavior is depending on the frequency.

In this work, we study the influence of the NaCl concentration on the exothermic and endothermic components of the ITT. The interest in studying the effect of this model salt on the ITT is of relevance because NaCl is being used in many applications using elastin-like polymers as an easy and convenient way to control T_t. However, the effect of NaCl on the ITT has been reported in the literature mainly as a phenomenological description,[11,35] and no real studies have been carried out to explain the actual effect of NaCl and, by extension, salts on this phenomenon. It is well known that salts cause a significant, concentration-dependent, decrease in T_t and an intriguing increase in the transition enthalpy (ΔH). In this work, we study the effect of NaCl on the endothermic and exothermic components of the ITT using TMDSC as a way to shed light on the physical basics of the influence of NaCl on the ITT.

Materials and Methods

ELP $(GVGVP)_{251}$ was prepared by recombinant DNA technology and expressed by *Escherichia coli* fermentation. Complete description of this procedure and essential characterizations of the gene product, $(GVGVP)_{251}$, have appeared detailed in the literature.[36]

DSC and TMDSC experiments were performed on a Mettler Toledo 822^e with liquid-nitrogen cooler. Calibration of both temperature and enthalpy was made with a standard sample of indium. In a typical DSC or TMDSC run, 25 μL of the solution was placed inside a standard 40 μL aluminum pan hermetically sealed. The same volume of water was placed in the reference pan.

DSC experiments were performed at 10 °C/min from 5 to 65 °C after 15 min at 5 °C. To follow the dependency of the thermal behavior of the polymer solution on polymer concentration, NaCl concentration was fixed at 0.86 M, while polymer concentration varied between 1 and 1000 mg/mL (per mL of solvent).

TMDSC was performed using a sinusoidal temperature function superimposed to a heating ramp. The conditions for the sweep in frequency were: heating ramp, $r = 1$ °C/min; amplitude, $A = 0.1$ °C; and the period, $P = 0.3-0.8$ min. 50 mg/mL (0.49 mM) aqueous solution of the polymer was prepared for a salt concentration ranging from 0 to 2.14 M to follow the dependency on NaCl concentration. The experiments were performed with a period of 0.6 min and were repeated four times. The error bars correspond to a confinement of 95%. Positive enthalpies represent endothermic processes, and negative enthalpies represent exothermic.

Results and Discussion

The ITT of the elastin-like polymer $(GVGVP)_{251}$ is shown in a conventional DSC experiment as an endotherm (Figure 1). When NaCl is added, the peak shifts to lower temperatures, while its area increases (Figure 1). Usually, the peak minimum is considered as the transition temperature (T_t) and the peak area can be identified with the enthalpy of the endotherm (ΔH). The quantitative dependency of these calorimetric parameters on NaCl concentration can be seen in Figure 2. According to that figure, there is a linear decrease in T_t versus the salt

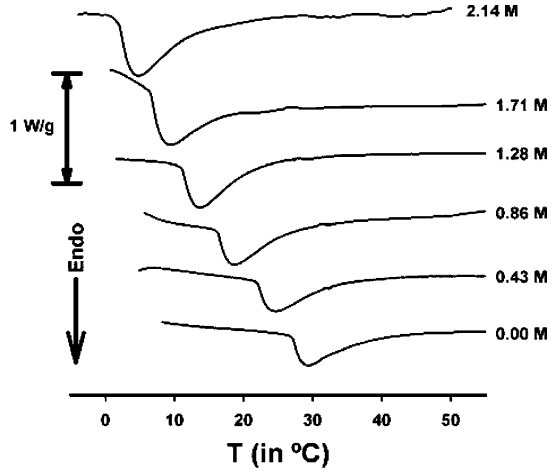

Figure 1. Conventional DSC thermogram of a 50 mg/mL $(GVGVP)_{251}$ polymer solution in the presence of cosoluted NaCl. [NaCl] concentrations are indicated in the plot.

