THE JOURNAL OF PHYSICAL CHEMISTRY B

Article

pubs.acs.org/JPCB

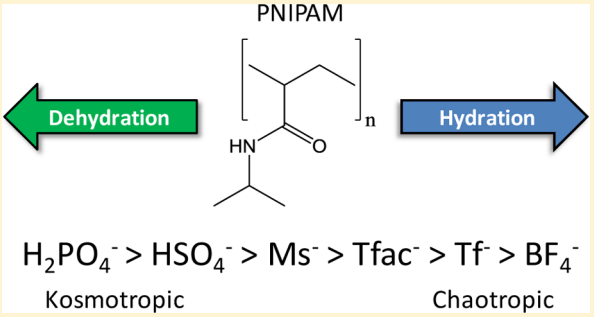
Phase Transition of Poly(*N*-isopropylacrylamide) in Aqueous Protic Ionic Liquids: Kosmotropic versus Chaotropic Anions and Their Interaction with Water

Natalie J. Debeljuh,[†] Alessandra Sutti,[‡] Colin J. Barrow,[†] and Nolene Byrne*[,‡]

[†]School of Life and Environmental Sciences, [‡]Institute for Frontier Materials, Deakin University, Geelong, Victoria 3216, Australia

Supporting Information

ABSTRACT: We have investigated the influence of a series of triethylammonium-based protic ionic liquid−water solutions on the lower critical solution temperature (LCST) of poly(*N*-isopropylacrylamide) (PNIPAM). We find that kosmotropic anions lower the LCST of PNIPAM more dramatically when compared with chaotropic anions. In addition, we have probed the solvent properties of the hydrated protic ionic liquid solutions using ^{1}H NMR, polarity measurements, and solvatochromic analysis of the Kamlet−Taft parameters, β and π^*. We find that the hydrogen bond character—more specifically, the interactions between water and pIL—is the dominant parameter responsible for lowering the LCST of PNIPAM. We have added choline dihydrogen phosphate (choline dhp) into this study on the basis of positive results from previously reported protein folding studies using this ionic liquid.



INTRODUCTION

Ionic liquids, ILs, consist entirely of ions with a melting point below 100 °C and are described as designer solvents because of their unlimited design flexibility. These unique liquids find application in a diverse range of fields from electrolyte[1] to biomolecule stabilization.[2] Previous studies have shown that ILs can dramatically increase the shelf life of certain proteins,[3] including the ability to highly concentrate the amount of protein present in the solution, as well as enhance thermal stability[2,4] and aid in refolding kinetics.[5] Our laboratory has been exploring the use of protic ionic liquids (pILs) for stabilization and modification of biomolecules, namely proteins.[3,6] PILs are a subclass of the IL[7] family formed by the neutralization of a Brønsted acid with a Brønsted base[8] and have an additional tunable feature as a result of the proton transfer, the proton activity.[6a] The proton activity can be linked to the pH of the pIL and has been used as a relative scale to select appropriate pILs for organic transformation reactions[9] and the solvation of biomolecules.[10] However, the proton activity is not the only parameter that determines protein stability and solubility.

Recently, the Hofmeister series has been used to describe the influence of ILs on biomolecule stabilization, in which the major attention has been centered on imidazolium-based cations in the aprotic IL family. Generally, the Hofmeister series is used to describe the impact salts have on protein solubility.[11] Salts can be described as either kosmotropes or chaotropes: kosmotropes are thought to enhance protein solubility and chaotropes reduce protein solubility.[12] The mechanism by which salts alter protein solubility has been linked to the ability of the salt to "make" or "break" water structure;[13] however, this explanation does not always explain observations, and other factors, such as specific ion binding and relative polarizabilities, have been implicated.[14] Recently, we studied the fibrilization of the Alzheimer's peptide, $A\beta_{16-22}$.[15] Fibrilization is directly linked to protein destabilization, and in the case of $A\beta_{16-22}$, destabilization of the peptide results in the formation of amyloid fibrils, which is a direct model for amyloid formation in Alzheimer's disease. We found that the stabilization of $A\beta_{16-22}$ monomers follows a reverse Hofmeister trend, in which kosmotropic anions enhance the fibrilization process. It therefore becomes important to understand which solvent parameters are contributing to this observation and how this can be used to correctly select appropriate pILs for biomolecule stabilization.

Poly(*N*-isopropylacrylamide), PNIPAM, is a thermosensitive polymer that has previously been shown to follow the Hofmeister trend, and the phase transition of this polymer is often used to describe protein folding/refolding processes.[16] It is well-known that PNIPAM develops a lower critical solution temperature (LCST) at 32 °C in aqueous solution,[17] which is the result of the polymer's switching from a soluble hydrophilic random coil at low temperatures to an insoluble hydrophobic globule at high temperatures.

Numerous studies have investigated ionic liquid interaction by solvatochromic analysis of the Kamlet−Taft parameters.[18]

Received: May 1, 2013
Revised: June 11, 2013
Published: June 12, 2013

dx.doi.org/10.1021/jp4043232 | *J. Phys. Chem. B* 2013, 117, 8430−8435

Lee et al.[19] describe how distinct variations in the hydrogen bond acceptor (HBA) basicity, β, and hydrogen bond donator (HBD) acidity, α, arise from modifying the methyl group on pyridinium-based ionic liquids. Furthermore, Nayak et al.[20] were able to measure the degree of hydrogen bonding and ion pairing within their ionic liquid systems in relation to how ionic liquids interact with polymers.

