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View Article Online
View Journal | View Issue

Cite this: *Phys. Chem. Chem. Phys.*, 2014, **16**, 335

Effects of some salts on H_2O as probed by a thermodynamic signature of glycerol: towards understanding the Hofmeister effects (VII)†

Yoshikata Koga*[ab] and Peter Westh[c]

Received 15th August 2013,
Accepted 22nd October 2013

DOI: 10.1039/c3cp53474j

www.rsc.org/pccp

The generality of the Hofmeister effects has been questioned of late, and doubts have been cast over their importance in understanding the specific ion effects on the chemistry and physics of biopolymers in aqueous solutions. Recent experimental evidence from modern non-linear spectroscopies points mostly to the direct interaction between the ion and the biopolymer in question that is more important for understanding the Hofmeister effects. On the other hand, our own contribution by higher order thermodynamical studies indicated that the effects of ions on H_2O itself may not be denied all together. Namely, we devised a methodology whereby the effect of an ion on H_2O is characterized by two orthogonal indices, hydrophobicity and hydrophilicity, by using a third order thermodynamic signature of hydrophobic 1-propanol (1P) as a probe, the 1P-probing methodology. The results indicated that the common anion ranking could be understood in terms of two indices, hydrophobicity and hydrophilicity of an individual ion. In the present work, we make an attempt at probing the effects of the same ions on H_2O by a typical hydrophile, glycerol (abbreviated as Gly in this article). Compared with the results of the 1P-probing methodology, we seek to determine how hydrophiles would react to the subtle modification of H_2O caused by the presence of an ion, since biopolymers are large amphiphiles with hydrophobic and hydrophilic surfaces. The results indicate that the Gly-probe is much less sensitive than the 1P-probe. We suggest therefore that it is the hydrophobic moieties of biopolymers that mainly give more conspicuous response to the modified H_2O by the presence of an ion.

Introduction

Ions and non-electrolyte solutes modify the molecular organization of H_2O in a specific manner. As one of their manifestations, they show marked differences in their solvent properties when used as mixed solvents. This was recognized back in 1887 by F. Hofmeister.[1–3] He ranked the effects of ions in the order from what reduces the solubility of lysozyme in aqueous solutions to what promotes it. Since then, almost the same ranking seems to apply to a large number of physical/chemical processes in aqueous solutions of biopolymers or colloids, particularly for anions. The left side of the ranking was named ''kosmotropes'' and the right ''chaotropes'' with Cl^- at about the null position.[4] Thus, at the zero-th approximation, it was generally regarded to be the effect of each ion on H_2O that dictates the overall properties of the ternary systems. More recent investigations, however, tend to point to direct ion–biopolymer interactions that are more important for the ion-specific effects. Indeed, modern non-linear higher order spectroscopic studies suggested that the bulk H_2O away from hydration shells of common ions was left unperturbed.[5–11] Hence, the Hofmeister effects must be due to direct interactions between the specific ion and the biopolymer in question. Furthermore, the reversals of the Hofmeister ranking have been observed by modifying the end groups of the biopolymer,[12] or by changing the solution compositions.[13–15] With these the Hofmeister effects may become non-existent. The close relation between the Hofmeister series of biopolymers and the lyotropic series of colloids has been long noted. Lyklema pointed out in analogy with colloid science that the Hofmeister series ought to be re-examined by taking into account the surface conditions, hydrophobicity or hydrophilicity, of biopolymers in question.[41] Meanwhile, Levin *et al.* claimed to have developed a theory of the surface density profile that could explain a variety of experimental results with a single adjustable parameter, and that could finally shed light on a century old enigma, the Hofmeister series.[42]

Nonetheless, the effects of ions and non-electrolyte solutes on H_2O are interesting in their own right and important for

[a] *Department of Chemistry, The University of British Columbia, Vancouver, B.C., Canada V6T 1Z1. E-mail: koga@chem.ubc.ca*
[b] *Suitekijuku, Vancouver, B.C., Canada V6R 2P5*
[c] *NSM Research Unit for Functional Biomaterials, Roskilde University, Roskilde, Denmark DK-4000*

† Electronic supplementary information (ESI) available. See DOI: 10.1039/c3cp53474j

fundamental investigations of the multicomponent aqueous solutions. We have recently devised what we call the 1-propanol (1P) probing methodology that was detailed elsewhere.[16,17] By applying it to aqueous solutions, we were able to characterize the effects of solutes, non-electrolytes and individual ions on H_2O using a pair of coordinates, hydrophobicity, a, and hydrophilicity, b, and thus to characterize the effect of a solute on a two-dimensional map with H_2O at the origin. For an individual species the former relates to its hydration number, n_H, and the latter to its effect on the degree of S–V cross fluctuation density (proportional to thermal expansivity) of the entire bulk of the solution.[18–21] Using this methodology, we found that there are five distinct classes of the effects of a solute on H_2O. In particular, the results of a series of studies on general ions by this methodology indicated that kosmotropes all belong either to ''hydration centers'' or ''hydrophobes'', both being interpreted as forming hydration shells around them, while chaotropes were found to be all ''hydrophiles'' (see below). Furthermore, the anion Hofmeister ranking matched the decreasing order of the distance from the origin for ''hydration centers'' and ''hydrophobes'' and then the increasing order of the distance for ''hydrophiles'' with the null point being H_2O itself. Cl^-, which is normally regarded as the null point, was found to belong to the ''hydration center'' and to be very close to the origin. According to our studies,[16,17] ''hydration centers'' were understood to form hydration shells but the bulk H_2O away from hydration shells is left unperturbed, while ''hydrophobes'' also form hydration shells with somewhat enhanced hydrogen bonding within them with concomitant reduction of the hydrogen bond probability of bulk H_2O.[20,21] ''Hydrophiles'', on the other hand, form hydrogen bonds directly with the hydrogen bond network of H_2O and retard the degree of fluctuation inherent in pure H_2O.[18,22,23] Thus, we suggested that the effects of ions on H_2O must remain important in understanding the Hofmeister effects. Of course, the individuality of chemical and physical properties of biopolymers is not unimportant. After all, they are large amphiphiles with hydrophobic and hydrophilic moieties. It would therefore be interesting to investigate how a hydrophobe and a hydrophile react to the modification of H_2O caused by the presence of an individual ion. The probe 1-propanol (1P) we have used so far is a typical ''hydrophobe'' in our classification.[16,17] The present work shows how a hydrophile, glycerol (abbreviated as Gly in this paper), would react to the modification of bulk H_2O caused by the presence of a specific ion following the earlier preliminary study.[24]

In dealing with aqueous solutions, particular consideration must be given to the composition. We earlier realized[20–22] that the solution properties are crucially dependent on the composition in general for aqueous solutions. We found that the aqueous solution generally consists of three distinctive regions, in each of which the mixing scheme (MS), the molecular level scenario of mixing, is qualitatively different from those of other regions. In the H_2O-rich region, H_2O is modified somewhat depending on the nature of the solute (the details of which were instrumental in classifying the solute into the five classes mentioned above)[16,17] but the basic integrity of liquid H_2O is retained inasmuch as the hydrogen bond network is connected fleetingly and yet permanently throughout the bulk. H_2O is here understood as a highly fluctuating hydrogen bonded assembly and yet hydrogen bonds are bond-percolated.[20,21,25,26] In the solute-rich region, the solute molecules tend to cluster together as in the pure state and H_2O interacts with such clusters as a single gas-like molecule. In the intermediate region, two kinds of clusters, one rich in H_2O and the other in solute, physically mix together. We name these three distinct mixing schemes Mixing Schemes I, II and III from the H_2O-rich end. The boundaries between the adjacent MSs are apparent from the anomalous behaviour of the third derivative thermodynamic quantity.[20–22] In special cases, the boundary could appear as liquid–liquid phase separation between MS I and MS II or precipitation of a solute at the MS II and MS III boundary.

