Lattice Model for Polymer Hydration: Collapse of Poly(*N*-isopropylacrylamide)

*Pierpaolo Bruscolini, Carla Buzano, Alessandro Pelizzola, Marco Pretti**

Istituto Nazionale per la Fisica della Materia (I.N.F.M.) and Dipartimento di Fisica, Politecnico di Torino, Corso Duca degli Abruzzi 24, I-10129 Torino, Italy

Summary: *Poly(N-isopropylacrylamide)* (PNIPAM) in dilute aqueous solution undergoes a *collapse* transition from coil to globule on *increasing* temperature. Such coil-to-globule collapse is usually considered analogous to the cold renaturation of small globular proteins. In this paper we propose a theoretical approach that is able to reproduce, in a semi-quantitative way, the unusual behavior of PNIPAM, and the observed thermodynamic properties. The procedure is based on two main steps: (i) the characterization of single monomer *hydration* thermodynamics, interpreted by a balance between the removal of monomer-monomer interactions and the addition of water-monomer interactions, and (ii) a simplified analysis of a *lattice* self-avoiding walk (SAW) model, which allows to account for the configurational entropy in a controlled way, and hence to relate the microscopic interactions to the „macroscopic" behavior of the polymer chain. The results show that the temperature dependence and magnitude of the interaction parameters that best fit experimental data validate a recently proposed qualitative interpretation of the mechanism of collapse transition for PNIPAM. The latter result turns out to be relevant to support the analogy with the cold renaturation of small globular proteins, and to clarify some important aspects of protein thermodynamics.

Introduction

PNIPAM is a simple synthetic homopolymer.[1,2] It has been shown experimentally that, in dilute aqueous solution, PNIPAM undergoes an unusual coil-globule collapse on *increasing* temperature. Such a phenomenon has been well characterized both from the thermodynamic[1,2] and from the structural point of view.[3] In particular calorimetric measurements have shown that the collapse of PNIPAM implies a heat capacity decrease, i.e. the heat capacity of PNIPAM in the collapsed state is significantly lower than in the expanded state.[1,2] A reason for the interest attracted by PNIPAM collapse is that similar thermodynamics characterizes the "cold" denaturation of small globular proteins. Thermodynamic properties of biological macromolecules, and particularly of proteins,[4] have great importance, together with geometrical structure, to understand the mechanisms by which they execute their biological functions.[5] In particular the heat capacity decrease affects the thermodynamics of protein folding and determines the stability of the native structure,[6,7] which turns out to be restricted to a precise temperature range. A high

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temperature ("warm") denaturation is usually observed, while an analogous low temperature ("cold") denaturation is sometimes observed and often extrapolated under the water freezing point, but the whole phenomenology is quite general.[8,9] Nevertheless proteins are quite complex heteropolymers and it is not easy to single out the causes of their behavior and to propose a theoretical model to explain it quantitatively. To this end a good strategy is to focus on simpler model systems presenting a similar behaviour.

Besides macroscopic (chain level) thermodynamics, the analogy of small globular proteins with PNIPAM has been recently supported by Graziano,[10] also on the basis of a microscopic (single monomer level) interpretation. The monomeric unit (NIPAM) is a chemical isomer of leucine (aminoacid residue), having a polar peptide group in the side chain, rather than in the backbone, and ten non-polar hydrogens (the structure is reported for instance in References [1,2]). One usually observes aggregation of PNIPAM chains just above the coil-globule transition temperature, which suggests that in the globule state most of the peptide groups present in the monomeric units are buried in the interior. Otherwise a large number of water molecules would cover the globule surface (being hydrogen-bonded to surface peptide groups), and such water molecules should be able to prevent aggregation and keep globules in solution. Graziano concludes that the coil-globule collapse of PNIPAM should involve the formation of intra-molecular hydrogen bonds (H-bonds) between buried peptide groups and the breaking of inter-molecular H-bonds between surface peptide groups and water molecules. The mechanism driving such collapse has to be explained by a subtle (enthalpic and entropic) balance between the two processes. These are not trivial from the thermodynamic point of view, mainly because of entropic effects related to the number of configurations of polymer chains and of water molecules. It would be very interesting to make the above arguments more quantitative (at least as far as orders of magnitude are concerned), in order to validate the molecular interpretation of PNIPAM collapse. Two main ingredients are necessary to this purpose: (i) a characterization of the temperature dependence of water-monomer and monomer-monomer interactions in terms of effective free energies, that take into account both enthalpic and entropic effects; (ii) a theory which is able to relate in a simple way microscopic interactions to the degree of compactness of the polymer chain (to detect the collapse) and to macroscopic thermodynamic properties (to allow a comparison with experimental data). To this end, Flory-like (mean field) theories of coil-globule transition could be very attractive, due to their simplicity: indeed they have been used so far to determine qualitative features of the collapse transition of PNIPAM. But, as shown both experimentally[3] and theoretically,[11] such theories give rise to some inconsistencies when applied to describe a compact (globular) chain. A recently proposed statistical treatment for a

