Effects of some salts on H2O as probed by a thermodynamic signature of glycerol: towards understanding the Hofmeister effects (VII)†

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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 H2O itself may not be denied all together. Namely, we devised a methodology whereby the effect of an ion on H2O 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 H2O 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 H2O 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 H2O by the presence of an ion.

Introduction

Ions and non-electrolyte solutes modify the molecular organization of H2O 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 H2O 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 H2O 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.11 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 H2O are interesting in their own right and important for

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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 H2O 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 H2O at the origin. For an individual species the former relates to its hydration number, nH, and the latter to its effect on the degree of the hydrogen bond probability of bulk H2O.20,21 ''Hydrophiles'', "hydrophobes'' also form hydration shells with somewhat enhanced 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 H2O.20,21 "Hydrophiles'', on the other hand, form hydrogen bonds directly with the hydrogen bond network of H2O and retard the degree of fluctuation inherent in pure H2O.18,22,23 Thus, we suggested that the effects of ions on H2O 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 H2O 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 H2O 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 realized20-22 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 H2O-rich region, H2O 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 H2O is retained inasmuch as the hydrogen bond network is connected fleetingly and yet permanently throughout the bulk. H2O is here understood as a highly fluctuating hydrogen bonded assembly and yet hydrogen bonds are bond-percolated.20,21,23,25,26

In the solute-rich region, the solute molecules tend to cluster together as in the pure state and H2O interacts with such clusters as a single gas-like molecule. In the intermediate region, two kinds of clusters, one rich in H2O and the other in solute, physically mix together. We name these three distinct mixing schemes Mixing Schemes I, II and III from the H2O-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 SO42−, OAc−, Cl−, and ClO4−. 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 H2O-rich region, MS I. This is based on our earlier findings that within this limited H2O-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 H2O 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 NH4+, (CH3)4N+(TMA+) and in addition NaCH3COO (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, $H^E_{\text{mix}}$ (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, $x_S$, $H^E_{\text{mix}}$ 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 H2O. (See Fig. 5 in the Appendix.) The peak top or the bend point that we name point X is where the integrity of liquid H2O is lost due to the presence of B at the value of $x_B$. We then observe how the $H^E_{\text{mix}}$ pattern changes as S is added while the characteristic pattern of $H^E_{\text{mix}}$ is retained. The induced changes, particularly those of the anomalous point, X, are indexed in two orthogonal directions in the graph of $H^E_{\text{mix}}$ 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 \( x_0 \)-axis) of point X per unit increase in \( x_0 \) is defined as hydrophobicity, \( a \). That of the southward shift (to the negative direction of \( H^{E}_{1P1P} \)-axis) is defined as hydrophilicity, \( b \). The shifts in both directions are found generally to be linear to \( x_0 \). By trying out a number of typical hydrophobes and hydrophilics 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 \( n_{H_2O} \), 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 \( 3.7 \pm 0.7 \) 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, \( H^{E}_{1P1P} \) is directly proportional to the partial molar \( S-V \) cross fluctuation density of 1P, \( \delta^{SV}_{1P1P} \), 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 \( H^{E}_{1P1P} \), its increase/decrease, is directly proportional to that of \( \delta^{SV}_{1P1P} \), \( H^{E}_{Gly} \), on the other hand, is only partially proportional to the equivalent \( \delta^{SV}_{Gly} \) with an extra constant term. \(^{23} \) Unless the behavior of the latter constant term is known, that of \( H^{E}_{Gly} \) cannot be directly connected to \( \delta^{SV}_{Gly} \). 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 hydrophilic reacts to the subtle modification of \( H_2O \) caused by the presence of ions within \( MS \), 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 \( \mu \)L syringe in a dry \( N_2 \) atmosphere for the titration calorimetry described below. \( NaCl \) (Merck, >99.8%), \( Na(CH_3)COO \) (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, \( x_0 \), immediately before use.

The excess partial molar enthalpy of Gly, \( H^{E}_{Gly} \), 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 \( H^{E}_{Gly} \) was estimated to be ±0.03 kJ mol\(^{-1} \).