In this context, our aim was to study the influence of a series of aqueous triethylammonium-based pILs on the LCST of PNIPAM. We have included choline dihydrogen phosphate (choline dhp) because this ionic liquid has been extensively studied in relation to protein stabilization. In addition, we discuss the other key solvent parameters, namely, hydrogen bond character and polarity, and scale these relative to the observations we observe with respect to biomolecule stabilization.

EXPERIMENTAL SECTION

Materials. Triethylamine (99%), choline hydroxide (45%), trifluoroacetic acid (99%), triflic acid (98%), hydrogen tetrafluoroboric acid (48%), mesylate (99.5%), dihydrogen phosphoric acid (99%), sodium hydrogen sulfate (98%), Nile Red (99%), 4-nitroaniline (99%), and deuterium oxide (99%) were purchased from Sigma Aldrich. *N*,*N*-diethyl-4-nitroaniline (99%) was purchased from Novachem. Poly(*N*-isopropylacrylamide) (98%) was purchased from Polysciences. All pIL dilutions were prepared using in-house Milli-Q water unless otherwise stated.

Synthesis of Protic Ionic Liquids. pILs were prepared as previously described.[8] The pILs synthesized include triethylammonium dihydrogen phosphate ($TeaH_2PO_4$), triethylammonium hydrogen sulfate ($TeaHSO_4$), triethylammonium mesylate (TeaMs), triethylammonium trifluoroacetate (TeaTfac), triethylammonium triflate (TeaTf), triethylammonium tetrafluoroborate ($TeaBF_4$), and choline dihydrogen phosphate (choline dhp). All pILs were thoroughly dried prior to use.

LCST Measurements. LCST measurements were collected by dynamic light scattering (DLS) on a Zetasizer Nanoseries (Malvern Instruments) equipped with a thermostatted sample chamber for maintaining the desired temperature within a temperature range of 0−70 °C. A 500 μL portion of sample containing 0.5% (w/w) PNIPAM was introduced into a disposable cuvette and parafilmed to eliminate solvent evaporation. Samples were stored on ice prior to analysis and were equilibrated at each temperature interval for 3 min over 64 accumulations per temperature point. The temperature was increased by increments of 0.3 °C.

^{1}H NMR Chemical Shift. NMR spectra were recorded on a commercial Bruker Ascend 500 MHz FT-NMR spectrometer. pIL samples were prepared in deuterium oxide and measured at 298 K. Proton chemical shifts were referenced relative to tetramethylsilane (TMS) in D_2O (internal capillary), and data were processed using Bruker software version 3.1.

Polarity Measurements. Because of the nature of the triethylammonium cation in our pILs, Reichardt's dye could not be used to determine pIL polarity.[21] Instead, polarity measurements were carried out using Nile Red. The limited ability of Nile red to sense the local polarity within ionic liquids is noted.[22] A stock solution of Nile Red (0.1 mol/L) was prepared in methanol and sonicated prior to use. A 10 μL portion of the stock solution was transferred to each pIL solution and allowed to evaporate under vacuum for 2 h. The λ_{max} of each pIL was measured at 298 K in a quartz cuvette by UV−vis spectrophotometry. The molar transition energy of Nile Red (E_{NR}) was calculated according to eq 1,

$$E_{NR} = \frac{hcN_A}{\lambda_{max}} \times 10^6 \quad (1)$$

where h is Planck's constant, c is the speed of light, N_A is Avogadro's number, and λ_{max} is the wavelength of maximum absorption of Nile Red.

Determination of β and π^* Kamlet−Taft Parameters. Stock solutions of 4-nitroaniline (1.2 mol/L) and *N*,*N*-diethyl-4-nitroaniline (1.2 mol/L) were prepared in methanol and sonicated prior to use. A 10 μL portion of the stock solution was transferred to each pIL solution and allowed to evaporate under vacuum. The λ_{max} of each sample was measured at 298 K in a quartz cuvette by UV−vis spectrophotometry. The hydrogen bond acceptor (HBA) basicity β values were determined according to eq 2,

$$\beta = \left(\frac{1.035\nu_{max}(\mathbf{2})}{1000\ \mathrm{cm}^{-1}}\right) - \left(\frac{\nu_{max}(\mathbf{1})}{1000\ \mathrm{cm}^{-1}}\right) + 2.64/2.8 \quad (2)$$

where $\nu_{max}(\mathbf{1})$ and $\nu_{max}(\mathbf{2})$ are the wavenumbers at maximum absorbance for 4-nitroaniline (**1**) and *N*,*N*-diethyl-4-nitroaniline (**2**) respectively.[23] Values of polarizability π^* were calculated according to eq 3,

$$\pi^* = \left(\frac{\nu_{max}(\mathbf{2})}{1000\ \mathrm{cm}^{-1}}\right) - 27.52/-3.182 \quad (3)$$

where $\nu_{max}(\mathbf{2})$ is the wavenumber at maximum absorbance for *N*,*N*-diethyl-4-nitroaniline (**2**).[24] Experimental conditions were optimized with methanol and water, and the values obtained were in good agreement with the literature[25] (see Table S1, Supporting Information). Random samples were selected for reproducibility of UV−vis measurements, and mean deviations for β and π^* parameters were calculated to be ±0.02.