SAW on a lattice, based on the Bethe approximation,[12] predicts the coil-globule transition in better agreement with Monte Carlo simulations, though keeping the simplicity of Flory-like models. In this work we apply this method to the PNIPAM case, with microscopic interactions extracted from experimental data on monomer calorimetry (water-monomer interactions) and from a simple model of intra-molecular H-bonding (monomer-monomer interactions).

Theoretical Background

In this section we consider a SAW on a lattice. The lattice coordination number is q. Each lattice site represents either a *chain segment* containing n monomeric units, or a *cluster of water molecules*. The two "chemical species" will be denoted respectively by M and W in the following. The grand-canonical potential of the system (per mole of sites) is

$$\Omega = U - TS - \mu_M \mathrm{P}_M - \mu_W \mathrm{P}_W , \tag{1}$$

where U and S are respectively the internal energy and entropy (per mole of sites), T is the absolute temperature, μ_M and μ_W are the chemical potentials (times Avogadro's number), and finally P_M and P_W denote the probabilities that a site is occupied by M or W. The internal energy includes interactions between chemical species lying on nearest-neighbor (NN) sites

$$U = \frac{q}{2}\left(E_{WW}\mathrm{P}_{WW} + 2E_{WM}\mathrm{P}_{WM} + E_{MM}\mathrm{P}_{MM}\right), \tag{2}$$

where $q/2$ is the number of NN contacts per site, E_{XY} is the interaction energy between chemical species X and Y (per mole of contacts) and P_{XY} is the probability that a pair of NN sites is occupied by X and Y respectively. Notice that P_{MM} includes only pairs of non consecutive segments along the chain. Site probabilities P_X can be derived from P_{XY} as marginal distributions in the following way

$$\mathrm{P}_W = \mathrm{P}_{WW} + \mathrm{P}_{WM} \tag{3}$$

$$\mathrm{P}_M = \frac{q}{q-2}\left(\mathrm{P}_{WM} + \mathrm{P}_{MM}\right), \tag{4}$$

where in the latter equation the pre-factor accounts for the fact that each segment has q–2 available contacts out of q NN sites. We can then write

$$U = qE\mathrm{P}_{WM} + \frac{q}{2}E_{WW}\mathrm{P}_W + \frac{q-2}{2}E_{MM}\mathrm{P}_M , \tag{5}$$

where

$$E = E_{WM} - \frac{E_{WW} + E_{MM}}{2} \tag{6}$$

is an effective water-segment contact energy, proportional to the well known Flory's χ parameter.[13] Finally, using the relationship $P_W = 1 - P_M$, the grand potential can be written in the following form