In the original experiment conducted by Hofmeister,[1,3] the first cloud points with 2 wt% lysozyme were determined in terms of the salt composition. They occurred at the mole fraction of 0.056, 0.03, 0.061 and 0.09, respectively, for the Na-salts of SO_4^{2-}, OAc^-, Cl^-, and ClO_3^-. From the description in this paper, it is not clear whether the first cloud point is phase separation or precipitation. We interpret his first cloud point as corresponding to the MS I and II boundary for safety, and we limit our attention to MS I of the multi-component aqueous solutions.

As detailed earlier,[16,17] the methodology we use is applicable only to the limited H_2O-rich region, MS I. This is based on our earlier findings that within this limited H_2O-rich region, MS I, the effects of ions are additive and that the effects of hydrophobic and hydrophilic moieties of amphiphiles are also additive. Similarly, for a multi-component system the effects of each solute are additive as long as the total mole fraction is small enough so that a body of liquid H_2O maintains its integrity.[16,17]

Here, following the previous Gly-probing study for Na-salts of some anions,[24] we apply it to Cl-salts of NH_4^+, $(CH_3)_4N^+$ (TMA^+) and in addition $NaCH_3COO$ (Na^+OAc^-). The latter was included, since we investigated recently how OAc^- works as a hydrophobe.[16,27]

The details of the probing methodology were described elsewhere.[16,17] Very briefly, one of the thermodynamic signatures, H_{BB}^E (defined below), for the probing component B in the ternary aqueous solution of B and the test sample S is determined as a function of the mole fraction of B, x_B, at a fixed initial mole fraction of S, x_S^0. H_{BB}^E shows the x_B-dependence pattern unique to the nature of B. For hydrophobic B, it displays a peak type and for a hydrophilic B a bend type anomaly reflecting a qualitative change in the molecular organization of H_2O. (See Fig. 5 in the Appendix.) The peak top or the bend point that we name point X is where the integrity of liquid H_2O is lost due to the presence of B at the value of x_B. We then observe how the H_{BB}^E pattern changes as S is added while the characteristic pattern of H_{BB}^E is retained. The induced changes, particularly those of the anomalous point, X, are indexed in two orthogonal directions in the graph of H_{BB}^E *vs.* x_B. Thus, the B-probing methodology is applicable only up to this mole fraction. The rate of westward shift (to the negative direction

of the x_B-axis) of point X per unit increase in x_S^0 is defined as hydrophobicity, *a*. That of the southward shift (to the negative direction of H_{BB}^E-axis) is defined as hydrophilicity, *b*. The shifts in both directions are found generally to be linear to x_S^0. By trying out a number of typical hydrophobes and hydrophiles for S, we catalogued the induced changes. Thus, we have a way to characterize the effect of an unknown solute S on H_2O using a pair of indices, *a* and *b*, and to display it in a two-dimensional map with H_2O defining its origin.

From the 1P-probing methodology, we drew the following conclusions for each ion studied here: Na^+, NH_4^+, and Cl^- are the "hydration centers" with the hydration number n_H, 5.2, 1 ± 1, and 2.3 ± 0.6, respectively, leaving the bulk H_2O away from hydration shells unperturbed.[16,17] The CH_3^- side of OAc– is a hydrophobe with the total hydration number 3.7 ± 0.7 and reducing progressively the hydrogen bond probability of bulk H_2O away from hydration shells. One out of 3.7 H_2O molecules hydrates the COO^- side of the ion as a hydration center without affecting the bulk H_2O.[16,27] TMA^+ was found to act as a hydrophile which forms hydrogen bonds directly with the hydrogen bond network of H_2O and to pin down the fluctuation inherent in liquid H_2O.[16,28]

As discussed in the Appendix, the Gly-probe has an intrinsic disadvantage in comparison with the 1P-probe. Namely, H_{1P1P}^E is directly proportional to the partial molar *S–V* cross fluctuation density of 1P, ${}^{SV}\delta_{1P}$, defined by eqn (2) and (3) in the Appendix. This signifies the effect of a solute on the mean square amplitude of the *S–V* cross fluctuation of bulk H_2O.[18,19] In other words, the mean square amplitude of the *S–V* cross fluctuation is monitored by perturbing the system by the infinitesimal increase of 1P. Thus, the behavior of H_{1P1P}^E, its increase/decrease, is directly proportional to that of ${}^{SV}\delta_{1P}$. H_{GlyGly}^E, on the other hand, is only partially proportional to the equivalent ${}^{SV}\delta_{Gly}$ with an extra constant term.[23] Unless the behavior of the latter constant term is known, that of H_{GlyGly}^E cannot be directly connected to ${}^{SV}\delta_{Gly}$. Another practical disadvantage of the Gly-probe is that its point X is not as conspicuous as that of the 1P-probe, since the latter displays a peak top, while the former a bend point. (See Fig. 5 and 6 in the Appendix.) With these disadvantages we attempt to investigate how a hydrophile reacts to the subtle modification of H_2O caused by the presence of ions within MS I, in comparison with a hydrophobe. Most of the solutes of biological significance are amphiphilic, and it would be important to investigate how hydrophobic and hydrophilic moieties respond to subtle modification caused by an ion while the basic integrity of liquid H_2O is retained. As mentioned above, we have found that within the limited H_2O-rich region the hydrophobic and hydrophilic moieties respond additively to H_2O modification.[16–23]

Experimental

Glycerol (abbreviated as Gly in this paper) (Sigma, >99%) was degassed *in vacuo* at 80 °C for about 30 min and then charged into a 1000 μL syringe in a dry N_2 atmosphere for the titration calorimetry described below. NH_4Cl (Merck, >99.8%), $N(CH_3)_4Cl$ (TMACl) (Merck, >98%) and $Na(CH_3COO)$ (NaOAc) (Sigma-Aldrich, >99.8%) were used to prepare stock solutions using Milli-Q water. The respective solutions were diluted to the desired initial mole fraction, x_S^0, immediately before use.

