Pressure Effect on the Hydration Properties of Poly(*N*-isopropylacrylamide) in Aqueous Solution Studied by FTIR Spectroscopy

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ABSTRACT: The hydration properties of poly(*N*-isopropylacrylamide) in aqueous solution were investigated by Fourier transform infrared spectroscopy as a function of high hydrostatic pressure and compared to the thermally induced changes. We show that although both pressure and temperature induce a phase separation the underlying mechanisms are fundamentally different. It is well documented that increasing the temperature above the lower critical solution temperature causes a dehydration of the hydrophilic and hydrophobic moieties. By contrast, high pressure enhances the hydration of the hydrophilic amide group. Moreover, pressure strengthens the weak C−H···O hydrogen bonds between the hydrophobic alkyl groups and water, although a reorganization of the water network around the hydrophobic groups occurs during the phase separation. From this it is concluded that PNiPA remains in a coillike state at high pressure. In addition, we suggest that PNiPA is a good model for the study of the hydration properties of proteins.

Introduction

Water-soluble polymers such as poly(*N*-isopropylacrylamide) (PNiPA) will, in dilute solutions, undergo a coil-to-globule transition in response to changes in temperature, pH, salt concentration, and the addition of cosolvents.[1,2] The collapse is then followed by the aggregation of single chains into larger particles. Its solubility in water arises from the potential of the amide group to form hydrogen bonds with water. In addition, it is assumed that water forms clathrate-like structures around the hydrophobic isopropyl group in the coil state.[3,4] Dehydration of the hydrophobic moieties is considered to be the major driving force for the coil-to-globule transition and subsequent aggregation.[2,4−6] A computer simulation of the coil-to-globule collapse indicated that its dynamics is dominated by the dynamics of water.[6] In other words, the hydrophobic collapse is not driven by the effective tendency of the hydrophobic groups to interact, but by a solvent-induced interaction of these groups. It is becoming increasingly recognized that solvent−solvent interactions are an essential part of the description of the phase behavior of water-soluble polymers.[6−8]

The purpose of the present study is to investigate the effect of high pressure on PNiPA in aqueous solution by Fourier transform infrared (FTIR) spectroscopy. It is possible to tune the strength of the hydrogen bond and to destabilize hydrophobic contacts by high hydrostatic pressure.[9−11] This is particularly useful because little is known about the relative importance of the hydrophilic vs the hydrophobic part of the polymer in the collapse and aggregation processes; the role of the clathrate-like water structure in the phase transition is still a matter of debate.[2] A previous high-pressure study attempted to assess the relative contribution of the hydrophobic effect to the aggregation of PNiPA.[9] The rationale for this experiment was that if the hydrophobic interaction is responsible for the polymer aggregation, it should be possible to dissociate the aggregate by high pressure. As this dissociation was not observed, it was concluded that factors other than the hydrophobic effect must contribute to the observed aggregation. Other cloud point studies[1] and a ^{1}H NMR study[12] have come to the same conclusion. For instance, Kunugi et al. determined the pressure−temperature phase diagram for PNiPA and found that at pressures below 150 MPa the cloud point temperature increases with increasing pressure.[1] Above 150 MPa the cloud point temperature decreases with increasing pressure. This suggests that the hydrophobic interaction cannot be the main driving force toward the aggregated state. However, what does cause the polymer to aggregate at high pressure remains elusive. Here FTIR spectroscopy provides a powerful tool to study the hydration properties of PNiPA. The temperature dependence of the carbonyl and alkyl stretching bands and amine bending bands has been investigated previously, and these bands were shown to be sensitive to changes in hydrogen bonding, i.e., hydration.[13,14] Thus, FTIR spectroscopy is capable of correlating hydration changes to a particular domain of the polymer. Throughout this work the temperature dependence of the infrared spectrum will be shown for purposes of comparison.