RESULTS AND DISCUSSION

The LCST for PNIPAM as a function of pIL concentration is shown in Figure 1.

It is seen that the hydrated pILs $TeaH_2PO_4$, $TeaHSO_4$, TeaTfac, and TeaMs all reduce the LCST as the concentration of the pIL increases. The choice of pIL anion on the LCST is dramatic, with a 20 °C difference observed between $TeaH_2PO_4$

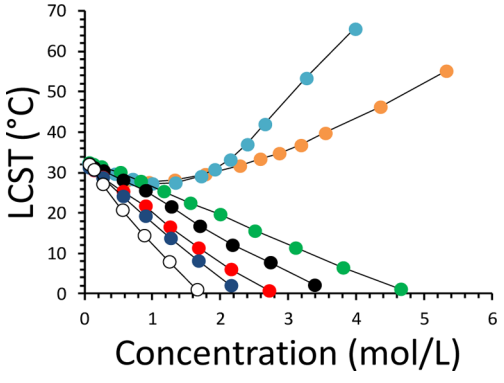


Figure 1. Lower critical solution temperatures of 0.5% (w/w) aqueous PNIPAM solution as a function of pIL anion; $TeaBF_4$ (pale blue), TeaTf (orange), TeaTfac (green), TeaMs (black), $TeaHSO_4$ (red), $TeaH_2PO_4$ (blue) and choline dhp (white).

dx.doi.org/10.1021/jp4043232 | J. Phys. Chem. B 2013, 117, 8430−8435

and TeaTfac at a fixed pIL concentration of 2 mol/L. Choline dhp has the greatest effect on the LCST of PNIPAM, indicating that choline dhp is the most effective at stabilizing the globular form. In comparison with aprotic ionic liquid studies involving PNIPAM, this trend is very different. For example, Nayak et al.[20] show that the aprotic ionic liquid BMIMOAc and $BMIMBF_4$ abruptly increase the LCST of PNIPAM at an ionic liquid volume fraction of 0.25 and 0.1, respectively. In our case, as the pIL concentration of $TeaH_2PO_4$, $TeaHSO_4$, and TeaMs continues to increase, we never see a flip in the phase transition temperature.

Interestingly, we observe this trend for all the fluorinated pILs investigated here. Both TeaTf and $TeaBF_4$ raise the LCST above that of PNIPAM in water, with $TeaBF_4$ having the greatest effect. TeaTfac also increases the LCST temperature above that in water, but requires concentrations >10.8 mol/L (data not shown). Previous reports using aprotic ILs with the BF_4^- anion[20,26] have reported an increase in the LCST and the presence of a UCST. We did not observe a UCST within the temperature range studied or across the entire pIL-water phase diagram for any of the fluorinated pILs investigated here. Wang et al.[27] previously reported that the LCST of PNIPAM in the aprotic IL $BMIMBF_4$ disappears at higher IL concentrations. This observation was attributed to the increase in intramolecular and intermolecular hydrogen bonds between the IL−water and polymer complex, which enhances the polymers stability at higher temperatures. Since we observe a similar increase in PNIPAM LCST as a function of fluorinated pIL concentration, we also agree with this explanation and further suggest that this stability exhibited by PNIPAM at higher temperature is caused by the increased hydrophobicity of the pIL, in which surrounding water molecules are being pulled away from the polymer.

Next, we turned our attention to the hydrogen bond interactions between water and pILs using ^{1}H NMR chemical shift analysis. Figure 2 shows the shift in the D_2O peak as a function of pIL.

It can be seen that the shift in the D_2O peak as a function of added pIL is very different, depending on the choice of pIL. The kosmotropic anions, $H_2PO_4^-$ and HSO_4^-, have the greatest impact on water. A significant downfield shift for D_2O is measured as a function of pIL, with $TeaH_2PO_4$ resulting in the greatest downfield shift for D_2O. The interaction of water and $TeaH_2PO_4$ is dominated by the anion, as measured by the significant shift observed for the $H_2PO_4^-$ anion probed using ^{31}P (see Figure S1, Supporting Information).

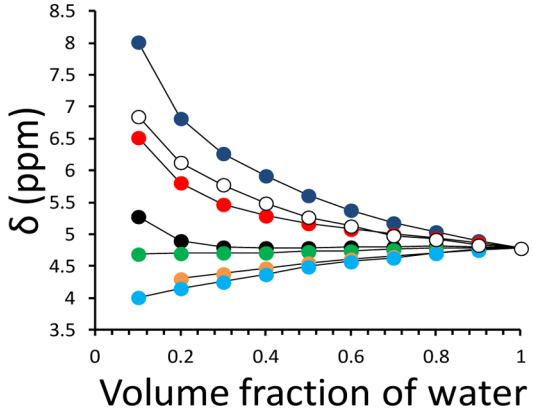


Figure 2. Proton chemical shift of water as a function of pIL concentration. $TeaH_2PO_4$ (blue), choline dhp (white), $TeaHSO_4$ (red), TeaMs (black), TeaTfac (green), TeaTf (orange), and $TeaBF_4$ (pale blue).