### Results and discussion

Fig. 1 shows the excess partial molar enthalpy of Gly, \( H^{E}_{Gly} \), in the ternary Gly–S–H\(_2O\) at a given initial mole fraction of S in the mixed solvent, \( x_0 \). The raw data are given in Table S1 in the ESL. \(^{†} \) While \( H^{E}_{Gly} \) becomes more endothermic as \( x_0 \) increases for \( TMACl \), Fig. 1(b), and \( NaOAc \), Fig. 1(c), that for \( NH_4Cl \) shows a similar behavior at the low \( x_0 \) range but becomes more exothermic at high \( x_0 \) within the \( x_0 \) range studied. But for all cases, the slopes of \( H^{E}_{Gly} \) against \( x_0 \) seem to become less as \( x_0 \) increases. To see these trends more clearly, we evaluate \( H^{E}_{Gly} \) defined as,

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H^{E}_{Gly} = N(\partial H^{E}_{Gly}/\partial n_{Gly}) = (1 - x_{Gly})/(\partial H^{E}_{Gly}/\partial x_{Gly}),
\]

at given \( x_0 = n_{Gly}/(n_{S} + n_{W}) \). 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 \( H^{E}_{Gly} \), we perform graphical differentiation as for \( H^{E}_{1P1P} \) without resorting to curve-fitting an analytical function to the \( H^{E}_{Gly} \) data. By this treatment the random error in \( H^{E}_{Gly} \) 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 \( H^{E}_{Gly} \) data are plotted in Fig. 2. Fig. 2(a) shows \( H^{E}_{Gly} \) for the binary Gly–H\(_2O\). It is apparent beyond the estimated uncertainty that the \( x_{Gly} \)-dependence pattern of \( H^{E}_{Gly} \) 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 measured another third derivative quantity, the partial molar \( S-V \) cross fluctuation density of Gly in Gly–H\(_2O\), \( \delta^{SV}_{Gly} \), 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...
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\(^2\) in that as S is added and \(x_0\) increases the break point X becomes more obscure to note in the \(H_{\text{Gly}}\) patterns.

Fig. 2(b)–(d) show the results for the ternary Gly–S–H\(_2\)O systems. The binary Gly–H\(_2\)O 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\(_2\)O is lost. We found from our earlier studies\(^2\) that up to point X the integrity of liquid H\(_2\)O is retained such that the hydrogen bond network is still connected throughout the bulk H\(_2\)O. It is this dilute concentration range where the probing methodology by 1P or Gly is applicable\(^3\). 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\(_2\)O 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\(_2\)O, respectively, but leave the bulk H\(_2\)O away from hydration shells unperturbed. At least the same ions were shown not to alter the bulk H\(_2\)O away from hydration shells by femto-second pump probe spectroscopic studies. OAc\(^-\) is a “hydrophobe” that is hydrated by a total of 3.7 molecules of H\(_2\)O. The hydrogen bond probability within the hydration shells is enhanced somewhat, but that of the bulk H\(_2\)O away from the hydration shells is reduced progressively. The bulk H\(_2\)O has not yet lost the hydrogen bond percolation until the system reaches point X\(^3\).

The distinction between hydration centers and hydrophobes was apparent in that the behavior of \(H_{\text{1P1P}}\) at \(x_{1P} = 0\) was

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**Fig. 1** (a) Excess partial molar enthalpy of Gly, \(H_{\text{Gly}}\), in Gly–S–H\(_2\)O (S = NH\(_4\)Cl) at 25 °C. (b) Excess partial molar enthalpy of Gly, \(H_{\text{Gly}}\), in Gly–S–H\(_2\)O (S = TMACl) at 25 °C. (c) Excess partial molar enthalpy of Gly, \(H_{\text{Gly}}\), in Gly–S–H\(_2\)O (S = NaOAc) at 25 °C.
different in the 1P-probing studies.\textsuperscript{16,17} Namely, for the hydration centers, the values of $H_{1P1P}^E$ 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$_4$Cl, they remained constant. For the present Gly-probing study, on the other hand, NH$_4$Cl does not seem to stay constant as is evident in Fig. 2(b). The previous Gly-probing study\textsuperscript{24} indicates the same observation for NaCl also. This discrepancy between the 1P- and Gly-probing methodologies could be related to our findings that $H_{1P1P}^E$ is directly proportional to the solute’s effect on the $S$–$V$ cross fluctuation density,\textsuperscript{16,17} while $H_{GlyGly}^E$ 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 $H_{GlyGly}^E$ at $x_{Gly} = 0$ as $x_0^S$ increases.