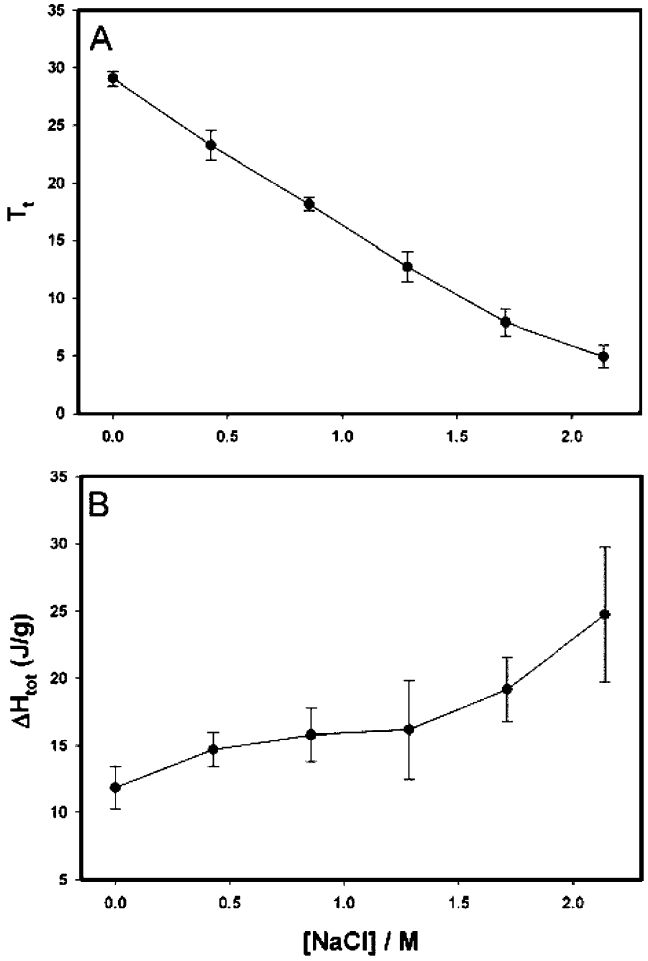

Figure 2. Influence of the salt concentration in the T_t (A) and ΔH (B) for a 50 mg/mL $(GVGVP)_{251}$ aqueous solution.

concentration. Only a slight deviation from this trend can be observed for high [NaCl]. On the other hand, the enthalpy shows a clear increase with the NaCl concentration (Figure 2B).

In conventional DSC, the endothermic peak taking place on heating the polymer solution has been attributed to the disruption of the water structures during the transition,[32] so an increase in this parameter must be understood as an increase in the number of ordered water structures of hydrophobic hydration or an increase in the state of order of them or both. Interestingly, these results are in contradiction with those shown by other polymers

that exhibit lower critical solution temperature (LCST), such as poly(*N*-isopropylacrylamide) (PNIPAM) or poly(vinyl methyl ether) (PVME). For those synthetic polymers, there is a decrease in the enthalpy when different salts, including NaCl, are present. This fact has been explained as being caused by the disruption of the ordered water surrounding the extended polymer chain, which changes from structured water to bulk water when heated above LCST.[37] This different behavior between the polymers that exhibit a LCST and the ELPs is likely due to the fact that the last ones show a more complex process involving not only the disruption of the water structures and the hydrophobic association but also an increment in order in the main chain with a subsequent stabilization. Therefore, although there is a certain trend in the literature to assume that elastin-like polymers are just one more member of the LCTS polymers, this could be fallacious because ELPs show quite different behavior under certain circumstances, such as the one remarked here.

TMDSC experiments were carried out to gain further insight on the actual effect of NaCl addition on the calorimetric parameters. This will provide supplementary clues on how the addition of NaCl influences the water structures or their interaction with the polymer chain.

In TMDSC, the endothermic peak of the ITT splits in two thermograms that represent overlapping processes, the non-reversing that is endothermic and the reversing that is exothermic, and their enthalpies depend on the frequency of the oscillation in the TMDSC experiment. At low periods (high frequency), both the hydration−dehydration and the folding−unfolding processes cannot follow the temperature program, then both appear in the non-reversing component, whereas no contribution is shown in the reversing component. On the contrary, at high periods (low frequencies), both processes can follow the temperature program and appear in the reversing component, so this component equals the total. However, for intermediate frequencies, the difference in kinetics[31] makes those processes that have an exothermic behavior, basically the folding, take place in the reversing component and those with endothermic behavior, basically the disruption of ordered water structures, take place in the non-reversing component.