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Materials and Methods

Materials. Poly(*N*-isopropylacrylamide) was obtained by free-radical polymerization of the corresponding monomer in dimethylformamide. The reaction was carried out at 70 °C under a nitrogen atmosphere using azobis(isobutyronitrile) (AIBN) as an initiator. The resulting polymer was precipitated by dropping the precooled reaction mixture slowly into a large volume of dry diethyl ether and was further purified on a Sephadex G-50 column eluted with water. It was dried under vacuum to constant weight at room temperature. The molar mass and the polydispersity index (M_w/M_n) were ~23 000 and ~1.7, respectively, as determined by gel permeation chromatography (in THF using polystyrene as a standard). PNiPA was dissolved in deuterated water (Cambridge Isotopes Inc., Andover, MA) at a concentration of 5.0 wt % and was incubated for ~16 h in order to enable all protons to exchange for deuterium.

Fourier Transform Infrared Spectroscopy. Infrared spectra were recorded with a Bruker IFS-66 FTIR spectrometer equipped with a liquid nitrogen cooled mercury cadmium telluride solid-state detector. The sample compartment was continuously purged with dry air. Typically, 250 interferograms were coadded after registration with a resolution of 2 cm^{-1}.

High hydrostatic pressure was generated in a diamond anvil cell (Diacell Products Ltd., UK). Barium sulfate was used as an internal pressure standard.[15] All pressure experiments were performed at room temperature.

Temperature measurements were performed using a transmission cell with CaF_2 windows (Graseby Specac, Orpington, UK) separated by a 50 μm Teflon spacer. The cell was placed into a heating jacket which is controlled by a Graseby Specac automatic temperature controller. The temperature increment was 0.2 °C/min.

Gaussian curve-fitting was performed using GRAMS/AI.7 (Thermo Galactic). The wavenumbers of the band centers found in the second-derivative spectra were used as the starting parameters.

Light Microscopy. The turbidity of the sample was followed in situ using an Olympus BH-2 light microscope with an ultralong working distance lens (20×). Images were acquired with a CCD camera (Sony) in combination with Snappy software (Play Inc.).

Results

Temperature Causes Dehydration of the Carbonyl Group Whereas Pressure Promotes the Formation of an Additional Hydrogen Bond. At 25 °C and 0.1 MPa the amide I′ band (the prime denotes the amides are deuterated) consists of a major peak at ~1626 cm^{-1} (89% of the band area) which is generally assigned to a carbonyl group that forms an intermolecular C═O···D−O−D hydrogen bond with water as this provides the origin of the solubility of PNiPA in water (Figure 1).[4,13] However, DFT calculations (in vacuo) and near-infrared spectroscopy (in D_2O) have provided evidence for the existence of intramolecular C═O···D−N hydrogen bonding in addition to C═O···D−O−D hydrogen bonding.[14,16] As the difference in the polymer−water and polymer−polymer hydrogen bond strength is relatively small (~4 cm^{-1}), these vibrations will overlap.[17] Curve fitting also indicates the presence of two further minor contributions at ~1604 (7%) and 1653 cm^{-1} (4%). These are attributed to an additionally hydrogen-bonded carbonyl and non-hydrogen-bonded carbonyl, respectively.[14] The assignment of the former is inferred from our pressure data (see below).

A pressure increase from 0.1 to 1073 MPa results in a loss of intensity and broadening of the amide I′ band (Figure 2A). The difference spectra, calculated by subtracting the spectrum at 0.1 MPa from each spectrum

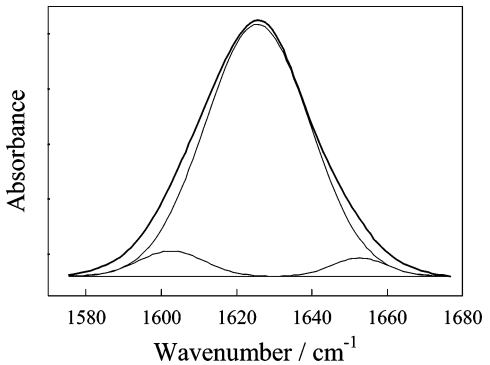

Figure 1. Amide I′ band of PNiPA at 25 °C and 0.1 MPa. The curves underneath the band are obtained by Gaussian curve-fitting.