The excess partial molar enthalpy of Gly, H_{Gly}^E, is determined by using a TAM III isothermal titration calorimeter (TA Instruments, New Castle, DE, USA) at 25.000 ± 0.005 °C. The titration procedure was modified to enable facile delivery of highly viscous Gly as described in the previous work.[24] Furthermore, a 30 min interval was given between successive titrations, in order to reduce a possible rheological effect of highly viscous Gly. The uncertainty in H_{Gly}^E was estimated to be ±0.03 kJ mol^{-1}.

Results and discussion

Fig. 1 shows the excess partial molar enthalpy of Gly, H_{Gly}^E, in the ternary Gly–S–H_2O at a given initial mole fraction of S in the mixed solvent, x_S^0. The raw data are given in Table S1 in the ESI.† While H_{Gly}^E becomes more endothermic as x_S^0 increases for TMACl, Fig. 1(b), and NaOAc, Fig. 1(c), that for NH_4Cl shows a similar behavior at the low x_{Gly} range but becomes more exothermic at high x_{Gly} within the x_{Gly} range studied. But for all cases, the slopes of H_{Gly}^E against x_{Gly} seem to become less as x_S^0 increases. To see these trends more clearly, we evaluate H_{GlyGly}^E defined as,[16,17,20–22]

$$H_{GlyGly}^E \equiv N(\partial H_{Gly}^E/\partial n_{Gly}) = (1 - x_{Gly})(\partial H_{Gly}^E/\partial x_{Gly}), \quad (1)$$

at given $x_S^0 = n_S/(n_S + n_W)$. In the ternary system Gly–S–H_2O, n_S is the molar amount of S, n_{Gly} that of Gly which alone increases little by little through titration, n_W that of H_2O, $N = n_{Gly} + n_S + n_W$, and $x_{Gly} = n_{Gly}/N$. Of course for the 1P-probe, the equivalent definition is given by replacing subscripts Gly by 1P. For evaluating H_{GlyGly}^E, we perform graphical differentiation as for H_{1P1P}^E without resorting to curve-fitting an analytical function to the H_{Gly}^E data. By this treatment the random error in H_{GlyGly}^E inevitably increases to ±1 kJ mol^{-1}, but there is no danger of introducing a systematic error by a wrong choice of the analytical function. It is practically impossible to find a correct function.

The resulting H_{GlyGly}^E data are plotted in Fig. 2. Fig. 2(a) shows H_{GlyGly}^E for the binary Gly–H_2O. It is apparent beyond the estimated uncertainty that the x_{Gly}-dependence pattern of H_{GlyGly}^E shows breaks in the slope at points X and Y at x_{Gly} = 0.073 and 0.14, respectively, indicated in the figure. The same behavior was observed in the previous Gly-probing study, though the x_{Gly} loci were at 0.08 and 0.015.[24] The existence of the breaks at points X and Y was confirmed recently[29] when we directly measured another third derivative quantity, the partial molar *S–V* cross fluctuation density of Gly in Gly–H_2O, ${}^{SV}\delta_{Gly}$, by differential pressure perturbation calorimetry.[30] Since this third derivative quantity is determined directly, we could take one more derivative graphically. The resulting fourth derivative quantity showed the onset of a step anomaly correctly at x_{Gly} = 0.076 and its end at 0.14 at 25 °C.[29] These should correspond to

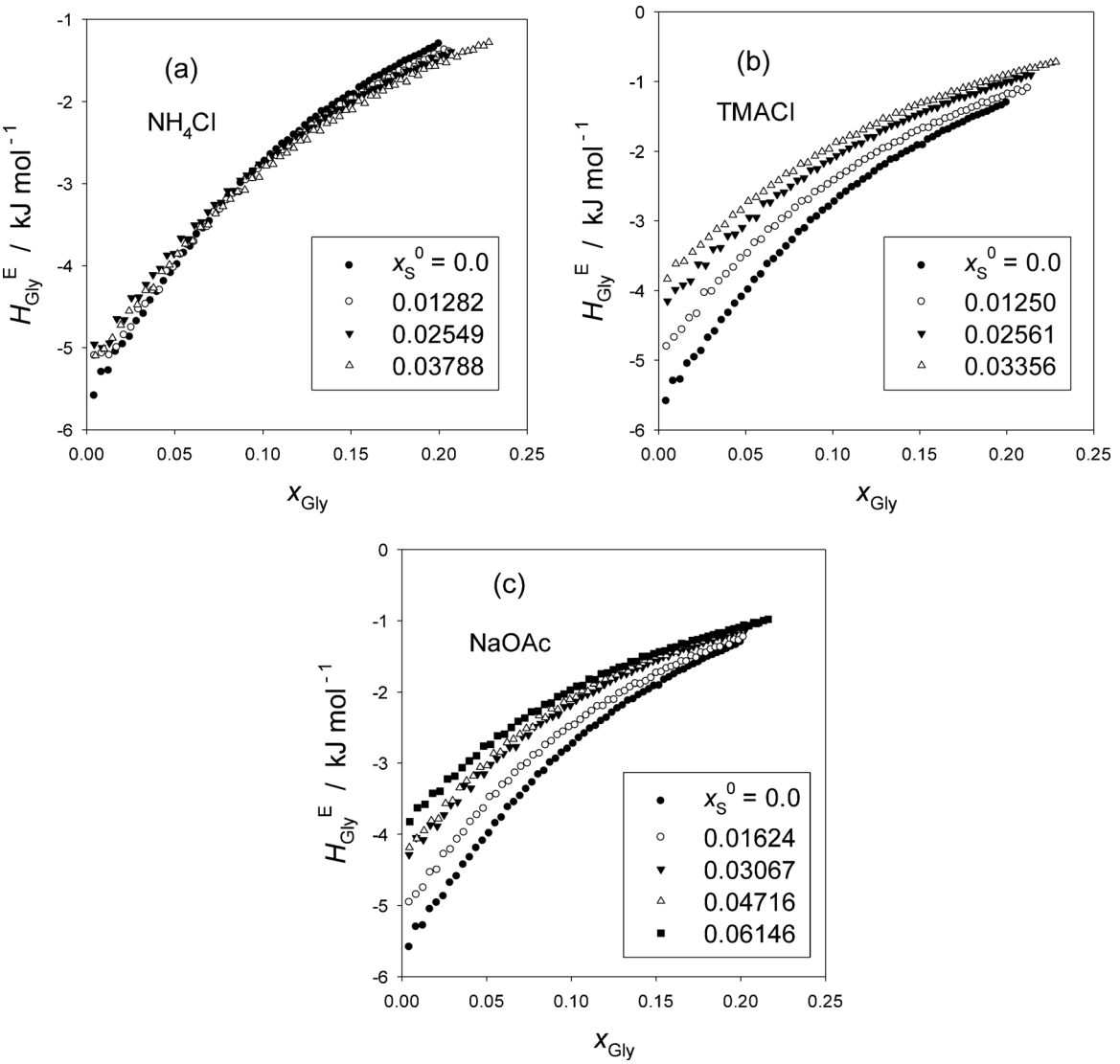

Fig. 1 (a) Excess partial molar enthalpy of Gly, H^E_{Gly}, in Gly–S–H_2O (S = NH_4Cl) at 25 °C. (b) Excess partial molar enthalpy of Gly, H^E_{Gly}, in Gly–S–H_2O (S = TMACl) at 25 °C. (c) Excess partial molar enthalpy of Gly, H^E_{Gly}, in Gly–S–H_2O (S = NaOAc) at 25 °C.

points X and Y in the third derivative quantity. As temperature increases, however, the step becomes progressively smaller and more obscure. The same observation was made in the previous Gly-probing study[24] in that as S is added and x_S^0 increases the break point X becomes more obscure to note in the H^E_{GlyGly} patterns.