$$\Omega = \Omega_0 + \Delta\Omega\,, \tag{7}$$

where

$$\Omega_0 = \frac{q}{2} E_{WW} - \mu_W \tag{8}$$

is easily recognized to be the grand potential of pure water, and

$$\Delta\Omega = qEP_{WM} - TS - \mu P_M \tag{9}$$

with

$$\mu = \mu_M - \frac{q-2}{2} E_{MM} + \Omega_0\,. \tag{10}$$

A model defined by a thermodynamic potential of the form of $\Delta\Omega$, containing only an effective water-segment interaction E and an effective segment chemical potential μ, has been previously investigated by approximating the conformational entropy S of the SAW by means of the Bethe approximation.[12,14] In this scheme, which takes into account correlations up to NN pairs, it is possible to write $S = S(P_M,P_{WM})$ and the equilibrium state of the system is determined by minimizing $\Delta\Omega$ with respect to the variational parameters P_M and P_{WM}. The minimization is performed numerically.[12] The resulting phase diagram is displayed in Figure 1a for the simple cubic lattice. A transition line separates a pure water phase with $P_M = 0$ from a polymerized phase with $P_M > 0$ (P_M is the order parameter). The transition is continuous for low values of E/RT (being R the ideal gas constant) and discontinuous for higher values, and the two regions are separated by a tricritical point. The polymerized phase in the limit of μ tending to the transition line represents an isolated chain in solution: the continuous transition region ($P_M \rightarrow 0^+$) corresponds to the coil state, while the discontinuous transition region corresponds to the globule state. The tricritical point represents the coil-globule collapse. In Figure 1b the behavior of P_M and

$$P_{W|M} \equiv \frac{q}{q-2} \frac{P_{WM}}{P_M} \tag{11}$$

at the transition line is reported as a function of E/RT. The latter parameter represents the conditioned probability that a segment is in contact with water in a given direction (again the pre-factor accounts for the fact that only q–2 contacts out of q bonds are available for each segment). The extended coil state is denoted by $P_{W|M} = 1$, while $P_M > 0$ denotes the globule state.

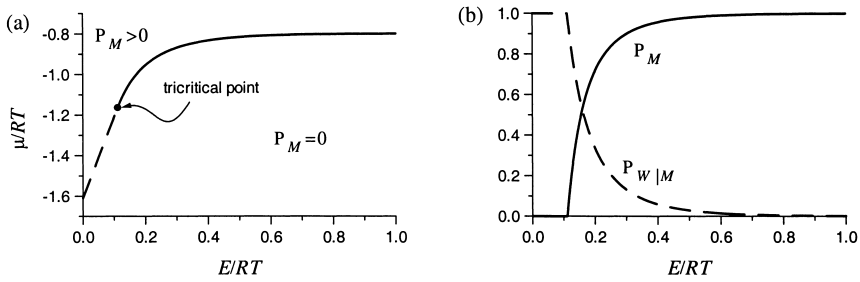

Figure 1. (a) Phase diagram in the plane μ/RT-E/RT for the simple cubic lattice ($q = 6$).
(b) Equilibrium probabilities at the transition line (polymerized phase) as a function of E/RT.

It is possible to show[14] that the tricritical point, that is the coil-globule collapse, occurs at

$$\frac{E}{RT} = \frac{1}{2}\log\frac{q-1}{q-2}. \tag{12}$$

Let us notice that on the transition line $\Delta\Omega = 0$, that is $\Omega = \Omega_0$ (the grand potential of the system coincides with that of pure water). Using the thermodynamic identity $\Omega = -PV$ (being P the applied pressure and V the lattice volume per mole of sites), and the fact that μ_M coincides with the Gibbs free energy per (mole of) segment, from Equation (10) the Helmholtz free energy (per mole of segment) turns out to be

$$F_M = \mu_M - PV = \mu + \frac{q-2}{2}E_{MM}, \tag{13}$$

where μ is now the chemical potential *at* the transition line. From the graph of Figure 1a, we see that

$$\mu = RTf\left(\frac{E}{RT}\right) \tag{14}$$

where the function f is known numerically. Equations (13) and (14) allow us to determine F_M, and hence all the other partial thermodynamic quantities, provided we know the interaction parameters E and E_{MM}.

Analysis of Interactions

In the case of PNIPAM in aqueous solution, the interaction parameters we have introduced in the previous section should be considered as *effective* energies, since they also contain important entropic contributions, which come out from degrees of freedom that are not explicitly taken into account, namely orientations of water molecules for E_{WM}, and of side

chains for E_{MM}. Such degrees of freedom can be taken into account in an approximate average way, by replacing interaction energies by temperature dependent effective free energies.