TMA$^+$ was found to act as a hydrophile\textsuperscript{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$_2$O progressively by breaking the proton donor–acceptor symmetry of liquid H$_2$O. Probably reflecting this, Fig. 2(c) shows a little sharper decrease in the $H_{GlyGly}^E$ value.
at \(x_{\text{gly}} = 0\), \(H_{\text{Gly}}^E(0)\), than the other two salts. NaOAc, on the other hand, with a hydrophobic OAc\(^-\) ion shows a decrease in \(H_{\text{Gly}}^E(0)\), the value of \(H_{\text{Gly}}^E\) at \(x_{\text{gly}} = 0\). This contrasts with the behavior of \(H_{1P1P}^E\) observed in the 1P-probing study, where the equivalent \(H_{1P1P}^E(0)\) increased.\(^{16,17}\)

To see these trends at \(x_{\text{gly}} = 0\) more clearly, the \(H_{\text{Gly}}^E\) data are extrapolated linearly to \(x_{\text{gly}} = 0\) and evaluated \(H_{\text{Gly}}^E(0)\) 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 \(S = \text{Na}_2\text{SO}_4\), 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 \(H_{\text{Gly}}^E\) and \(H_{\text{Gly}}^E\) for \(S = \text{Na}_2\text{SO}_4\) are also given as Fig. S3(a) and (b) (ESI).