at higher pressure, show that this effect is due to a loss of intensity at ~1626 cm^{-1} and the appearance of a new peak at ~1606 cm^{-1} (Figure 2B). The latter peak shifts to a lower wavenumber (1601 cm^{-1}) as pressure is further increased. A red shift of the C═O peak frequency is usually due to the formation of a hydrogen bond because the hydrogen bond reduces the electron density of the carbonyl C═O bond and thus its frequency.[13] This suggests that the new peak is a consequence of the formation of a second hydrogen bond since the peak at ~1626 cm^{-1} already represented a hydrogen-bonded carbonyl group. The second hydrogen bond will be formed with water instead of another polymer molecule as intermolecular polymer−polymer interaction can be excluded on steric grounds.[16] The formation of an additional hydrogen bond to water is not uncommon in proteins where a similar shift of ~20 cm^{-1} is observed for solvent-exposed amides of protein α-helices.[18] Such a shift is far greater than expected for the simple physical effect of pressure or temperature on the C═O vibration ($|\Delta\nu|$ = 4.8 cm^{-1}/263 K).[19] Note that the band at ~1626 cm^{-1} does not provide information on the relative ratio of C═O···D−O−D to C═O···D−N hydrogen bonds or any change therein under pressure.

Figure 2C shows a plot of the band shift as a function of pressure. The transition midpoint is found at 300 ± 20 MPa, and the volume change associated with the process is −55 ± 1.5 mL mol^{-1}, which is comparable with the volume changes observed for protein unfolding.[20] The transition is reversible with no apparent hysteresis. However, a hybrid two-dimensional correlation analysis reveals that a hysteresis does exist, as in the case of the thermally induced phase separation.[21] The pressure-induced phase separation was detected in situ by light microscopy simultaneously with the acquisition of FTIR spectra. Interestingly, the phase separation is observed at 168 ± 50 MPa, which corresponds to the onset of the transition observed by FTIR spectroscopy (Figure 2C). This is similar to DSC experiments where the onset temperature of the endothermic peak corresponds to the lower critical solution temperature (LCST).[14] Likewise, NMR relaxation experiments indicate a broad transition interval[12] compared to the rather sharp changes commonly seen when plotting the turbidity of the polymer solution. This phenomenon is due to the fact that the initial phase separation involves only a fraction of the polymer chains that is too small to be detected by FTIR spectroscopy. As the pressure further increases, more chains will undergo phase separation and the concentration of the polymer in the polymer-rich phase will increase.[22,23] Thus, the infrared spectral