Fig. 2(b)–(d) show the results for the ternary Gly–S–H_2O systems. The binary Gly–H_2O system data are represented by two straight lines and its point X is indicated by a hollow X on the line. Point X is an important point that indicates the end of the dilute solution regime where the integrity of liquid H_2O is lost. We found from our earlier studies[20,23,31] that up to point X the integrity of liquid H_2O is retained such that the hydrogen bond network is still connected throughout the bulk H_2O. It is this dilute concentration range where the probing methodology by 1P or Gly is applicable.[16,17] Thus, it is unfortunate that with the present data at hand the loci of point X are not located with confidence.

We thus approach differently. From the previous 1P-probing methodology, we found how each solute, a non-electrolyte or an individual ion, modifies H_2O within the respective MS I. As mentioned above, Na^+, NH_4^+ and Cl^- belong to the class of "hydration centers" that are hydrated by 5.2, 1 and 2.3 molecules of H_2O, respectively, but leave the bulk H_2O away from hydration shells unperturbed. At least the same ions were shown not to alter the bulk H_2O away from hydration shells by femto-second pump probe spectroscopic studies.[5] OAc^- is a "hydrophobe" that is hydrated by a total of 3.7 molecules of H_2O. The hydrogen bond probability within the hydration shells is enhanced somewhat, but that of the bulk H_2O away from the hydration shells is reduced progressively. The bulk H_2O has not yet lost the hydrogen bond percolation until the system reaches point X.[16,17,21]

The distinction between hydration centers and hydrophobes was apparent in that the behavior of H^E_{1P1P} at x_{1P} = 0 was

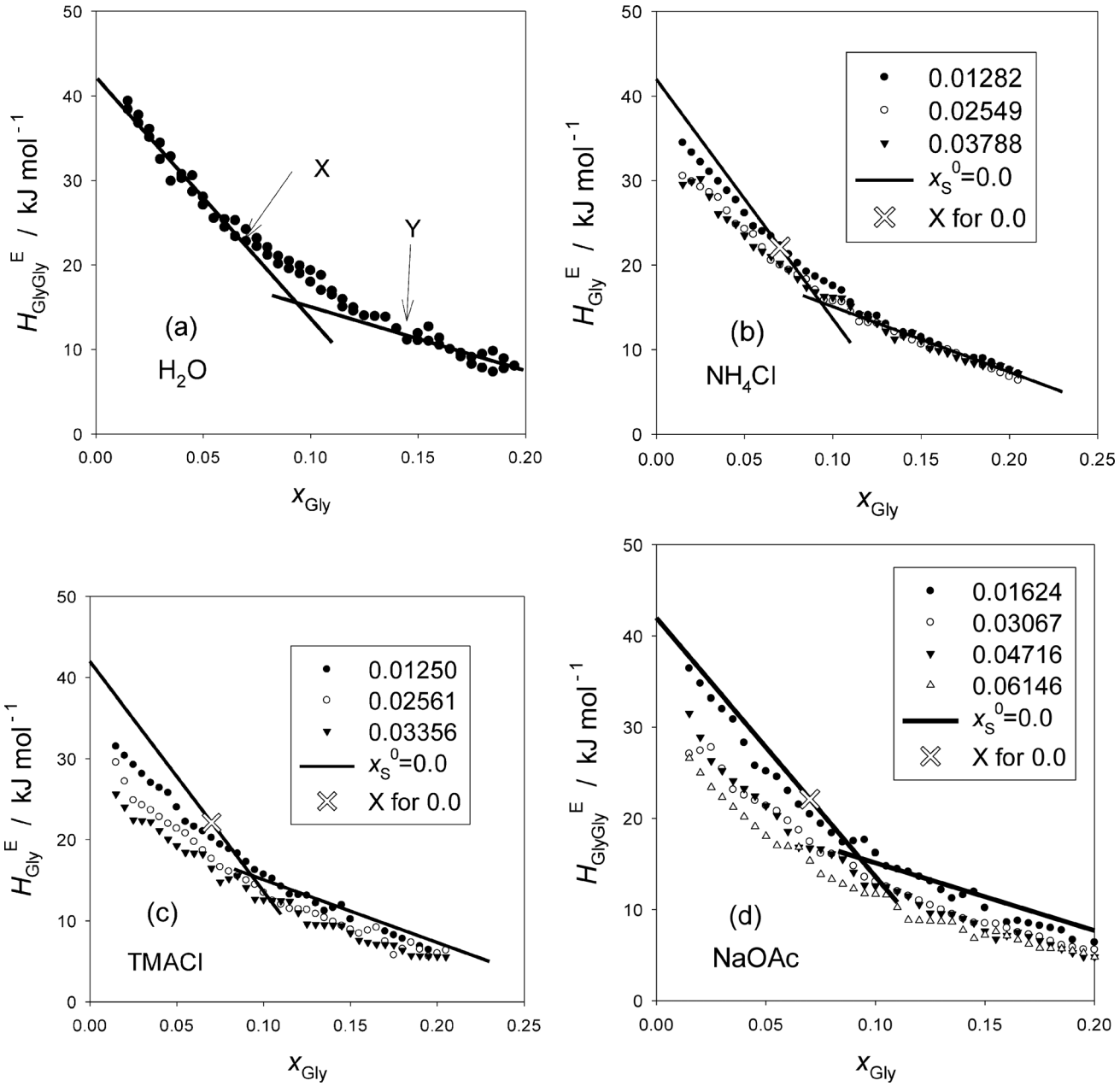

Fig. 2 (a) Gly–Gly enthalpic interaction, H^{E}_{GlyGly}, in binary Gly–H_2O at 25 °C. The value of point X for the binary Gly–H_2O, x^{0}_{Gly}, was found to be 0.073 and that of Y 0.14. (b) Gly–Gly enthalpic interaction, H^{E}_{GlyGly}, in Gly–S–H_2O at 25 °C for S = NH_4Cl. (c) Gly–Gly enthalpic interaction, H^{E}_{GlyGly}, in Gly–S–H_2O at 25 °C for S = TMACl. (d) Gly–Gly enthalpic interaction, H^{E}_{GlyGly}, in Gly–S–H_2O at 25 °C for S = NaOAc.

different in the 1P-probing studies.[16,17] Namely, for the hydration centers, the values of H^{E}_{1P1P} remain constant and independent of x^{0}_{S}, while they increased as x^{0}_{S} increased for the hydrophobes. Hence for salts consisting of counter ions in the "hydration center" such as, NaCl, and NH_4Cl, they remained constant. For the present Gly-probing study, on the other hand, NH_4Cl does not seem to stay constant as is evident in Fig. 2(b). The previous Gly-probing study[24] indicates the same observation for NaCl also. This discrepancy between the 1P- and Gly-probing methodologies could be related to our findings that H^{E}_{1P1P} is directly proportional to the solute's effect on the *S–V* cross fluctuation density,[16,17] while H^{E}_{GlyGly} is partially proportional with an extra constant term as discussed above and in the Appendix. The latter constant term, the origin of which is yet to be elucidated, may be responsible for the observed downward shift of H^{E}_{GlyGly} at x_{Gly} = 0 as x^{0}_{S} increases.