Interestingly, little to no shift for the D_2O peak is observed with increasing TeaMs concentration. This suggests that the water-pIL hydrogen network is dependent on the choice of pIL, with this difference being reflected in the LCST of PNIPAM. The pILs with the greatest impact on water (kosmotropic) lower the LCST of PNIPAM more effectively. It should be noted that although choline dhp has the greatest impact on lowering the LCST of PNIPAM, the shift in the D_2O peak is slightly less than that measured for $TeaH_2PO_4$. The enhanced lowering of the LCST of PNIPAM in the presence of choline dhp can be best explained by the combined influence of the kosmotropic $H_2PO_4^-$ anion and the hydroxyl group on the cation. In our experiment, we observed a slight upfield shift of the D_2O peak with increased TeaTf and $TeaBF_4$ concentrations. It is not surprising that TeaTf and $TeaBF_4$ display weak water interactions because of their strong hydrophobic character. This is reflected also on the LCST of PNIPAM, with the hydrophobic anions increasing the LCST. The shift of water in the presence of the acid precursors was measured (see Figure S2, Supporting Information). In all cases, water was shown to shift downfield, with the largest shift observed for the methanesulfonic acid, and the least shift for the phosphoric acid. This trend is opposite that of the pILs−water trend, suggesting that the water−pIL interactions are different from the acid−water interactions, most likely because of the neutralization of the acid proton, which is available to interact with water in the acid precursor state.

The polarizability of each pIL solution was measured using the *N*,*N*-diethyl-4-nitroaniline dye. It has been reported that when the charge surrounding the anion of an IL becomes delocalized over more atoms, the polarizability (π^*) decreases as a result of a decrease in the strength of the Coulombic interactions between the solute and dye.[18a] Figure 3a and b show the Kamlet−Taft π^* values of the nonfluorinated and fluorinated pILs, respectively.

At low water content, all pILs measured a π^* value similar to that observed for nonaqueous molecular solvents and aprotic ILs.[18a,19,25b,28] Both hydrophobic character and size of the anion is known to impact the polarizability values. We observe that as the hydrophobic character of the pIL is increased, the polarizability of the solution is decreased. It is also observed that smaller tetrahedral anions, such as $H_2PO_4^-$, HSO_4^-, BF_4^-, and Ms^- have a larger π^* value, which is in agreement with previous trends.[29] As the water content is increased, the value for π^* changes significantly, depending on the anion in the pIL. Both $TeaH_2PO_4$ and $TeaHSO_4$ have π^* values similar to water at $\phi_{water} > 0.3$, suggesting a waterlike environment, even in the concentrated pIL state. A study by Cammarata et al.[30] describes that depending on the type of anion, water either interacts strongly or is present largely as bulk water. Since water involves icelike structured pools and disordered zones, the addition of a highly ordered anion such as $H_2PO_4^-$ or HSO_4^- may cause the unordered zones to become more ordered.[6b] This, being the result of hydrogen bonding between the anion and water molecules, could suggest why we see a waterlike behavior in the kosmotropic pILs, which becomes more evident in higher pIL concentrations.

TeaMs shows a clear step change at $\phi_{water} > 0.8$, and at these concentrations, the system becomes more waterlike. Both TeaTf and $TeaBF_4$ show low polarizabilities compared with

dx.doi.org/10.1021/jp4043232 | J. Phys. Chem. B 2013, 117, 8430−8435

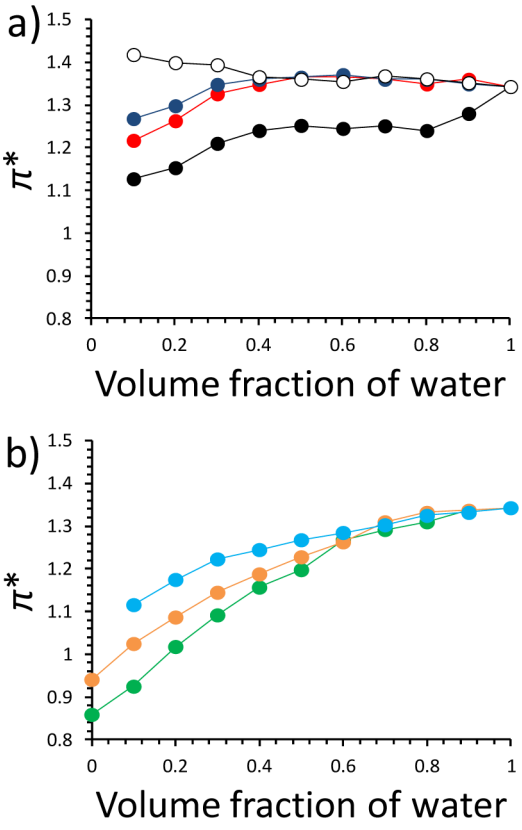


Figure 3. Kamlet–Taft π^* parameter for (a) nonfluorinated pILs TeaMs (black), $TeaHSO_4$ (red), $TeaH_2PO_4$ (blue), and choline dhp (white) and (b) fluorinated pILs $TeaBF_4$ (pale blue), TeaTf (orange), and TeaTfac (green).