Since the data points for \(H_{\text{Gly}}^E\) at \(x_{\text{gly}} < 0.015\) 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. \(H_{\text{Gly}}^E(0)\) decreases as \(x_S^0\) increases, in contrast to the increase in \(H_{1P1P}^E(0)\), the value of \(H_{1P1P}^E\) at \(x_{1P} = 0.16,17\). This is only natural due to a geometrical reason. \(H_{1P1P}^E\) increases from \(x_{1P} = 0\) to point \(X\), while \(H_{\text{Gly}}^E\) decreases down to its point \(X\). A hydrophobic sample \(S\) will shift the \(H_{\text{Gly}}^E\) pattern including point \(X\) towards west, a smaller value of \(x_B\) (for \(B = 1P\) or Gly). Since a number, \(n_{\text{H}_2\text{O}}\), of \(H_2\text{O}\) 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 \(H_2\text{O}\) was found to be several times slower than that of bulk \(H_2\text{O}\).\(^3\) This westward shift will inevitably result in an increase in the value of \(H_{\text{Gly}}^E\) for a line with a positive slope (for \(B = 1P\)) and a decrease for that with a negative slope (for \(B = \text{Gly}\)), unless there is a mechanism to pin down \(H_{\text{BB}}^E(0)\) 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 \(H_{\text{Gly}}^E(0)\) 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 “hydrophilic”, shows a marginal decrease in \(H_{\text{Gly}}^E(0)\) 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, \(\text{NH}_4\text{Cl}\) and \(\text{NaCl}\), \(H_{\text{Gly}}^E(0)\) decreases slightly, by just above the uncertainty upon increasing \(x_S^0\). They showed no change in \(H_{1P1P}^E(0)\) 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 \(H_{\text{Gly}}^E(0)\) in spite of the fact that the total hydration numbers are different; \(n_{\text{H}} = 7.5\) for \(\text{NaCl}\) and 3.3 for \(\text{NH}_4\text{Cl}\). This could also hint that the Gly-probe is not as sensitive as the 1P-probe towards subtle modification of \(H_2\text{O}\) caused by the presence of \(S\). NaOAc, containing a hydrophobic anion, shows no difference in the decrease of \(H_{\text{Gly}}^E(0)\) with those of hydration centers. \(\text{Na}_2\text{SO}_4, \text{SO}_4^{2-}\) being a hydration center at \(x_{1P} = 0\) found by the 1P-probing,\(^{16,17}\) also shows the same trend. NaBr, Nal and NaSCN, consisting of \(\text{Na}^+\) and a hydrophilic anion with its hydrophilicity increasing in the order of \(\text{Br}^- < \text{I}^- < \text{SCN}^-\), do not show any difference among themselves nor from the hydration center group. TMACl is the only salt that stands
out in terms of its decrease in $H_{E_{Gly}}^B (0)$, 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. The decrease of $H_{E_{Gly}}^B (0)$ 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 $H_{E_{Gly}}^B$ and $x_{S}^{B_{Gly}}$. Now that point X for the present $H_{E_{Gly}}^B$ is hard to identify, we proceed our analysis by calculating the point X in the $H_{E_{Gly}}^B$ pattern assuming that the shifts of $x_{S}^{Gly}$ (X) and $H_{E_{Gly}}^B$ (X) are both linear to $x_{S}^0$ 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 $x_{S}^0$ to $x_{Gly}$ (X) = 0, $x_{S}^0 [0]$ is equal to 1/$n_{H} + 1$, and using the $x_{Gly}$, locus of point X for the binary Gly–H$_2$O determined in Fig. 2(a), we calculated the $x_{Gly}$-loci of point X at given $x_{S}^0$, 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 ESL.\(^{†}\) We then read off the value of $H_{E_{Gly}}^B$ in Fig. 2(b)–(d) for the present data and equivalent graphs of $H_{E_{Gly}}^B$ against the previous work\(^{24}\) at the calculated point X, $x_{Gly}$ (X). The $H_{E_{Gly}}^B$ 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 $H_{E_{Gly}}^B$ (X) is estimated to be ±2 kJ mol$^{-1}$. For all other salts in Fig. 4(a) except for Na$_2$SO$_4$ and TMACl, $H_{E_{Gly}}^B$ (X) may be regarded as remaining constant and independent of $x_{S}^0$, taking into account the estimated uncertainty, ±2 kJ mol$^{-1}$. For Na$_2$SO$_4$, $H_{E_{Gly}}^B$ (X) clearly increases as $x_{S}^0$ 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”.\(^{16,12\}}\) 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$_2$O 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 $H_{E_{B}}^B$ at point X is independent of the identity of the probe B. Namely, as $x_{B}$ increases and hence the available bulk H$_2$O decreases, there must be some inherent mechanisms due only to SO$_4^{2−}$ to reduce the hydrogen bond probability of bulk H$_2$O. Self-aggregation of SO$_4^{2−}$ could be a reason, as observed for urea above $x_{S}^0 > 0.08$ (S = urea),\(^{33}\) for the 1-butyl-2,3-dimethylimidazolium cation at $x_{S}^0 > 0.006^{14}$ and for the 1-butyl-3-methylimidazolium cation at $x_{S}^0 > 0.014^{15}$. However, they all show a sudden decrease in the slope of $H_{E_{B}}^B (X)$ vs. $x_{S}^0$ in

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$_2$O by the present Gly-probing, $H_{E_{Gly}}^B (X)$ at the presumed point X was read off the graph of $H_{E_{Gly}}^B$, Fig. 2(b)–(d). An assumption was made that the $x_{Gly}$-locus of point X is also linear to $x_{S}^0$ as the case of the 1P-probing.