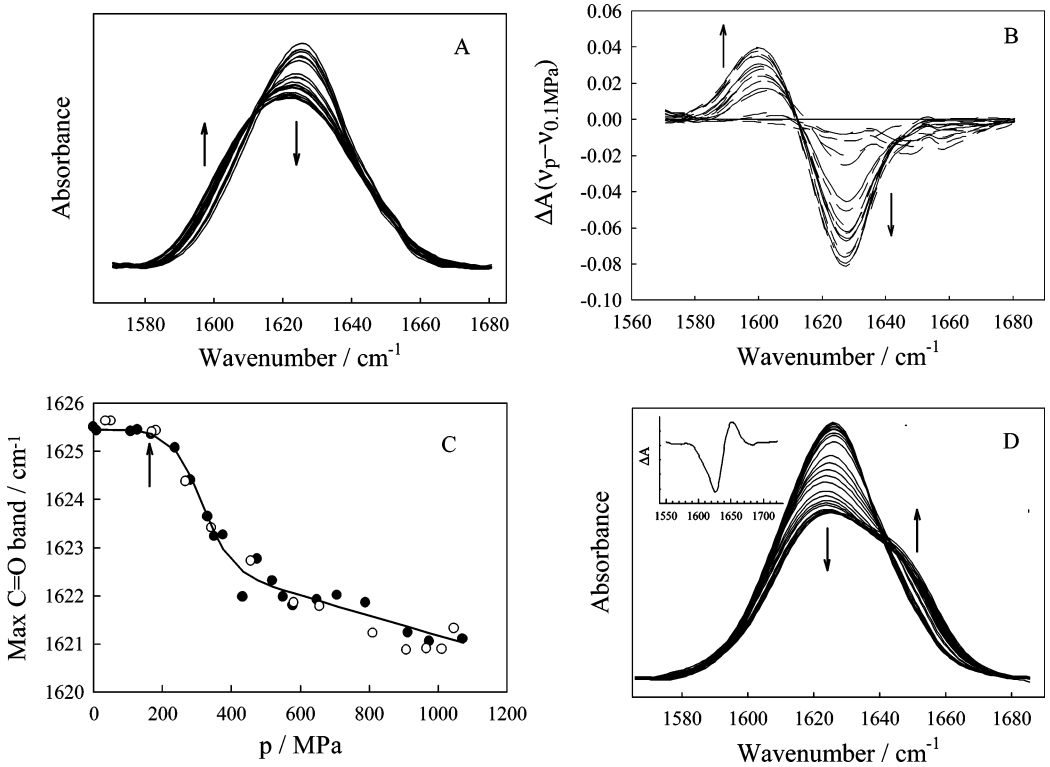

Figure 2. Spectral variations in the C=O stretching band of PNiPA as a function of pressure at 25 °C (A) and the corresponding difference spectra (B). The arrows indicate the direction of the changes as pressure increases. (C) Plot of the position of the amide I′ band maximum as a function of pressure (●, compression; ○, decompression). The arrow indicates the LCSP (as observed by light microscopy). (D) The C=O band changes as a function of temperature at 0.1 MPa. The inset shows the difference spectrum of the amide I′ band at 53 °C and the amide I′ band at 25 °C.

changes report on what triggers the collapse of the polymer chains. The system remains phase separated at high pressure (1073 MPa).

In contrast to pressure, the intensity loss at ~1626 cm^{-1} is accompanied by the formation of a peak at ~1652 cm^{-1} when the temperature is raised (Figure 2D, at 0.1 MPa). The latter is characteristic of the free, non-hydrogen-bonded carbonyl.[14] This demonstrates that the temperature-induced coil-to-globule transition is accompanied by a partial loss of hydrogen bonds involving the carbonyl group.[13] Similar to pressure the LCST corresponds to the onset of the observed transition (T_{onset} = 33 °C).

The differences between temperature and pressure effects are also observed in the amide II′ band where a temperature increase causes a red shift of the band and pressure induces a slight blue shift (data not shown). The opposite shift of the amide I′ and II′ bands can be explained by the nature of the underlying vibration. The amide II′ absorption band originates mainly from the N−H bending vibration (Table 1). Here hydrogen bond formation will increase the force constant of the vibration and thus shift the amide II′ to a higher wavenumber and vice versa when a hydrogen bond is broken.[13]

Pressure- vs Temperature-Induced Changes in the C−H Stretching Bands. A number of experimental studies and quantum mechanical calculations have shown that the C−H stretching band of an alkyl group undergoes a blue shift when the alkyl group interacts with water, forming a weak C−H···O hydrogen bond.[24−28] The blue shift indicates a strengthening of the C−H···O hydrogen bond and originates from a shortening of the C−H bond length. This behavior is opposite to that of the classical hydrogen bond, and its underlying mechanism is not well understood.[25,26] Thus, IR spectra of the C−H stretching regions can provide important information concerning the hydration of alkyl groups in PNiPA. The various C−H stretching absorption bands in the infrared spectrum and their assignments are summarized in Table 1.

Table 1. Observed Infrared Band Positions and Assignments of PNiPA in D_2O[13,14]

band position (cm^{-1})	assignment
2982	antisymmetric C−H stretching[a]
2940	antisymmetric C−H stretching of $-CH_2$[b]
2882	symmetric C−H stretching[a]
1626	amide I′ (C=O stretching)
1450	amide II′ (N−H bending)
1391	CH_3 symmetric deformation[a]
1371	CH_3 symmetric deformation[a]
1133	CH_3 rocking[a]

[a] Vibrational mode associated with the methyl group of the isopropyl group. [b] Vibrational mode associated with the backbone.