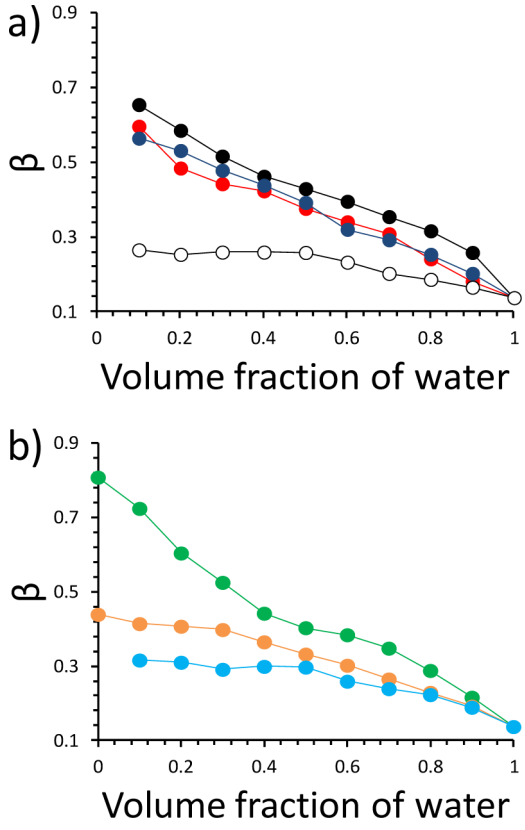


Figure 4. Kamlet–Taft β parameter for (a) nonfluorinated pILs TeaMs (black), $TeaHSO_4$ (red), $TeaH_2PO_4$ (blue), and choline dhp (white) and (b) fluorinated pILs $TeaBF_4$ (pale blue), TeaTf (orange), and TeaTfac (green).

other pILs in this study, supporting the weak water interactions measured by ^{1}H NMR. Choline dhp shows the highest polarizability, and at high concentrations, the polarizability is greater than water. This is an interesting outcome and will be the subject of further investigations. It should be noted that Zhang et al. have shown that NTf2-containing nonprotic-bearing multiple hydroxyl groups have polarities similar to the pILs reported here.[31]

The HBA basicity of each pIL as a function of water is shown in Figure 4a and b for the nonfluorinated and fluorinated pILs, respectively. The hydrogen-bond-accepting (HBA) basicity of a solvent can be measured by the Kamlet–Taft parameter, β. The β parameter describes the hydrogen-bond-accepting (HBA) basicity of the cation with the solvatochromic probe dye and is largely determined by the nature of the anion.[29] Generally, large β values are associated with a strong basic character of the cation toward the probe dye. The hydrogen-bond-donating (HBD) α acidity values could not be obtained because of the nature of the triethylammonium cation.[21]

From Figure 4a, we see a very small difference in the HBA basicity of the Tea^+ cation when coupled to the $H_2PO_4^-$, HSO_4^-, and Ms^- anions. The β values increase linearly in all three pILs as the volume fraction of water is reduced. The larger β value at ϕ_{water} = 0.1 implies that Tea^+ has a greater tendency to accept protons from the probe dye when coupled with Ms^- than with $H_2PO_4^-$. This was interesting since the ΔpK_a is lower for $TeaH_2PO_4$. We also investigated the HBA for the primary and secondary amines ethylammonium and diethylammonium with the Ms^- anion (see Figure S3, Supporting Information) and found that the primary amine has a lower β value across the dilution series. This implies that EaMs is more protonated than the tertiary TeaMs, which is in agreement with previous work completed by Stoimenovski et al.[32] The low β parameter measured for choline dhp is consistent with aprotic ionic liquid values,[18a,19,28,29,33] suggesting strong ion interactions.

The β values for fluorinated pILs TeaTfac, TeaTf and $TeaBF_4$ (Figure 4b) show a much greater spread at $\phi_{water} > 0.5$. The largest β value in our study was measured for TeaTfac, which indicates that the Tea^+ cation strongly accepts a proton from the probe dye. This implies that this pIL is the least protonated in the series investigated here. As the hydrophobic character of these pILs increases from $Tfac^-$ to BF_4^-, we see that the Tea^+ cation becomes a weaker hydrogen bond acceptor.

In relation to PNIPAM, low β values have been associated with weak anion interactions with the amide of PNIPAM.[20] In our study, TeaMs displays a larger β value than $TeaH_2PO_4$ and $TeaHSO_4$, supporting the LCST trend observed. The $Tfac^-$ anion is also more likely to interact with PNIPAM because its conjugate acid is interacting strongly with the probe dye. There have been several instances in which ILs designed with the BF_4^- anion have demonstrated low β values.[20,34] The interaction between BF_4 and PNIPAM is considered weak because of competitive hydrogen bonding between the cation and BF_4 for the polymer. In our case, the low β values measured for $TeaBF_4$ is evidence of this weak anion–PNIPAM interaction.