As far as water-segment energy is concerned, we can relate it to the single monomer hydration free energy, by means of interpolation formulas to experimental data. Unfortunately hydration data are not available in the literature for NIPAM monomer and we then consider leucine,[15,16] an aminoacid residue with the same chemical composition. NIPAM has the polar peptide group in the side chain rather than in the backbone,[1,2] but a detailed experimental analysis[15,16] suggests that hydration effects should be additive down to the scale of chemical groups, and hence a different assembly of the same groups into molecules should not make a significant difference as far as hydration is concerned. Accordingly in Reference [17] it is shown that hydration thermodynamic parameters can be well approximated, for a polymer in an expanded coil state (i.e. when most monomers are hydrated), by the sum of single monomer contributions, and this is why we are somehow allowed to employ data from single monomer measurements to describe polymer hydration at a microscopic level. Let us consider an expanded chain in aqueous solution: the internal energy is approximated by Equation (2) with $P_{MM} \approx 0$. Using also Equations (3) and (4) with the same approximation, we can easily write

$$U \approx \frac{q}{2} E_{WW} P_W + (q-2)\left(E_{WM} - \frac{E_{WW}}{2} \right) P_M . \tag{15}$$

It is then evident that the partial segment energy is represented by the factor which multiplies P_M in the total energy expression, and this should coincide, according to the above discussion, with the hydration free energy of n (mole of) monomers, that is

$$n\Delta F^\circ = (q-2)\left(E_{WM} - \frac{E_{WW}}{2} \right) \tag{16}$$

(we shall denote single monomer hydration thermodynamic quantities by a $^\circ$ superscript). In the following we assume to be allowed to neglect pressure-volume terms and to use indifferently Helmholtz and Gibbs hydration free energies ($\Delta F^\circ \approx \Delta G^\circ$). To interpolate experimental data, we assume a linear temperature dependence of the (constant pressure) hydration molar heat capacity

$$\Delta C^\circ \approx \Delta C_0^\circ + \Delta C_0'^\circ (T - T_0), \tag{17}$$

where ΔC_0° is the hydration heat capacity at the reference temperature T_0 and $\Delta C_0'^\circ$ is its derivative (with respect to temperature). By simple integration we easily obtain

$$\Delta G^\circ = \Delta H^\circ - T\Delta S^\circ \tag{18}$$

with

$$\Delta H^\circ = \Delta H_0^\circ + \Delta C_0^\circ (T - T_0) + \frac{1}{2}\Delta C_0'^\circ (T - T_0)^2 \tag{19}$$

$$\Delta S^\circ = \Delta S_0^\circ + \Delta C_0'^\circ (T - T_0) + (\Delta C_0^\circ - T_0 \Delta C_0'^\circ) \log \frac{T}{T_0}, \tag{20}$$

where ΔH_0° and ΔS_0° are respectively hydration enthalpy and entropy at the reference temperature. Figure 2b shows that a linear fit for the heat capacity (Figure 2a) yields a satisfactory interpolation on the whole temperature range of interest.

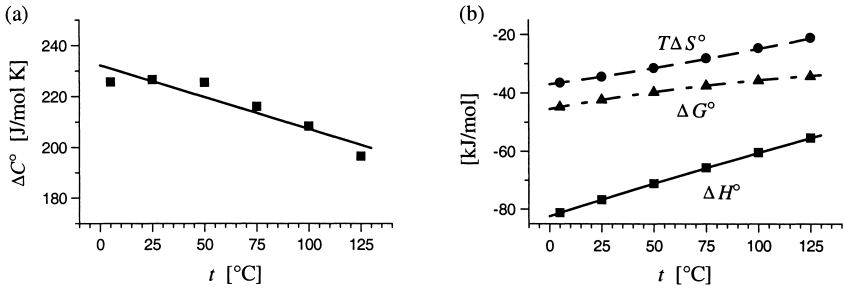

Figure 2. Hydration molar heat capacity (a), enthalpy, entropy and free energy (b) for leucine. Symbols denote experimental data;[15,16] lines are obtained by interpolation formulas described in the text, with ΔC_0° = 226 J/mol K, $\Delta C_0'^\circ$ = −0.249 J/mol K^2, ΔH_0° = −76.690 kJ/mol, and ΔS_0° = −115 J/mol K. The reference temperature is T_0 = 298 K.

It can be observed that, mainly due to the polar peptide group, the hydration free energy is negative, meaning that NIPAM is quite a hydrophilic monomer. Hence the stability of a globular state needs the presence of additional attractive interactions between monomers (Van der Waals forces and H-bonds), which is in agreement with Graziano's interpretation of PNIPAM collapse,[10] and also with Privalov and Makhatadze's results about polypeptides.[15,16] Let us notice that also the hydration entropy is negative, due to ordering of water molecules in the surroundings of solute molecules.