TMA^{+} was found to act as a hydrophile[16,17,28] that forms hydrogen bonds directly with the existing (momentarily but perpetually) hydrogen bond network keeping the hydrogen bond connectivity intact. But it reduces the degree of fluctuation inherent in pure H_2O progressively by breaking the proton donor–acceptor symmetry of liquid H_2O. Probably reflecting this, Fig. 2(c) shows a little sharper decrease in the H^{E}_{GlyGly} value

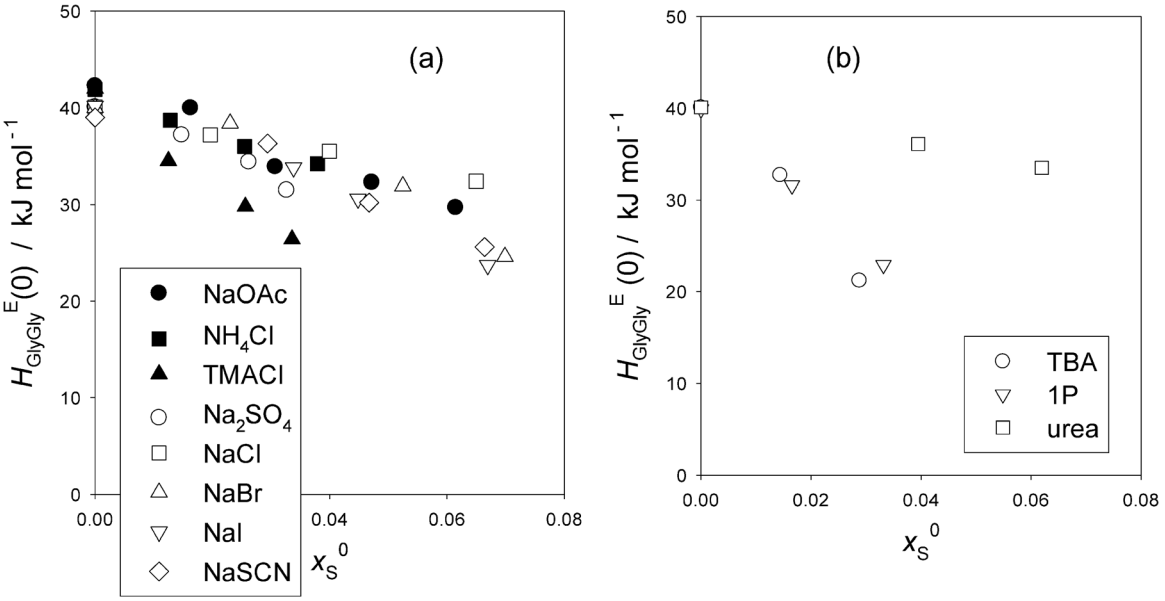

Fig. 3 (a) The values of H^{E}_{GlyGly} at the infinite dilution, x_{Gly} = 0.0, H^{E}_{GlyGly} (0) against x_S^0 for various salts (S). The uncertainty is estimated to be ±2 kJ mol^{-1}. Filled symbols; this work, and hollow symbols; ref. 24. (b) The values of H^{E}_{GlyGly} at the infinite dilution, x_{Gly} = 0.0, H^{E}_{GlyGly} (0) against x_S^0 for non-electrolytes. The data are taken from the previous Gly-probing study.[24] The uncertainty is estimated to be ±2 kJ mol^{-1}.

at x_{Gly} = 0, H^{E}_{GlyGly} (0), than the other two salts. NaOAc, on the other hand, with a hydrophobic OAc^- ion shows a decrease in H^{E}_{GlyGly} (0), the value of H^{E}_{GlyGly} at x_{Gly} = 0. This contrasts with the behavior of H^{E}_{1P1P} observed in the 1P-probing study, where the equivalent H^{E}_{1P1P} (0) increased.[16,17]

To see these trends at x_{Gly} = 0 more clearly, the H^{E}_{GlyGly} data are extrapolated linearly to x_{Gly} = 0 and evaluated H^{E}_{GlyGly} (0) values. The results are plotted in Fig. 3(a). Also shown in the figure are the equivalent plots with hollow symbols taken from the previous Gly-probing study.[24] For S = Na_2SO_4, the raw data were not used for the analysis in ref. 24. We reproduce the data here with the permission of the original authors as Table S2 in the ESI.† Both graphs of H^{E}_{Gly} and H^{E}_{GlyGly} for S = Na_2SO_4 are also given as Fig. S3(a) and (b) (ESI†). Since the data points for H^{E}_{GlyGly} at $x_{Gly} < 0.015$ are not available for all cases, the uncertainty in the extrapolated results could amount to ±2 kJ mol^{-1}. Fig. 3(b) shows the same plots for non-electrolyte samples. In the latter figure, two typical cases for hydrophobes, TBA and 1P, are shown. H^{E}_{GlyGly} (0) decreases as x_S^0 increases, in contrast to the increase in H^{E}_{1P1P} (0), the value of H^{E}_{1P1P} at x_{1P} = 0.[16,17] This is only natural due to a geometrical reason. H^{E}_{1P1P} increases from x_{1P} = 0 to point X, while H^{E}_{GlyGly} decreases down to its point X. A hydrophobic sample S will shift the H^{E}_{BB} pattern including point X towards west, a smaller value of x_B (for B = 1P or Gly). Since a number, n_H, of H_2O molecules are used up for hydration, and they are not available for the probe B to interact, point X will be reached at a lesser value of x_B. Indeed, the dynamics of the hydrating H_2O was found to be several times slower than that of bulk H_2O.[5] This westward shift will inevitably result in an increase in the value of H^{E}_{BB} for a line with a positive slope (for B = 1P) and a decrease for that with a negative slope (for B = Gly), unless there is a mechanism to pin down H^{E}_{BB} (0) at a constant value. This is what happens for the 1P-probing, B = 1P, for ''hydration centers''. Going back to Fig. 3(b), it is surprising that the decreases in H^{E}_{GlyGly} (0) for both TBA and 1P show no difference, although TBA is a stronger hydrophobe than 1P.[16,17,20,21] This could indicate whether the Gly-probing is not as sensitive as 1P-probing or the effect of stronger TBA might be compensated for by its effect on the extra constant term discussed in the Appendix. Urea, a ''hydrophile'', shows a marginal decrease in H^{E}_{GlyGly} (0) upon increasing its initial mole fraction, x_S^0. This could be understood by the fact that the hydrophilicity indices determined by the 1P-probing are similar for urea and the probe Gly; the values of b being −1210 and −1180 respectively.[16]