The effects of pressure and temperature on the C−H stretching vibrations are compared in Figure 3. Increasing the temperature above the LCST causes a (cooperative) red shift of the C−H stretching bands arising from both the isopropyl group in the side chain and the backbone CH_2 without introducing any significant changes in the band shape (Figure 3A). The red shift results from a dehydration of the hydrophobic moiety, mainly the isopropyl group.[13]

Conversely, when pressurized, the peaks display a linear shift to a higher frequency with a discontinuity around the lower critical solution pressure (LCSP) (Figure 4). The slopes ($d\nu/dp$) of the lines before and after the discontinuity are on the order of 5−14 cm^{-1}/GPa. These values are similar to those found for 1-acetamido-3-(2-pyrimidinyl)imidazolium bromide and

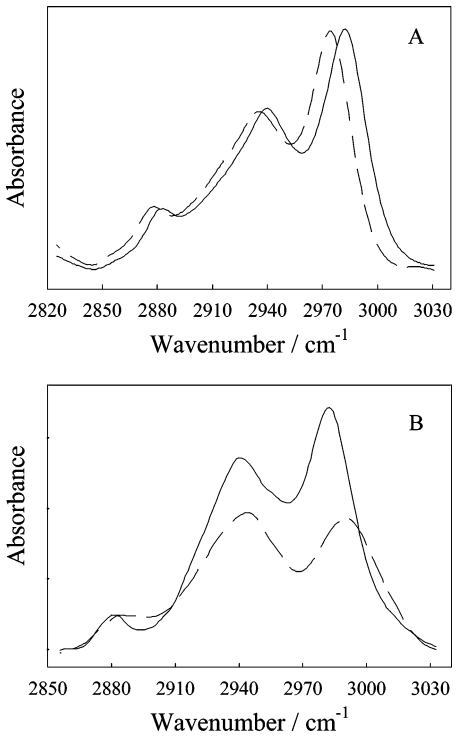

Figure 3. IR absorption spectrum of PNiPA in the C−H stretching region (2850−3040 cm^{-1}): (A) temperature effect at 25 and 53 °C and (B) pressure effect at 0.1 and 1073 MPa. Solid lines represent the low-pressure/temperature conditions.

tert-butyl alcohol and were suggested to be indicative of a strengthening of the C−H···O hydrogen bonds with increasing pressure.[27,29] The discontinuity corresponds to the transition region (168−400 MPa) observed in the carbonyl vibration mode (Figure 2) and suggests that the C−H stretching vibration does not respond to pressure in a homogeneous manner in this pressure region, as discussed below. In addition, pressure distorts the band shape by diminishing the peak intensity and broadening the peaks (Figure 3B). The decreased intensity results from the formation of new C−H···O hydrogen bonds[25] whereas the broadening is indicative of an increased inhomogeneity in the population. The latter supposedly arises from a reorganization of the hydrogen bond network and/or geometry.[30] This conclusion is reenforced by the sharp contrast with the thermal effect which merely induces a red shift that reflects a dehydration of the hydrophobic moiety.[13] A similar pressure effect has been observed previously for *tert*-butyl alcohol in aqueous solution and was interpreted in terms of a broader distribution of *tert*-butyl alcohol molecules with variable degrees of hydration at high pressure.[27] The isopropyl group deformation band undergoes similar changes (Figure 5).

Discussion

We have investigated the effect of pressure on the hydration properties of PNiPA in aqueous solution. By monitoring the behavior of the C=O and C−H stretching bands, it is demonstrated that pressure and temperature have an antagonistic effect on the hydration. Raising the temperature induces the well-known dehydration of both the hydrophobic and hydrophilic groups, thereby causing collapse and aggregation of the polymer chains, whereas under pressure the polymer chain becomes more hydrated. This is consistent with a recent molecular dynamics simulation that demonstrated that