We now have to provide a characterization also for intra-molecular interactions, that is the segment-segment energy. According to Graziano, it should be related to internal H-bonding, and we can assume that the Van der Waals energy is just an additive constant. In this case specific experimental data are not available, thus we propose an extremely simplified model based on fluctuating H-bonds between side chains. We compute the partial partition function and hence the effective interaction energy, which turns out to be a function of two parameters, namely enthalpy and entropy of H-bond breaking, that are approximately independent of

temperature. Let us consider an ideal globular state in which every segment has $q-2$ contacts to other segments, each (mole of) contact yielding an interaction energy E_{MM}. Each NIPAM monomer possesses one donor and one acceptor in its peptide group, and can then form two H-bonds at most, even if a bond is obviously shared by two monomers. Assuming that in the ideal globule all H-bonds can be formed, and also that intra-segment H-bonds are not allowed, we can write

$$n\Delta F^* = -\frac{q-2}{2} E_{MM}, \tag{21}$$

where ΔF^* is the free energy of breaking a (mole of) H-bond. Let us now suppose to describe the single monomer as immersed in a cluster of similar monomers, which do not interact with one another. Let us also remind again that NIPAM can form at most two H-bonds. The cluster partition function thus reads

$$Z = N_0 + N_1 \exp\frac{E_{HB}}{RT} + N_2 \exp\frac{2E_{HB}}{RT}, \tag{22}$$

where N_k is the number of cluster configurations with k formed bonds and E_{HB} is the energy of breaking a (mole of) bond. The breaking free energy can be written as a free energy difference with respect to an ideal case in which bonds cannot be formed. We then have

$$\Delta F^* = \frac{1}{2} RT \log\frac{Z}{Z_0} \tag{23}$$

where the 1/2 factor avoids double counting, and

$$Z_0 = N_0 + N_1 + N_2 \tag{24}$$

is the trivial partition function of the non-bonding case. Assuming that only the last term in the partition sum (22) is significant, we can write

$$\Delta F^* = \Delta U^* - T\Delta S^*, \tag{25}$$

where

$$\Delta U^* \approx E_{HB} \tag{26}$$

$$\Delta S^* \approx \frac{1}{2} R \log\frac{Z_0}{N_2}. \tag{27}$$

The breaking energy ΔU^* and entropy ΔS^* are then approximately independent of temperature. Let us finally notice that the assumption of a dominant term in the partition function is justified, with the values of ΔU^* and ΔS^* found in the following (see caption of Figure 3), in the hypothesis $N_0 >> N_1$ and $N_0 >> N_2$, which is reasonable because of the high directivity of H-bonds.

We have thus provided a characterization of both intra-molecular (water-monomer) and inter-molecular (monomer-monomer) interactions. Using Equations (6), (16) and (21), we finally obtain the effective water-monomer energy E (to be used as input parameter for the lattice model) in the following form

$$E = \frac{n}{q-2}\left(\Delta F^\circ + \Delta F^*\right), \tag{28}$$

where ΔF° is known from experiments, while ΔF^* contains the two unknown parameters ΔU^* and ΔS^*, which will be determined to fit experimental data. As previously mentioned, from the knowledge of E it is possible to determine also the temperature dependence of μ via Equation (14), whence from Equations (13) and (21) the polymer partial free energy (per mole of monomer)

$$F_\wp = \frac{F_M}{n} = \frac{RT}{n} f\left(\frac{E}{RT}\right) - \Delta F^*. \tag{29}$$

Taking into account Equations (28) and (25), it is then easy to compute the partial heat capacity

$$C_\wp = -T\frac{d^2 F_\wp}{dT^2} = \frac{1}{q-2}\Delta C^\circ f'\left(\frac{E}{RT}\right) - \frac{n}{(q-2)^2}\frac{\left(\Delta U^\circ + \Delta U^*\right)^2}{RT^2} f''\left(\frac{E}{RT}\right), \tag{30}$$

where ΔC° and $\Delta U^\circ \approx \Delta H^\circ$ are defined by Equations (17) and (19) respectively, the function f is known numerically (see Theoretical Background section), f' and f'' are its first and second derivatives respectively, and E is defined by Equations (28), (25), (18), (19), (20). It is easy to see that $C_\wp$ is a function of temperature and of the parameters q, n, ΔU^*, ΔS^*, that have to be determined to perform a comparison with experimental results available for PNIPAM specific heat.[1,2]