Only the contribution of the water of hydrophobic hydration and the hydrophobic association of the polymer chains have been considered here to account for the exothermic and endothermic components found in the transition enthalpy. In principle, the contribution of hydrophilic hydration and the formation and breaking of hydrogen formed both intramolecularly or with water in the hydrophilic parts of the polymer, mainly around the amide bond, could be also present. However, the number of water molecules involved in ordered hydrophobic hydration structures has been previously estimated to be around 100 water molecules per pentamer by dielectric relaxation[11] or 170 water molecules per pentamer by DSC experiments.[17] Therefore, due to the huge amount of water molecules involved in the formation of hydrophobic hydration, as compared to those presumably involved in the interaction with the amide bonds in the polymer backbone, the calorimetric contribution of those is likely negligible.

Figure 3 shows ΔH_{rev} (in absolute values) as a function of the period at different salt concentrations. The curves show a maximum ΔH_{rev}, which takes places at $P_M = 0.6$ min for the three NaCl concentrations tested. Therefore, we can assume that this period is the one that yields a maximum split of both components. Additionally, an increase in ΔH_{rev} can be also observed in that figure with the addition of NaCl.

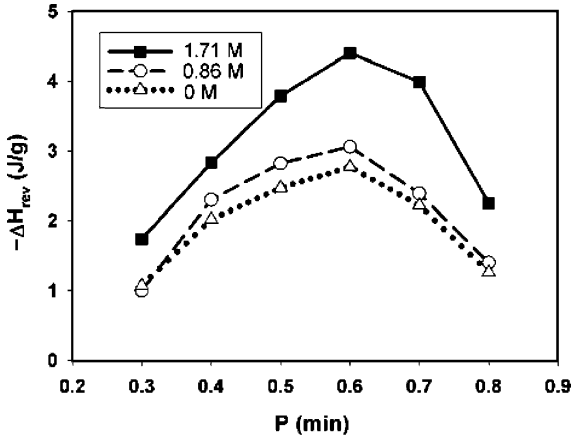

Figure 3. ΔH_{rev} as a function of the oscillation period for a 50 mg/mL $(GVGVP)_{251}$ aqueous sample with [NaCl] = 0.86 and 1.71 M (heating ramp, $r = 1$ °C/min; amplitude, $A = 0.1$ °C).

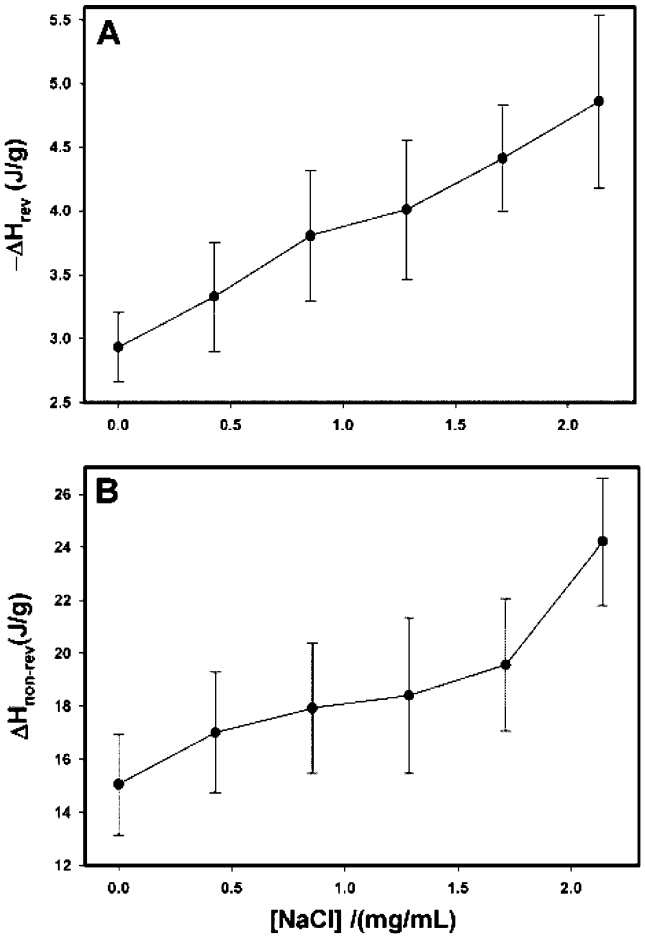

Figure 4. Enthalpy components obtained from TMDSC experiments of a 50 mg mL^{-1} $(GVGVP)_{251}$ aqueous solution in the presence of NaCl as a function of [NaCl] (heating ramp, $r = 1$ °C/min; amplitude, $A = 0.1$ °C; period, $P = 0.6$ min). (A) Enthalpy of the reversing component. (B) Enthalpy of the non-reversing component. Positive enthalpies indicate endothermic processes.