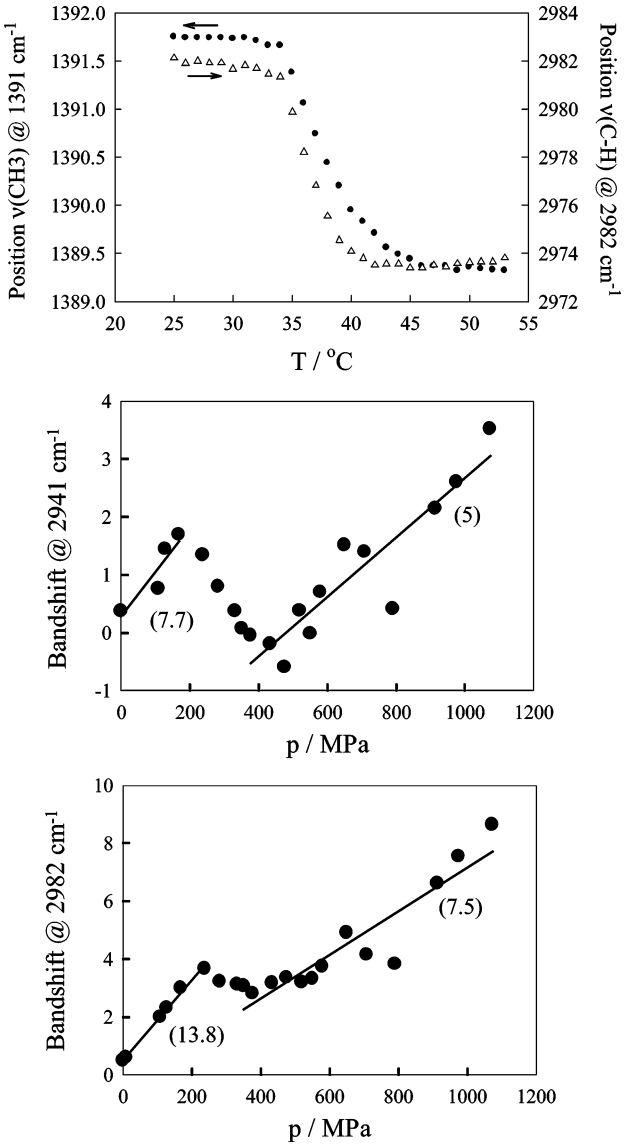

Figure 4. Effect of temperature and pressure on the position of the C−H stretching peaks. Numbers in brackets represent the slopes (cm^{-1}/GPa) calculated by linear regression. The band assignments are given in Table 1.

the average number of water molecules surrounding a hydrophobic polymer site increases with pressure and that the hydrated state of a polymer chain is stabilized by the energetically favorable state of water in the first hydration shell surrounding the hydrophobic groups.[7] Indeed, pressure is known to destabilize hydrophobic contacts by stabilizing a solvent-separated pair of hydrophobic groups relative to the contact pair.[10,31] Our finding is also in agreement with Otake et al., who found that the specific volume of a PNiPA solution does not change as a function of pressure.[13] (A positive volume change was anticipated due to the release of hydration water surrounding the hydrophobic groups if the hydrophobic interaction were important.) Thus, as the polymer becomes more hydrated at higher pressures, it seems unlikely that it will collapse into a globule at all. It has been shown for systems such as polystyrene in tetrahydrofuran and partially hydrolyzed polyacrylamide in water that the polymer chain dimensions are often independent of pressure under good solvent conditions.[32] Our conclusion is also corroborated by the fact that proteins can be unfolded under pressure, indicating