Finally, solvent polarity is also known to play an important role in influencing the LCST of PNIPAM,[35] so we have

dx.doi.org/10.1021/jp4043232 | J. Phys. Chem. B 2013, 117, 8430–8435

investigated the polarity of our pILs as a function of water using Nile Red. The molar transition energies (E_{NR}) were calculated from the λ_{max} of each pIL according to eq 1. The E_{NR} values measured for $TeaHSO_4$ and $TeaH_2PO_4$ suggest they have a polarity similar to that of water (201.7 kJ mol^{-1}),[36] whereas TeaTfac can be compared to low-molecular-weight alcohols, such as ethanol (218.2 kJ mol^{-1})[36] (see Figure S4, Supporting Information). The small polarity variation measured in $TeaH_2PO_4$ and $TeaHSO_4$ as a function of added water further supports the strong kosmotropic nature of these anions and the strong water structuring effects, which exist for these pILs. The polarity of $TeaBF_4$ could not be numerically determined because of band-splitting of the main absorption band. Because of the long wavelength shift of Nile Red in $TeaBF_4$, we presume this pIL is more polar than $TeaHSO_4$ (see Figure S5, Supporting Information); however, it has been reported that band-splitting of the main absorption band is associated with low solvent polarity, such as in the case of cyclohexane.[37]

CONCLUSION

We found that triethylammonium pILs consisting of the kosmotropic anions $H_2PO_4^-$ and HSO_4^- were able to reduce the LCST of PNIPAM more effectively than the chaotropic anions. Choline dhp was shown to stabilize the globular form of PNIPAM the greatest. The fluorinated anions investigated here enhanced the chaotropic nature of the pILs. The pILs showed a trend similar to previous reports investigating aprotic ionic liquids, in which an increase in the LCST above that measured in water was observed only at increasing fluorinated pIL concentrations. The increase in the LCST when using fluorinated pILs is likely due to the presence of an enhanced hydration layer around the polymer caused by unfavorable water−pIL interactions. We explored the solvent properties of the hydrated pIL solutions and suggest that the water−pIL interactions are responsible for the differences observed in the LCST trend for the different pILs investigated here. ^{1}H NMR showed that the kosmotropic anions interact strongly with water, whereas no interaction was observed for the chaotropic anion. The hydrogen network of the hydrated pIL solution is likely the most important solvent property for protein stabilization.

ASSOCIATED CONTENT

Supporting Information

Additional figures regarding ^{31}P NMR chemical shifts, Kamlet−Taft β parameters, Nile Red polarity measurements as a function of water content, and UV−vis absorbance spectra. This material is available free of charge via the Internet at http://pubs.acs.org.

AUTHOR INFORMATION

Corresponding Author

*E-mail: nolene.byrne@deakin.edu.au.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

This research was supported by the Australian Research council and Deakin University.

REFERENCES

(1) (a) Galinski, M.; Lewandowski, A.; Stepniak, I. Ionic liquids as electrolytes. *Electrochim. Acta* **2006**, *51*, 5567−5580. (b) Gale, R. J.; Osteryoung, R. A. The electrical double layer at mercury in room temperature aluminum chloride: 1-butylpyridinium chloride ionic liquids. *Electrochim. Acta* **1980**, *25*, 1529.

(2) Fujita, K.; MacFarlane, D. R.; Forsyth, M. Protein solubilising and stabilising ionic liquids. *Chem. Commun.* **2005**, 4804−4806.

(3) Byrne, N.; Wang, L.-M.; Belieres, J.-P.; Angell, C. A. Reversible folding−unfolding, aggregation protection, and multi-year stabilization in high concentration protein solutions using ionic liquids. *Chem. Commun.* **2007**, *26*, 2714−2716.

(4) (a) Noritomi, H.; Minamisawa, K.; Kamiya, R.; Kato, S. Thermal stability of proteins in the presence of aprotic ionic liquids. *J. Biomed. Sci. Eng.* **2011**, *4*, 94−99. (b) Mann, J. P.; McCluskey, A.; Atkin, R. Activity and thermal stability of lysozyme in alkylammonium formate ionic liquids− influence of cation modification. *Green Chem.* **2009**, *11*, 785−792. (c) Baker, S. N.; McCleskey, T. M.; Pandey, S.; Baker, G. A. Fluorescence studies of protein thermostability in ionic liquids. *Chem. Commun.* **2004**, *8*, 940−941.

(5) (a) Lange, C.; Patil, G.; Rudolph, R. Ionic liquids as refolding additives: *N*′-alkyl and *N*′-(ω-hydroxyalkyl) *N*-methylimidazolium chlorides. *Protein Sci.* **2005**, *14* (10), 2693−2701. (b) Summers, C. A.; Flowers, R. A. Protein renaturation by the liquid organic salt ethylammonium nitrate. *Protein Sci.* **2000**, *9*, 2001−2008.