Fig. 3(a) shows that for the hydration center salts, NH_4Cl and NaCl, H^{E}_{GlyGly} (0) decreases slightly, by just above the uncertainty upon increasing x_S^0. They showed no change in H^{E}_{1P1P} (0) in the 1P-probing results.[16,17] This decrease could also be due to an unknown effect on the extra constant term discussed above. Furthermore, there seems to be no difference among all these two hydration center salts in their x_S^0-dependence of H^{E}_{GlyGly} (0) in spite of the fact that the total hydration numbers are different; n_H = 7.5 for NaCl and 3.3 for NH_4Cl. This could also hint that the Gly-probe is not as sensitive as the 1P-probe towards subtle modification of H_2O caused by the presence of S. NaOAc, containing a hydrophobic anion, shows no difference in the decrease of H^{E}_{GlyGly} (0) with those of hydration centers. Na_2SO_4, SO_4^{2-} being a hydration center at x_{1P} = 0 found by the 1P-probing,[16,17] also shows the same trend. NaBr, NaI and NaSCN, consisting of Na^+ and a hydrophilic anion with its hydrophilicity increasing in the order of $Br^- < I^- < SCN^-$, do not show any difference among themselves nor from the hydration center group. TMACl is the only salt that stands

Table 1 The values of x_{Gly} at the presumed point X calculated using the total hydration number, n_H(tot), obtained by the 1P-probing methodology, and the value of x_{Gly} at the observed point X for the binary Gly–H_2O by the present Gly-probing. H^E_{GlyGly} at the presumed point X was read off the graph of H^E_{GlyGly}, Fig. 2(b)–(d). An assumption was made that the x_{Gly}-locus of point X is also linear to x^0_S as the case of the 1P-probing

Salt	n_H		x^0_S	x_{Gly} (X)	H^E_{GlyGly} (X)
NH_4Cl	+	1	0	0.073	21.1
	−	2.3	0.01282	0.06898	21
	(tot)	3.3	0.02549	0.06500	21
			0.03788	0.06111	21
TMACl	+	0	0	0.073	21.1
	−	2.3	0.01250	0.06999	20
	(tot)	2.3	0.02561	0.06683	18
			0.03356	0.06492	17
NaOAc	+	5.2	0	0.073	21.1
	−	3.7	0.01624	0.06126	23
	(tot)	8.9	0.03067	0.05084	22
			0.04716	0.03892	23
			0.06146	0.02852	23

out in terms of its decrease in H^E_{GlyGly} (0), as hydrophiles are expected to do. The hydrophilicity index of TMA^+ is b = –1180, while those of other hydrophilic anions are −920, –2050, and –2800 respectively.[16] Thus, TMA^+ is only modestly hydrophilic, and yet the decrease of H^E_{GlyGly} (0) stands out. This must be due to the weaker effect of the counter ion Cl^- than Na^+. The hydration number, n_H, for Cl^- is 2.3, while that for Na^+ is 5.2. But it is more likely that all these observations among salts could be due to the effect of each S on the extra constant term in the proportionality between H^E_{GlyGly} and the ${}^{SV}\delta_{Gly}$.

Now that point X for the present H^E_{GlyGly} is hard to identify, we proceed our analysis by calculating the point X in the H^E_{GlyGly} pattern assuming that the shifts of x_{Gly} (X) and H^E_{GlyGly} (X) are both linear to x^0_S as was the case for the 1P-probing methodology.[16,17] (X) indicates the respective coordinates at point X. Noting that the extrapolated value of x^0_S to x_{Gly} (X) = 0, x^0_S (0) is equal to $1/(n_H + 1)$, and using the x_{Gly} locus of point X for the binary Gly–H_2O determined in Fig. 2(a), we calculated the x_{Gly}-loci of point X at given x^0_S, which are listed in Table 1 for the present data. The same data treatment is applied to the previous Gly-probing study,[24] and listed in Table S4 in the ESI.† We then read off the value of H^E_{GlyGly} in Fig. 2(b)–(d) for the present data and equivalent graphs of H^E_{GlyGly} against x_{Gly} for the previous work[24] at the calculated point X, x_{Gly} (X). The H^E_{GlyGly} (X) values are also listed in Table 1 and Table S4 (ESI†), and plotted in Fig. 4(a) for salts, and in Fig. 4(b) for non-electrolytes. The uncertainty of the resulting H^E_{GlyGly} (X) is estimated to be ±2 kJ mol^{-1}.

For all other salts in Fig. 4(a) except for Na_2SO_4 and TMACl, H^E_{GlyGly} (X) may be regarded as remaining constant and independent of x^0_S, taking into account the estimated uncertainty, ±2 kJ mol^{-1}. For Na_2SO_4, H^E_{GlyGly} (X) clearly increases as x^0_S increases. This is an interesting and important finding. From the 1P-probing, $SO_4{}^{2-}$ was found to belong to a special case of the "hydration center".[16,32] As the mole fraction of the probe 1P, x_{1P}, increases, both S and 1P together were found to reduce the hydrogen bond probability of bulk H_2O just as a hydrophobe stronger than the probe 1P does, while in the absence of 1P (*i.e.* at x_{1P} = 0), $SO_4{}^{2-}$ alone acts as purely a hydration center. Thus the present finding suggests that the increase in H^E_{BB} at point X is independent of the identity of the probe B. Namely, as x_B increases and hence the available bulk H_2O decreases, there must be some inherent mechanisms due only to $SO_4{}^{2-}$ to reduce the hydrogen bond probability of bulk H_2O. Self-aggregation of $SO_4{}^{2-}$ could be a reason, as observed for urea above x^0_S > 0.08 (S = urea),[33] for the 1-butyl-2,3-dimethylimidazolium cation at x^0_S > 0.006[34] and for the 1-butyl-3-methylimidazolium cation at x^0_S > 0.014.[35] However, they all show a sudden decrease in the slope of H^E_{BB} (X) *vs.* x^0_S in