<table>
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<tr>
<th>Salt</th>
<th>$n_{H}$</th>
<th>$x_{S}^0$</th>
<th>$x_{Gly}$ (X)</th>
<th>$H_{E_{Gly}}^B$ (X)</th>
</tr>
</thead>
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<tr>
<td>NH$_4$Cl</td>
<td>+</td>
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<td>0</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2.3</td>
<td>0.01282</td>
<td>0.06898</td>
</tr>
<tr>
<td></td>
<td>(tot)</td>
<td>3.3</td>
<td>0.02549</td>
<td>0.06500</td>
</tr>
<tr>
<td>TMACl</td>
<td>+</td>
<td>0</td>
<td>0</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>2.3</td>
<td>0.01250</td>
<td>0.06999</td>
</tr>
<tr>
<td></td>
<td>(tot)</td>
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<td>0.02561</td>
<td>0.06683</td>
</tr>
<tr>
<td>NaOAc</td>
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<td>5.2</td>
<td>0</td>
<td>0.073</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>3.7</td>
<td>0.01624</td>
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<tr>
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<td>0.06146</td>
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Fig. 4 (a) The values of $H_{E_{Gly}}^B$ at presumed point “X”, $H_{E_{Gly}}^B$ (X) against $x_{S}^0$ 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 $H_{E_{Gly}}^B$ at presumed point “X”, $H_{E_{Gly}}^B$ (X), against $x_{S}^0$ for non-electrolytes. Evaluated using the data in the previous Gly-probing study.\(^{24}\) The uncertainty is estimated to be ±2 kJ mol$^{-1}$. 

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\({}^{1}\) The issue of Gly-probing methodology has been raised in the recent work of the authors. The issue of S, taking into account the estimated uncertainty, 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”.\(^{16,12\}}\) 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$_2$O 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 $H_{E_{B}}^B$ at point X is independent of the identity of the probe B. Namely, as $x_{B}$ increases and hence the available bulk H$_2$O decreases, there must be some inherent mechanisms due only to SO$_4^{2−}$ to reduce the hydrogen bond probability of bulk H$_2$O. Self-aggregation of SO$_4^{2−}$ could be a reason, as observed for urea above $x_{S}^0 > 0.08$ (S = urea),\(^{33}\) for the 1-butyl-2,3-dimethylimidazolium cation at $x_{S}^0 > 0.006^{14}$ and for the 1-butyl-3-methylimidazolium cation at $x_{S}^0 > 0.014^{15}$. However, they all show a sudden decrease in the slope of $H_{E_{B}}^B (X)$ vs. $x_{S}^0$ in...
the respective 1P-probing studies. For Na2SO4 aqueous solutions, a dielectric relaxation study suggests the formation of H2O separated cation–anion pairing as its concentration increases,16 but this would also reduce $H_{\text{BB}}^E (X)$ rather than increase as observed here. We rather speculate that as the availability of un-hydrated bulk H2O decreases, SO4$_2^-$ ions may start to interact more strongly with the existing hydrogen bond network of bulk H2O rather than just forming hydration shells. This may be due to the fact that SO4$_2^-$ ions presumably spread O atoms out in four tetragonal directions. As a result, the average hydrogen bond probability of bulk H2O is reduced progressively. Recent studies using modern non-linear spectroscopic techniques aided by MD simulations37,38 revealed how the ClO$_4^-$ ion exchanges hydrogen bonds from a H2O molecule with another in concentrated aqueous solutions of about 0.1 mole fraction. At this concentration, there are therefore hardly any H2O molecules left to study the state of bulk H2O away from hydration shells. Similar studies on SO4$_2^-$ in H2O could provide an important clue with more dilute aqueous solutions so that the state of bulk H2O away from hydration shells could be studied.

In the case of TMACl, TMA$^+$ being hydrophilic, slightly more so than Gly16,28 and Cl$^-$ being a weak hydration center,16,17 the decrease in $H_{\text{GlyGly}}^E (X)$ reflects the effect of TMA$^+$ and indicates the reduction in the effect of the solute on the degree of fluctuation in the bulk H2O.