(6) (a) Angell, C. A.; Byrne, N.; Belieres, J.-P. Parallel developments in aprotic and protic ionic liquids: physical chemistry and applications. *Acc. Chem. Res.* **2007**, *40* (11), 1228−1236. (b) Debeljuh, N.; Barrow, C. J.; Henderson, L. C.; Byrne, N. Structure inducing ionic liquids−enhancement of alpha helicity in the Abeta(1−40) peptide from Alzheimer's disease. *Chem. Commum.* **2011**, *47*, 6371−6373. (c) Byrne, N.; Rodoni, B.; Constable, F.; Varghese, S.; Davis, J. H. Enhanced stabilization of the tobacco mosaic virus using protic ionic liquids. *Phys. Chem. Chem. Phys.* **2012**, *14* (29), 10119−10121. (d) Akanbi, T. O.; Barrow, C. J.; Byrne, N. Increased hydrolysis by *Thermomyces lanuginosus* lipase for omega-3 fatty acids in the presence of a protic ionic liquid. *Catal. Sci. Technol.* **2012**, *2* (9), 1839−1841.

(7) Wilkes, J. S.; Wasserscheid, P.; Welton, T. *Ionic liquids in synthesis*, 2nd ed.; Wiley-VCH: Weinheim: 2008; pp 1−6.

(8) Belieres, J.-P.; Angell, A. C. Protic ionic liquids: preparation, characterization and proton free energy level representation. *J. Phys. Chem. B* **2007**, *111*, 4926−4937.

(9) (a) Henderson, L. C.; Byrne, N. Rapid and efficient protic ionic liquid-mediated pinacol rearrangements under microwave irradiation. *Green Chem.* **2011**, *13*, 813. (b) Gordon, C. P.; Byrne, N.; McCluskey, A. A facile, protic ionic liquid route to *N*-substituted 5-hydroxy-4-methyl-3-oxoisoindoline-1-carboxamides and *N*-substituted 3-oxoisoindoline-4-carboxylic acids. *Green Chem.* **2010**, *12*, 1000−1006.

(10) Byrne, N.; Angell, A. C. Protein unfolding, and the "Tuning In" of reversible intermediate states, in protic ionic liquids. *J. Mol. Biol.* **2008**, *378*, 707−714.

(11) (a) Constantinescu, D.; Weingartner, H.; Herrmann, C. Protein denaturation by ionic liquids and the Hofmeister series: A case study of aqueous solutions of Ribonuclease A. *Angew. Chem., Int. Ed.* **2007**, *46*, 8887−8889. (b) Weingartner, H.; Cabrele, C.; Herrmann, C. How ionic liquids can help to stabilize native proteins. *Phys. Chem. Chem. Phys.* **2012**, *14*, 415−426.

(12) Hofmeister, F. Zur lehre der wirkung der salze. *Arch. Exp. Pathol. Pharmakol* **1888**, *24*, 247−260.

(13) Marcus, Y. Effect of ions on the structure of water: Structure making and breaking. *Chem. Rev.* **2009**, *109*, 1346−1370.

(14) Lo Nostro, P.; Ninham, B. W.; Milani, S.; Lo Nostro, A.; Pesavento, G.; Baglioni, P. Hofmeister effects in supramolecular and biological systems. *Biophys. Chem.* **2006**, *124*, 208−213.

(15) Debeljuh, N.; Barrow, C. J.; Byrne, N. The impact of ionic liquids on amyloid fibrilization of $A\beta_{16-22}$: tuning the rate of fibrilization using a reverse Hofmeister strategy. *Phys. Chem. Chem. Phys.* **2011**, *13*, 16534−16536.