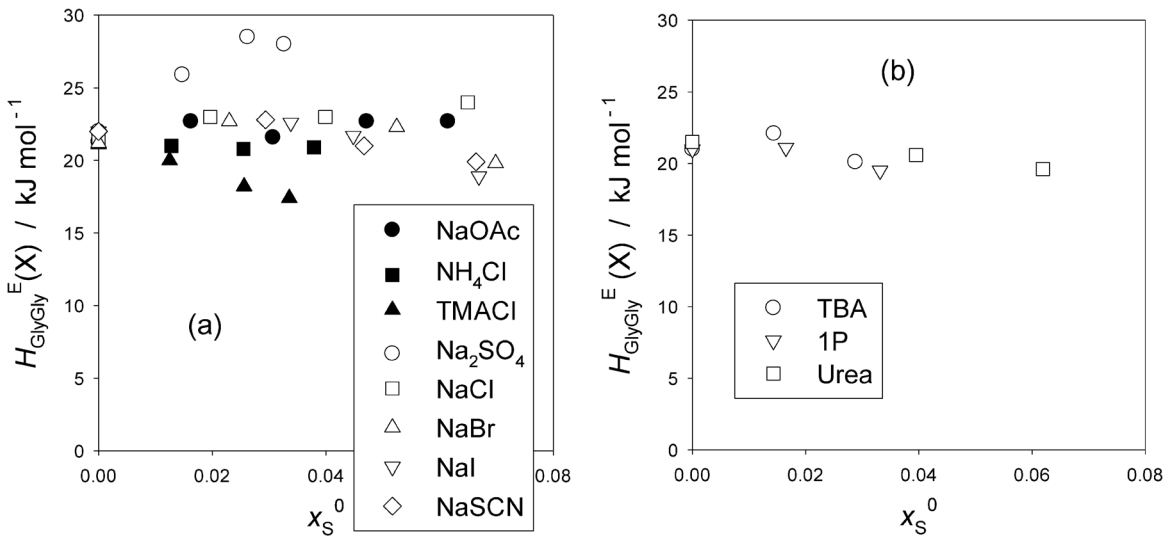

Fig. 4 (a) The values of H^E_{GlyGly} at presumed point "X", H^E_{GlyGly} (X) against x^0_S for various salts (S). Filled symbols; present work and hollow symbols; ref. 24. The uncertainty is estimated to be ±2 kJ mol^{-1}. (b) The values of H^E_{GlyGly} at presumed point "X", H^E_{GlyGly} (X), against x^0_S for non-electrolytes. Evaluated using the data in the previous Gly-probing study.[24] The uncertainty is estimated to be ±2 kJ mol^{-1}.

the respective 1P-probing studies. For Na_2SO_4 aqueous solutions, a dielectric relaxation study suggests the formation of H_2O separated cation–anion pairing as its concentration increases,[36] but this would also reduce $H^E_{BB}(X)$ rather than increase as observed here. We rather speculate that as the availability of un-hydrated bulk H_2O decreases, SO_4^{2-} ions may start to interact more strongly with the existing hydrogen bond network of bulk H_2O rather than just forming hydration shells. This may be due to the fact that SO_4^{2-} ions presumably spread O atoms out in four tetragonal directions. As a result, the average hydrogen bond probability of bulk H_2O is reduced progressively. Recent studies using modern non-linear spectroscopic techniques aided by MD simulations[37,38] revealed how the ClO_4^- ion exchanges hydrogen bonds from a H_2O molecule with another in concentrated aqueous solutions of about 0.1 mole fraction. At this concentration, there are therefore hardly any H_2O molecules left to study the state of bulk H_2O away from hydration shells. Similar studies on SO_4^{2-} in H_2O could provide an important clue with more dilute aqueous solutions so that the state of bulk H_2O away from hydration shells could be studied.

In the case of TMACl, TMA^+ being hydrophilic, slightly more so than Gly[16,28] and Cl^- being a weak hydration center,[16,17] the decrease in $H^E_{GlyGly}(X)$ reflects the effect of TMA^+ and indicates the reduction in the effect of the solute on the degree of fluctuation in the bulk H_2O.

For NH_4Cl and NaCl, the constituent ions are all hydration centers. Hence, these salts do not alter the bulk H_2O away from hydration shells, and hence the effect of the solute on the degree of the *S–V* cross fluctuation should remain constant independent of x^0_S. This is exactly what we observe in Fig. 4(a). For the 1P-probe, however, not only at point X but also at $x_B = 0$ the values of H^E_{BB} were found to remain constant. For the present Gly-probe, the values of $H^E_{GlyGly}(0)$ at $x_{Gly} = 0$ do not remain constant, as shown in Fig. 3(a). OAc^-, on the other hand, was found to act as a hydrophobe with the total n_H = 3.7 and to reduce the hydrogen bond probability of bulk H_2O to the same degree as the probe 1P.[16,17,27] Fig. 4(b) indicates the behavior of typical hydrophobes, TBA and 1P. They are hydrated by 20 and 29 H_2O molecules, respectively,[16] and reduce the hydrogen bond probability of bulk H_2O away from hydration shells, more so for TBA than 1P. As a consequence, the effect of the solute on the degree of *S–V* cross fluctuation density increases due to a decrease in the negative contribution. Namely, as the hydrogen bond probability of liquid H_2O decreases, the chances for local and instantaneous formation of highly hydrogen bonded patches which contributes negatively to the *S–V* cross fluctuation decrease. Thus the net fluctuation increases, which should manifest in an increase in H^E_{BB}. It was indeed the case for the 1P-probing, B = 1P, and the value of $H^E_{BB}(X)$ is larger for TBA at point X than for 1P.[16] Fig. 4(b) shows, on the other hand, that for the Gly-probing, the values of $H^E_{GlyGly}(X)$ remain constant, independent of x^0_S for both hydrophobes. Similarly, the values of $H^E_{GlyGly}(X)$ for NaOAc remain constant as observed in Fig. 4(a).

The remaining three Na-salts are made of hydrophilic anions as found by the 1P-probing.[16,17] The hydrophilicity is stronger in the order of $SCN^- > I^- > Br^-$, the values of hydrophilicity being $b = -2800$, -2050, and -920 respectively.[16] In spite of the almost three-fold difference, the distinction in the x^0_S-dependence of $H^E_{GlyGly}(X)$ among them is not apparent. Thus, the Gly-probe appears to be insensitive to the difference in the modified H_2O by hydrophiles as well as by hydrophobes. Or it could be due to the constant additive term in the partial proportionality of the partial molar *S–V* cross fluctuation and H^E_{GlyGly} that makes the $H^E_{GlyGly}(X)$ appear insensitive.

Thus, while the extra constant term in the partial proportionality between H^E_{GlyGly} and ${}^{SV}\delta_{Gly}$ must be measured and its nature ought to be elucidated, we suggest that the behavior of H^E_{GlyGly} is not entirely inconsistent with the effects of S on H_2O deduced by the 1P-probing methodology.[16,17] It is clear, however, that the Gly-probe is not so sensitive as the 1P-counterpart. This would have an important implication for understanding the Hofmeister rankings, in that it is the hydrophobic part of a biological polymer that will respond more strongly to the slight modification of liquid H_2O caused by the presence of an ion.