Results and Discussion

The previous sections provide a way to relate lattice model parameters (q and n) and intra-molecular H-bonding parameters (ΔU^* and ΔS^*) to the thermodynamic properties of PNIPAM in dilute acqueous solution and to indicators of its compactness, as functions of temperature. We can now evaluate the above parameters in order to reproduce the heat capacity behavior experimentally observed for PNIPAM.[12,13] First of all we take the coordination number to be $q = 6$ (simple cubic lattice), while the stiffness parameter is fixed to $n = 15.5$ (a realistic value for PNIPAM). The former assumption is somehow arbitrary, but we shall verify that it does not heavily affect the results. The latter assumption is based on experimental evidence: in

Reference [3] we see gyration radii $R_g \approx 125–135$ nm for two PNIPAM samples with molecular weights $M \approx 10.8–12.1\times10^6$ g/mol at the Θ-point. Assuming that in this case the polymer behaves like an ideal coil,[13] we have that the average end-to-end distance is $\sqrt{6}$ times the gyration radius, and equals $l\sqrt{nN}$, where l is the real distance between monomeric units along the chain, nl is the effective (Kuhn) segment length, and $N = M/m$ (being $m \approx 113$ g/mol the molecular weight of NIPAM monomer) is the polymerization index. From PNIPAM structure[1,2] we can estimate $l \approx 0.254$ nm, and easily obtain

$$n = \frac{6m}{M}\frac{R_g^2}{l^2} \approx 15.2–15.8\,. \tag{31}$$

Internal H-bonding parameters ΔU^* and ΔS^* are determined numerically by imposing two constraints. First we require that the collapse occurs at the experimentally measured temperature[1,2] $t_c \approx 34.3$ °C ($T_c \approx 307.45$ K). From Equations (12), (28) and (25) we have

$$\Delta U^* - T_c\Delta S^* = RT_c\frac{q-2}{2n}\log\frac{q-1}{q-2} - \Delta F^\circ\big|_{T=T_c}\,, \tag{32}$$

where $\Delta F^\circ \approx \Delta G^\circ$ is defined by Equations (18), (19), (20). Secondly we require that the theory reproduces also the heat capacity decrease from coil to globule, which is one the most important thermodynamic features both of PNIPAM collapse and of protein cold renaturation, as mentioned in the Introduction. Precisely we impose that the specific heat difference between $T_{\text{coil}} = 307$ K (just before the peak) and $T_{\text{globule}} = 323$ K (when the specific heat is stabilized after the peak) equals the experimentally measured value[2] for a high molecular weight case (our model describes the limit of infinite chain). We then have

$$\frac{C_\wp\big|_{T=T_{\text{coil}}} - C_\wp\big|_{T=T_{\text{globule}}}}{m} = 0.44 \text{ cal / g K}\,, \tag{33}$$

where $C_\wp$ is defined by Equation (30). Solving Equations (32) and (33) yields $\Delta U^* \approx 67.8$ kJ/mol and $\Delta S^* \approx 86.1$ J/mol K. The partial specific heat $C_\wp/m$ predicted by the theory with this parameter choice is shown in Figure 3a as a function of temperature, together with experimental results.[1] In Figure 3b equilibrium probabilities (defined in the Theoretical Background section) are displayed: a coil to globule collapse on increasing temperature is observed, followed by a globule-coil expansion at high temperature. Theoretical predictions turn out to be in quite a good agreement with experimental results, but some comments are in order. First of all we remind that the validity of our results is only semi-quantitative, due to the extremely simplified nature of the model, the approximate statistical treatment, and the presence of a parameter not directly related to experiments, such as the coordination number

q. Nevertheless the theoretical results correctly reproduces all the qualitative features of PNIPAM collapse on increasing temperature, that is a peak in the specific heat, and the specific heat decrease on going from coil to globule.