The actual dependency of ΔH_{rev} at P_M on NaCl concentration has been plotted in Figure 4A, whereas the enthalpy dependency of the non-reversing component has been plotted in Figure 4B. This increase in enthalpy keeps the ratio $\Delta H_{tot}/\Delta H_{rev}$ around −3.7 and $\Delta H_{nonrev}/\Delta H_{rev}$ around −4.8.

As it can be seen in that figure, both the endothermic and the exothermic components increase as NaCl concentration increases. Because the endothermic component is related to the

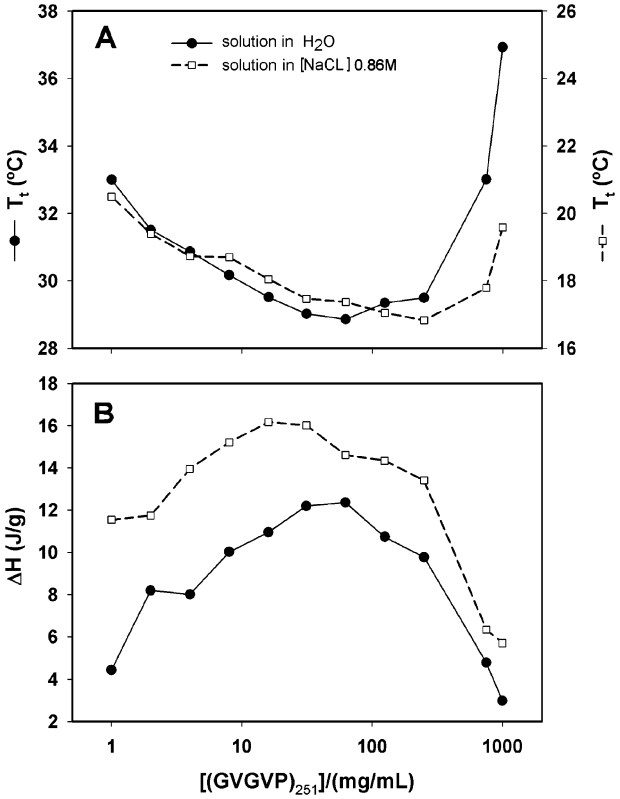

Figure 5. Dependence of T_t and ΔH on $[(GVGVP)_{251}]$ (concentration: mg/(mL of solvent)) as obtained by conventional DSC with (−□−) and without NaCl cosolution (−●−). (A) Transition temperature (the curve for the cosolution with NaCL has been plotted in a different *y*-axis for better comparison of the polymer concentration effect); (B) total enthalpy of the endotherm (per grams of polymer).

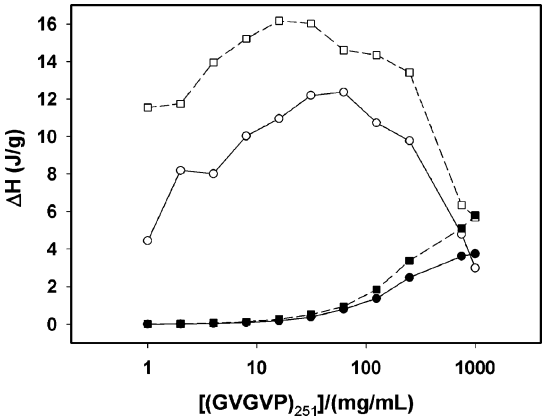

Figure 6. ΔH (per grams of polymer and per grams of water) of $(GVGVP)_{251}$ water solutions, with and without NaCl cosolution, as a function of $[(GVGVP)_{251}]$. (−□−) with NaCl and per gram of polymer; (−○−) without NaCl and per gram of polymer; (−■−) with NaCl and per gram of water; (−●−) without NaCl and per gram of water.

disruption of the water structures during the ITT, the effect of NaCl cannot be explained as a disruption of this structured water because that would have the opposite effect on ΔH_{nonrev}. As discussed before, that fact is just the opposite from that found for polymers that exhibit LCST.