For NH4Cl and NaCl, the constituent ions are all hydration centers. Hence, these salts do not alter the bulk H2O 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 $x_O$. This is exactly what we observe in Fig. 4(a). For the 1P-probe, however, not only at point X but also at $x_P = 0$ the values of $H_{\text{BB}}^E$ were found to remain constant. For the present Gly-probe, the values of $H_{\text{GlyGly}}^E (0)$ at $x_{\text{Gly}} = 0$ 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 $n_{\text{H}} = 3.7$ and to reduce the hydrogen bond probability of bulk H2O 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 H2O molecules, respectively,16 and reduce the hydrogen bond probability of bulk H2O 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 H2O 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 $H_{\text{BB}}^E$. It was indeed the case for the 1P-probing, $B = 1P$, and the value of $H_{\text{BB}}^E (X)$ 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 $H_{\text{GlyGly}}^E (X)$ remain constant, independent of $x_O$ for both hydrophobes. Similarly, the values of $H_{\text{GlyGly}}^E (X)$ 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 $x_O$-dependence of $H_{\text{GlyGly}}^E (X)$ among them is not apparent. Thus, the Gly-probe appears to be insensitive to the difference in the modified H2O by hydrophilic as well as by hydrophobic. Or it could be due to the constant additive term in the partial proportionality of the partial molar $S$–$V$ cross fluctuation and $H_{\text{GlyGly}}^E (X)$ that makes the $H_{\text{GlyGly}}^E (X)$ appear insensitive.

Thus, while the extra constant term in the partial proportionality between $H_{\text{GlyGly}}^E$ and $SV\delta_{1P}$ must be measured and its nature ought to be elucidated, we suggest that the behavior of $H_{\text{GlyGly}}^E$ is not entirely inconsistent with the effects of S on H2O 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 H2O caused by the presence of an ion.

Appendix

Fig. 5 shows the plots of $H_{1P1P}^E$ and $SV\delta_{1P}$ for the binary 1P–H2O system. The ordinate for $SV\delta_{1P}$ is scaled by a single factor $\xi$. The definition of $H_{1P1P}^E$ is given in eqn (1) in the main text except for swapping subscripts Gly with 1P. $H_{1P1P}^E$ 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

$$SV\delta \equiv \langle (\Delta S') (\Delta V') \rangle / k (V') = T\xi_{1P}. \tag{2}$$

![Fig. 5](image-url) The partial molar $S$–$V$ cross fluctuation density of 1P, $SV\delta_{1P}$, and the enthalpic 1P–1P interaction, $H_{1P1P}^E$, in 1P–H2O at 25 °C. The ordinate for $SV\delta_{1P}$ is scaled by a single factor. Reproduced with permission from ref. 18.

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 ⟨S⟩ and ⟨V⟩ respectively. k is the Boltzmann constant, zp thermal expansivity and Vm the molar volume of the solution. This quantity is important for studying H2O and aqueous solutions in that it contains a negative contribution due to putative formation of ice-like patches in H2O, which contributes negatively to the S–V cross fluctuation density, SVδ. Its partial molar derivative is defined taking into account the fact that SVδ is an intensive quantity as

\[ SV_1P \delta = N(\overline{c}SV\delta /\overline{\rho}_1P) = (1 - x_{1P})(\overline{c}SV\delta /\overline{\rho}_1P). \]  

SV1Pδ so defined is regarded as the effect of solute 1P on the S–V cross fluctuation density of the entire system. Of course, SVGly1Pδ is also defined by swapping the subscript 1P with Gly.

What Fig. 5 indicates then is that H1P–1P1P and SV1Pδ are directly proportional with a single factor z within the dilute region up to point X; namely,

\[ H_{1P1P} = zSV_1P \delta. \]  

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 H2O.18,19 This finding was instrumental in devising the 1P-probing methodology.16,17 The initial increase in H1P1P up to point X is related to a net increase in SV1Pδ due to the decrease in the negative contribution in SVδ because the hydrogen bond probability of bulk H2O is reduced progressively by the presence of a hydrophobic solute 1P.20,21,31

The equivalent quantities of binary Gly–H2O, H GlyGly and SVGly are plotted in Fig. 6. The raw data of zp determined by dilatometry40 are used to calculate SVδ. A clear distinction is evident between Fig. 5 and 6 in their xP-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 xP-dependence pattern of H GlyGly to distinguish “hydrophobes” and “hydrophiles”. Furthermore, H GlyGly is only partially proportional to SVGly up to point X. Namely,

\[ H_{GlyGly}^E = \eta SV_{Gly} + \zeta, \]

with appropriate constants, η and ζ. Thus the Gly–Gly interaction is only partially proportional with an extra constant, 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 GlyGly and SVGly is necessary.

Acknowledgements

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References

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