(16) (a) Graziano, G. On the temperature-induced coil to globule transition of poly-*N*-isopropylacrylamide in dilute aqueous solutions. *Int. J. Biol. Marcomol.* **2000**, *27*, 89−97. (b) Ptitsyn, O. B.; Kron, A. K.; Eizner, Y. The models of the denaturation of globular proteins. II. Hydrophobic interactions and conformational transition in poly-(methacrylic acid). *J. Polym. Sci. Part C* **1968**, *16*, 3509−3517.
(17) Heskins, M.; Guillet, J. E. Solution properties of poly(*N*-isopropylacrylamide). *J. Macromol. Sci. Pure Appl. Chem.* **1968**, *2*, 1441−1455.
(18) (a) Crowhurst, L.; Mawdsley, P. R.; Perez-Arlandis, J. M.; Salter, P. A.; Welton, T. Solvent−solute interactions in ionic liquids. *Phys. Chem. Chem. Phys.* **2003**, *5*, 2790−2794. (b) Ab Rani, M. A.; Brant, A.; Crowhurst, L.; Dolan, A.; Lui, M.; Hassan, N. H.; Hallett, J. P.; Hunt, P. A.; Niedermeyer, H.; Perez-Arlandis, J. M.; Schrems, M.; Welton, T.; Wilding, R. Understanding the polarity of ionic liquids. *Phys. Chem. Chem. Phys.* **2011**, *13* (37), 16831−16840.
(19) Lee, J.-M.; Ruckes, S.; Prausnitz, J. M. Solvent polarity and Kamlet−Taft parameters for ionic liquids containing a pyridinium cation. *J. Phys. Chem. B* **2008**, *112*, 1473−1476.
(20) Nayak, P. K.; Hathorne, A. P.; Bermudez, H. Critical solution behaviour of poly(*N*-isopropylacrylamide) in ionic liquids/water mixtures. *Phys. Chem. Chem. Phys.* **2013**, *15*, 1806−1809.
(21) Ogihara, W.; Aoyama, T.; Ohno, H. Polarity measurements for ionic liquids containing dissociable protons. *Chem. Lett.* **2004**, *33* (11), 1414−1415.
(22) Jin, H.; O'Hare, B.; Dong, J.; Arzhantsev, S.; Baker, G. A.; Wishart, J. F.; Benesi, A. J.; Maroncelli, M. Physical properties of ionic liquids consisting of the 1-butyl-3-methylimidazolium cation with various anions and the bis(trifluoromethylsulfonyl)imide anion with various cations. *J. Phys. Chem. B* **2007**, *112* (1), 81−92.
(23) Kamlet, M. J.; Taft, R. W. The solvatochromic comparison method. I. The β-scale of solvent hydrogen-bond acceptor (HBA) basicities. *J. Am. Chem. Soc.* **1975**, *98*, 377−383.
(24) Kamlet, M. J.; Abboud, L. J.; Taft, R. W. The solvatochromic comparison. Method. 6. The π^* scale of solvent polarities. *J. Am. Chem. Soc.* **1977**, *99*, 6027.
(25) (a) Reichardt, C., *Solvents and solvent effects in organic chemistry*, 3rd ed.; Wiley-VCH: Weinheim: 2003. (b) Shukla, S. K.; Khupse, N. D.; Kumar, A. Do anions influence the polarity of protic ionic liquids? *Phys. Chem. Chem. Phys.* **2012**, *14*, 2754−2761. (c) Persson, I. Solvent and complex formation in strongly solvating solvents. *Pure Appl. Chem.* **1986**, *58*, 1153−1161.
(26) (a) Kumar, A.; Reddy, P. M.; Venkatesu, P. Polyacrylic acid polymer modulates the UCST-type phase behavior of ionic liquid and water. *RSC Adv.* **2012**, *2*, 6939−6947. (b) Reddy, P. M.; Venkatesu, P. Ionic liquid modifies the lower critical solution temperature (LCST) of poly(*N*-isopropylacrylamide) in aqueous solution. *J. Phys. Chem. B* **2011**, *115*, 4752−4757.
(27) Wang, Z.; Wu, P. The influence of ionic liquid on phase separation of poly(*N*-isopropylacrylamide) aqueous solution. *RSC Adv.* **2012**, *2*, 7099−7108.
(28) Muldoon, J. M.; Gordon, C. M.; Dunkin, I. R. Investigations of solvent−solute interactions in room temperature ionic liquids using solvatochromic dyes. *J. Chem. Soc., Perkin Trans.* **2001**, *2*, 433.
(29) Jelicic, A.; Garcia, N.; Lohmannsroben, H.-G.; Beuermann, S. Prediction of the ionic liquid influence on propagation rate coefficients in methyl methacrylate radical polymerizations based on Kamlet−Taft solvatochromic parameters. *Macromolecules* **2009**, *42*, 8801−8808.
(30) Cammarata, L.; Kazarian, S. G.; Salter, P. A.; Welton, T. Molecular states of water in room temperature ionic liquids. *Phys. Chem. Chem. Phys.* **2001**, *3*, 5192−5200.
(31) Zhang, S.; Qi, X.; Ma, X.; Lu, L.; Deng, Y. Hydroxyl ionic liquids: the differentiating effect of hydroxyl on polarity due to ionic hydrogen bonds between hydroxyl and anions. *J. Phys. Chem. B* **2010**, *114* (11), 3912−3920.
(32) Stoimenovski, J.; Izgorodina, E. I.; MacFarlane, D. R. Ionicity and proton transfer in protic ionic liquids. *Phys. Chem. Chem. Phys.* **2010**, *12*, 10341−10347.
(33) Bini, R.; Chiappe, C.; Mestre, V. L.; Pomelli, C. S.; Welton, T. A rationalization of the solvent effect on the Diels−Alder reaction in ionic liquids using multiparameter linear solvation energy relationships. *Org. Biomol. Chem.* **2008**, *6* (14), 2522−2529.
(34) Kodama, K.; Tsuda, R.; Niitsuma, K.; Tamura, T.; Ueki, T.; Kokubo, H.; Watanabe, M. Structural effects of polyethers and ionic liquids in their binary mixtures on lower critical soltuion temperature liquid-liquid phase separation. *Polym. J.* **2011**, *43*, 242−248.
(35) (a) Kolaric, B.; Sliwa, M.; Vallee, R. A. L.; Van der Auweraer, M. Polymer−dye interactions as a tool for studying phase transitions. *Colloids Surf. A* **2009**, *338*, 61−67. (b) Kumar, A. C.; Bohidar, H. B.; Mishra, A. K. The effect of sodium cholate aggregates on thermoreversible gelation of PNIPAM. *Colloids Surf. B* **2009**, *70*, 60−67.
(36) Carmichael, A. J.; Seddon, K. R. Polarity study of some 1-alkyl-3-methylimidazolium ambient-temperature ionic liquids with the solvatochromic dye, Nile Red. *J. Phys. Org. Chem.* **2000**, *13*, 591−595.
(37) Davis, M. M.; Hetzer, H. B. Titrimetric and equilibrium studies using indicators related to Nile Blue A. *Anal. Chem.* **1966**, *38*, 451−461.