As it has been mentioned before,[35] the mechanism by which NaCl affects the ITT cannot be a nonspecific electrostatic type nor the direct ion binding to specific groups on the peptide chains, because the side chains of this polymer are essentially uncharged and mainly apolar. According to the presented data and taking into account the behavior accounted in the literature for different ELPs showing different mean polarities, the effect of the increase in [NaCl] in the thermal parameter is totally equivalent to an increase in the hydrophobicity of the polymer chain, which, as it has been shown before, causes the decrease in T_t, the increase in ΔH,[1] and the simultaneous increase (in absolute figures) of both ΔH_{nonrev} and ΔH_{rev}.[32] Therefore, the effect of NaCl could be understood as an offset effect of the solvent polarity. In this sense, an increase in [NaCl] could cause an increase in the polarity of the solvent, and that higher difference in polarity with respect to the hydrophobic moieties of the polymer causes more and more ordered structures surrounding the polymer chains.

Previous works have shown that the dependency of the thermal parameters on the polymer concentrations, for a wide concentration range going from excess to deficiency of water, can be used to estimate the quality of hydrophobic hydration and number of molecules per pentamer implied in that.[17] In this work, a similar study has been carried out with and without the presence of NaCl (Figure 5). As both enthalpies in the components of the TMDSC increase when the total enthalpy increases, only experiments with conventional DSC have been carried out. As it is seen in Figure 5A, there is an increment in T_t for high and low polymer concentrations being the minimum of T_t between 40 and 125 mg/mL. Along with these increases in T_t, there is a decrease in ΔH (Figure 5B). Both increases in T_t and decreases in ΔH are caused by different effects. For high polymer concentrations, the increment in T_t and decrease in ΔH are due to the deficiency of water. In that water deficiency state, only the most stable hydrophobic hydration structures are formed, which causes a logical increase in T_t, while ΔH decreases.[17] On the other hand, the increase in T_t with the decrease in polymer concentration has been proved by turbidimetry and DSC.[11,38,39] That increase in T_t fits well with the logarithm of the concentration ($T_t = m \ln(C) + b$) in the work of Meyer et al.[38] The data presented here also fit that function for low concentrations being $m = -0.97$ and -0.62 °C for the solution without and with NaCl, respectively, so these parameters also depend on the salt concentration. In addition to the increase in T_t, there is a decrease in ΔH with the decrease in polymer concentration. That behavior can be explained by a decrease in an interchain cooperativity that could take place under dilute solutions. This lack of efficient intrachain cooperativity would hamper the folding process, causing the observed thermal effects.

It can be also seen in Figure 5 that the polymer solution in the presence of NaCl shows lower T_t and higher ΔH than the solution without NaCl for all of the polymer concentrations. This fact would indicate that there is not a real competition for water between the apolar moieties and the salt ions.

If the enthalpy is presented per grams of water, and assuming that for high concentrations all of the available water forms part of the hydration of the polymer and there is not bulk water, it is possible to see the effect of NaCl on those structures (Figure 6). The transition enthalpy per gram of water does not reach a horizontal line. This fact clearly indicates that there are several types of water structures with different stability and, accordingly, different "melting" enthalpies.[17] Also, as the enthalpy per gram of water for the solution with NaCl is higher than that for the solution without NaCl, there exists a clear effect of the presence of NaCl that can be interpreted as an increase in the quality of the ordered hydrophobic hydration.

Conclusions

The influence of NaCl in the ITT of a model ELP can be followed by calorimetric TMDSC and conventional DSC. The different results gathered in this work showed that the presence of NaCl causes a concentration-dependent decrease in T_t and increase in ΔH. According to TMDSC experiments, both exothermic and endothermic components comprising the DSC endothermic peak increase as [NaCl] increases. That, in addition to data coming from conventional DSC in water deficient solutions, points to the interpretation of the effect of NaCl as causing a better organization of the polymer in the folded state and a more extense and better structured corona of hydrophobic hydration surrounding the apolar moieties in the extended state of the polymer chain. Finally, these results can also shed light on the mechanisms that govern the ITT, pointing out the differences between this mechanism and the one shown by other polymers showing LCST.

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