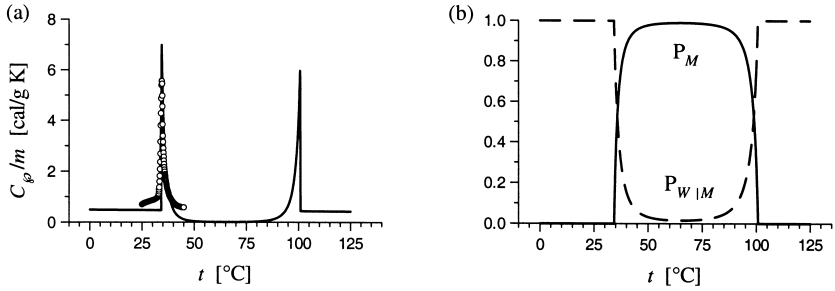

Figure 3. (a) Partial specific heat of high molecular weight ($M \approx 7000$ kg/mol) PNIPAM in aqueous solution[1] (circles), compared to theoretical predictions (solid line), obtained by Equation (30) with $q = 6$, $n = 15.5$, $\Delta U^* = 67.8$ kJ/mol, and $\Delta S^* = 86.1$ J/mol K.
(b) Equilibrium probabilities as a function of temperature.

Extending calculations to a wider temperature range with respect to experiments, it turns out that a globule to coil expansion is predicted at high temperature (just over the water boiling point), accompanied by another specific heat peak. This transition is not observed experimentally but the fact that it is predicted by the theory is significant: the theoretical transition temperature $t_t \approx 101$ °C is in good (qualitative) agreement with an extrapolation proposed by Graziano,[10] which predicts $t_t \sim 100 \div 133$ °C, for PNIPAM samples with molecular weights in the range $M \approx 11.2 \div 7000$ kg/mol.[13] Intra-molecular interaction parameters ΔU^* and ΔS^* obtained by the fit turn out to be reasonable to characterize H-bonding, even if they are overestimated in principle: in fact ΔU^* includes Van der Waals contributions, while the description of intra-molecular H-bonding neglects cooperativity, thus overestimating ΔS^*. Let us also briefly address the issue of kinetic energy, which is not taken into account by the lattice model. Reasonably it should give a heat capacity contribution (per mole of monomer) of the order of R. Dividing by the monomer molecular weight we would obtain a specific heat contribution of the order of $R/m \approx 0.0176$ cal/g K, which is clearly negligible. It is finally possible to verify that the coordination number does not heavily affect the predicted H-bonding parameters: for $q = 12$ we obtain $\Delta U^* \approx 65.4$ kJ/mol and $\Delta S^* \approx 78.3$ J/mol K. The same remark holds for the warm transition temperature (the cold one is fixed by Equation (32)), namely for $q = 12$ we have $t_t \approx 127$ °C.

Conclusion

In this paper we have proposed a theoretical approach to describe the collapse of PNIPAM in dilute aqueous solution. Such collapse is unusual because it occurs on increasing temperature, and it has been conjectured that this fact is related to the peculiar thermodynamics of hydration (water-monomer interaction) and intra-molecular H-bond formation (monomer-monomer interactions).[10] We have characterized such interactions via effective temperature-dependent free energies. Moreover we have applied a generalized mean-field (Bethe) approach to a lattice description of the polymer configurational statistics, in order to relate microscopic interactions to macroscopic properties of PNIPAM chains. The Bethe approximation is one step beyond ordinary Flory-like theories, and should be more suitable to describe a collapsed phase, because of its ability to take into account pair correlations in an exact way.[12] By means of this procedure, we have determined the partial specific heat of PNIPAM in solution as a function of temperature and of two parameters, namely enthalpy and entropy of intra-molecular H-bond formation. We have computed these two parameters to reproduce the collapse temperature and the corresponding specific heat decrease, measured in experiments.[1,2] After these two simple requirements, it turns out that the theoretical specific heat curve correctly reproduces a peak (corresponding to the collapse) and a lower specific heat in the collapsed phase. The agreement with experimental data is mainly qualitative, but orders of magnitude are correct. Moreover we find it meaningful that also the computed microscopic parameters ΔU^* and ΔS^* have the right order of magnitude to characterize intra-molecular interaction based on H-bond formation. This result contributes to validate Graziano's explanation[10] of the molecular mechanism of PNIPAM collapse, which would turn out to be specially relevant, if it could be applied to explain also the thermodynamics of globular proteins. It is actually in agreement with the results of Privalov and Makhatadze,[17] who suggested, after a detailed analysis of experiments on polypeptides, that hydration gives the major contribution to the heat capacity increment, and that the leading interactions stabilizing the compact state are intra-molecular H-bonds.

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