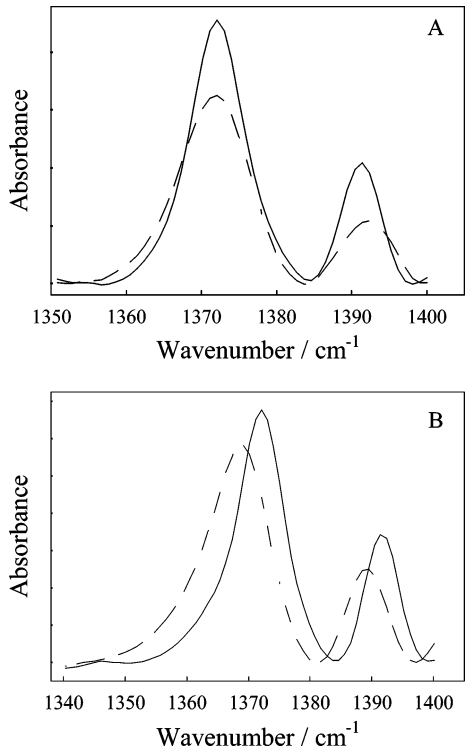

Figure 5. IR absorption spectrum of PNiPA in the $-C(CH_3)_2$ deformation region (1350−1400 cm^{-1}): (A) pressure effect at 0.1 and 1073 MPa and (B) temperature effect at 25 and 53 °C. Solid lines represent the low-pressure/temperature conditions.

that the unfolded (hydrated) state has a lower volume than the native state. We have previously shown that pressure and cold unfolding of proteins are mechanistically similar processes.[33] Since the coil-to-globule transition represents the inverse of the cold unfolding, it is not surprising that pressure does not induce such a transition.

In a recent study van Zanten and co-workers observed the phase separation of poly(ethylene-*co*-1-butene) in dimethyl ether under high-pressure conditions where the polymer−solvent interaction was favorable.[34] These authors concluded that the observed behavior should be attributed to the compressibility of the solution rather than to polymer−polymer interactions. It is likely that in the present case changes in water−water interactions are responsible for the phase separation of PNiPA in water at high pressure. Computational studies have shown previously that water−water interactions provide an important driving force for the behavior of polymer chains.[6−8] In addition, changes in the hydrogen bond structure of water are important in determining the strength of the hydrophobic interaction and explain why the hydrophobic interaction is destabilized at high pressure.[35] It is noteworthy that other water-soluble polymers such as poly(ethylene oxide) and poly(*N*-vinyl-2-pyrrolidone) do undergo a chain collapse with increasing pressure, albeit that the decrease in radius is rather small.[36,37] This suggests that the nature of the polymer determines to what extent the solvent can pack around its chain as the solvent density changes.

Spectral Changes of PNiPA Are Reminiscent of Protein Unfolding. It has not gone unnoticed that the opposite peak shifts seen here for an increase in pressure or temperature in the amide I′ region are also observed for proteins during thermal and pressure unfolding[38,39] or folding as in the case of a coillike elastin-derived peptide.[40] The thermodynamic[41,42] and spectroscopic (this work) similarities suggest that the underlying molecular interactions are the same. This suggests that the pressure- or temperature-induced spectroscopic changes in the amide I′ band of proteins cannot be purely interpreted in terms of conformational changes and that there is an influence of the hydration state. This is supported by a recent molecular simulation study on the unfolding of a α-helical peptide.[43]

Conclusion

Macroscopically pressure and temperature both induce a phase separation of PNiPA in aqueous solution above the LCSP and LCST, respectively. However, on the microscopic level the effects of pressure and temperature are very different. Increasing the temperature above the LCST causes a dehydration of the hydrophilic and hydrophobic moieties, enabling the latter to be involved in intra- and intermolecular interactions within and between polymer chains. By contrast, high-pressure further enhances the hydration of the hydrophilic amide group and strengthens the C−H···O hydrogen bonds between the hydrophobic alkyl groups and water. It is suggested that under pressure the polymer chain remains in a coillike conformation. Presumably, the origin of the phase separation is to be found in the changes in solvent−solvent interaction, the analysis of which will be the focus of future work.

We have also hinted at the resemblance in spectral behavior of PNiPA and proteins, suggesting that the spectral changes observed by FTIR spectroscopy may contain a significant contribution from changes in hydration in addition to structural changes. As such, PNiPA is a good model for the study of the hydration properties of proteins as a function of a physical or chemical perturbation. It has the advantage that the spectroscopic properties can be easily interpreted in terms of hydrogen bonding without the interference of conformational changes that contribute to the complexity of the amide I′ band of proteins.

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