Appendix

Fig. 5 shows the plots of H^E_{1P1P} and ${}^{SV}\delta_{1P}$ for the binary 1P–H_2O system. The ordinate for ${}^{SV}\delta_{1P}$ is scaled by a single factor ξ. The definition of H^E_{1P1P} is given in eqn (1) in the main text except for swapping subscripts Gly with 1P. H^E_{1P1P} signifies the 1P–1P interaction in terms of enthalpy in the solution. The *S–V* cross fluctuation density, ${}^{SV}\delta$, is defined as,[18,19,23]

$$ {}^{SV}\delta \equiv \langle(\Delta S)(\Delta V)\rangle/k\langle V\rangle = T\alpha_p. \qquad (2) $$

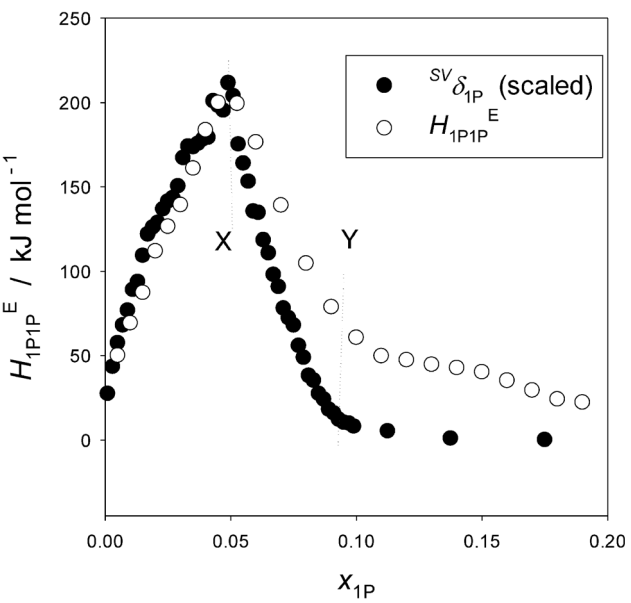

Fig. 5 The partial molar *S–V* cross fluctuation density of 1P, ${}^{SV}\delta_{1P}$, and the enthalpic 1P–1P interaction, H^E_{1P1P}, in 1P–H_2O at 25 °C. The ordinate for ${}^{SV}\delta_{1P}$ is scaled by a single factor. Reproduced with permission from ref. 18. Copyright (1999), NRC Research Press, National Research Council of Canada, Ottawa.

ΔS and ΔV are the variation of the instantaneous value of S and V in a coarse grain containing a fixed number of molecules from their ensemble average $\langle S\rangle$ and $\langle V\rangle$ respectively. k is the Boltzmann constant, α_p the thermal expansivity and V_m the molar volume of the solution. This quantity is important for studying H_2O and aqueous solutions in that it contains a negative contribution due to putative formation of ice-like patches in H_2O, which contributes negatively to the S–V cross fluctuation density, ${}^{SV}\delta$. Its partial molar derivative is defined taking into account the fact that ${}^{SV}\delta$ is an intensive quantity as,[39]

$$ {}^{SV}\delta_{1P} \equiv N(\partial {}^{SV}\delta/\partial n_{1P}) = (1 - x_{1P})(\partial {}^{SV}\delta/\partial x_{1P}). \quad (3)$$

${}^{SV}\delta_{1P}$ so defined is regarded as the effect of solute 1P on the S–V cross fluctuation density of the entire system. Of course, ${}^{SV}\delta_{Gly}$ is also defined by swapping the subscript 1P with Gly.

What Fig. 5 indicates then is that H^E_{1P1P} and ${}^{SV}\delta_{1P}$ are directly proportional with a single factor ξ within the dilute region up to point X; namely,

$$ H^E_{1P1P} = \xi {}^{SV}\delta_{1P}. \quad (4)$$

As discussed at some length elsewhere,[18,19] the 1P–1P enthalpic interaction and the effect of 1P on the S–V cross fluctuation density of the entire solution share the same fundamental cause and thus the enthalpic interaction is operative *via* bulk H_2O.[18,19] This finding was instrumental in devising the 1P-probing methodology.[16,17] The initial increase in H^E_{1P1P} up to point X is related to a net increase in ${}^{SV}\delta_{1P}$ due to the decrease in the negative contribution in ${}^{SV}\delta$ because the hydrogen bond probability of bulk H_2O is reduced progressively by the presence of a hydrophobic solute 1P.[20,21,31]

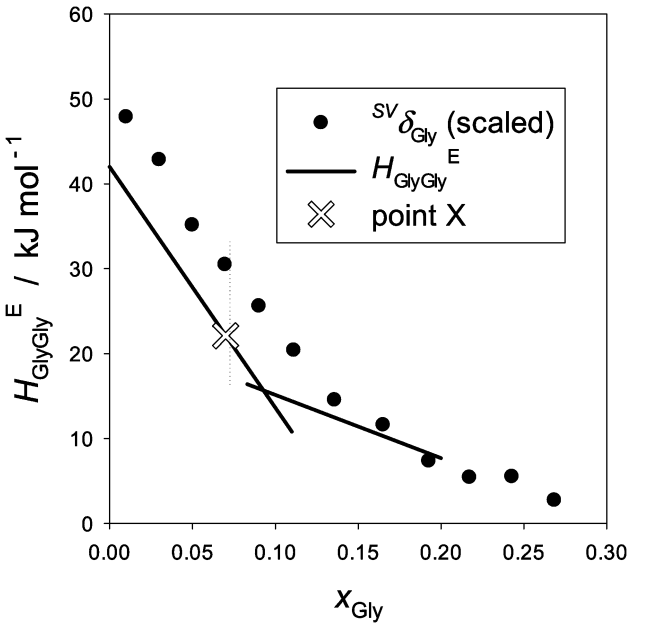

Fig. 6 The partial molar S–V cross fluctuation density of Gly, ${}^{SV}\delta_{Gly}$, and the Gly–Gly enthalpic interaction, H^E_{GlyGly}, in binary Gly–H_2O at 25 °C. The ordinate for ${}^{SV}\delta_{Gly}$ is scaled by a single factor. The data of α_p were taken from ref. 41. H^E_{GlyGly} data are represented by two straight lines as shown in Fig. 2(b) *etc.*

The equivalent quantities of binary Gly–H_2O, H^E_{GlyGly} and ${}^{SV}\delta_{Gly}$ are plotted in Fig. 6. The raw data of α_p determined by dilatometry[40] are used to calculate ${}^{SV}\delta$. A clear distinction is evident between Fig. 5 and 6 in their x_B-dependence patterns. The former pattern is unique to a hydrophobic solute, 1P, and the latter to a hydrophilic solute, Gly. Or rather, we used this qualitative difference in the x_B-dependence pattern of H^E_{BB} to distinguish "hydrophobes" and "hydrophiles". Furthermore, H^E_{GlyGly} is only partially proportional to ${}^{SV}\delta_{Gly}$ up to point X. Namely,

$$ H^E_{GlyGly} = \eta {}^{SV}\delta_{Gly} + \zeta, \quad (5)$$

with appropriate constants, η and ζ. Thus the Gly–Gly interaction is only partially proportional with an extra constant term, ζ, the nature and property of which are not yet elucidated. Further investigations on its T- or p-dependencies or the effect of the third component on these constants are required. For this purpose, systematic determination of H^E_{GlyGly} and ${}^{SV}\delta_{Gly}$ is necessary.

Acknowledgements

This work was supported by the Carlsberg Foundation.

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