The Effects of Chloride Salts of Some Cations on the Molecular Organization of H_2O. Towards Understanding the Hofmeister Series. II

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We use the thermodynamic behaviour of 1-propanol (1P) as a probe in ternary 1P–salt–H_2O systems to elucidate the effect of a salt on the molecular organization of H_2O. For salts, we have chosen $CaCl_2$, NH_4Cl, and $(CH_3)_4NCl$ (TMAC). Having fixed the counter anion at Cl^-, we compare here mainly the effects of chosen cations on H_2O. Together with an earlier study on NaCl, we found that Ca^{2+}, Na^+, and NH_4^+ are hydrated by a number of H_2O molecules and leave the bulk H_2O away from the hydration shell unperturbed. The hydration numbers were found to be 6.4 ± 1.6, and 1.2 ± 0.4, for Ca^{2+} and NH_4^+, respectively with the hydration number 5.2 for Na^+, the result of a simulation study, chosen as a reference. Thus, a salting out (also referred to as structure making, stabilizing, or kosmotropic) tendency would decrease in the order; $Ca^{2+} > Na^+ > NH_4^+$. TMA^+, on the other hand, showed a more hydrophilic characteristics than the probing 1P. Thus, TMA^+ is rather chaotropic (salting in, structure breaking or destabilizing) within this methodology.

We have developed a methodology for elucidating the effect of a solute on the molecular organization of H_2O, using the detailed thermodynamic behavior pertaining to 1-propanol (abbreviated as 1P) as a probe in ternary 1P–solute–H_2O.[1–9] This methodology was shown to be powerful in distinguishing particularly clearly the quantitative and the qualitative differences between hydrophobic and hydrophilic solutes.[9] In our recent paper called paper I,[1] we applied the same methodology to learn the effects of sodium salts of SO_4^{2-}, $OCOCH_3^-$ (OAc^-), Cl^-, ClO_4^-, and SCN^- on the molecular organization of H_2O. We discussed the effect of each anion in relation to the well accepted ranking of the Hofmeister series.[10–13] We showed that a kosmotrope (salting-out, structure making, or stabilizing agent) acts as a stronger hydration center than Cl^- or a hydrophobe, while a chaotrope (salting-in, structure-breaking, or destabilizing agent) was hydrophilic as urea,[6] and polyols.[7,9,14] Here, we study the effects of the chloride salts of $(CH_3)_4N^+$ (TMA^+), NH_4^+, and Ca^{2+} on the molecular organization of H_2O. Together with an earlier similar study for NaCl,[1,8] we discuss the effects of four cations on the molecular organization of H_2O, with the counter anion fixed at Cl^-.

We showed earlier, using the present methodology, that one Na^+ and one Cl^- are hydrated by 7.5 molecules of H_2O and that the bulk H_2O away from the hydration shell remains unaffected by the presence of the ions.[1,8] A recent first principle simulation indicated that the hydration number of Na^+ is 5.2 and that the bulk H_2O outside of the hydration shell is not affected by the presence of Na^+.[15] Since the conclusions are similar at least for Na^+, we tentatively take this hydration number, 5.2, for Na^+ as a reference standard. Hence, we assigned 2.3 for that of Cl^- in our work.[1,8] This analysis is continued below where we will make a reasonable estimate for uncertainty in the hydration numbers. We note that there are many estimates for hydration numbers by a variety of methods under a variety of assumptions. For example, the hydration number for Cl^- is assigned zero,[16,17] while that for Na^+ ranges from 3.9[16] to 0.2.[17] We adopt here, however, 5.2 for the hydration number for Na^+ within the present methodology.

Methodology

Our methodology was described in some detail in paper I.[1] Briefly, we determine experimentally the excess partial molar enthalpy of 1-propanol (1P), H_{1P}^E, in the ternary 1P–salt–H_2O system. H_{1P}^E is determined as the response in H^E of the entire system by perturbing the amount of 1P, n_{1P}, only. Hence, H_{1P}^E shows the actual contribution of 1P to the system. Or H_{1P}^E represents the actual thermodynamic situation of 1P in the system in terms of H^E. We measure H_{1P}^E accurately and in small increments in the mole fraction of 1P, x_{1P}. We are thus able to calculate within a few percent what we call the 1P–1P interaction in terms of enthalpy, $H_{1P\text{–}1P}^E$, defined as,

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$$H^{E}_{1P-1P} \equiv N\left(\frac{\partial H^{E}_{1P}}{\partial n_{1P}}\right) = (1 - x_{1P})\left(\frac{\partial H^{E}_{1P}}{\partial x_{1P}}\right), \qquad (1)$$

graphically without resorting to any fitting function. $N = n_{1P} + n_S + n_W$, where n_S, and n_W are the amounts of the chosen salt and H_2O, respectively. This quantity shows the response of H^E_{1P}, the enthalpic situation of 1P in the mixture, on perturbing the amount of 1P. Hence, H^E_{1P-1P}, a third derivative of G, signifies the 1P–1P interaction in terms of enthalpy.

We earlier devised the normalized entropy–volume cross fluctuation, ${}^{SV}\Delta$, using thermal expansivity, α_p, which is a second derivative of G.[18] Namely,

$${}^{SV}\Delta \equiv RT\alpha_p/V_m, \qquad (2)$$

where V_m is the molar volume. ${}^{SV}\Delta$ contains information about the peculiarity of liquid H_2O. Unlike normal liquids, in which fluctuation with volume increase is consistently associated with entropy increase, H_2O molecules fluctuate with volume increase associated with entropy decrease due to hydrogen-bond formation, in addition to the normal liquid behavior. Thus, the behavior of ${}^{SV}\Delta$ reflects this well-known peculiarity of H_2O. We then define and evaluate the partial molar normalized fluctuation of 1P as,

$${}^{SV}\Delta_{1P} \equiv (1 - x_{1P})(\partial^{SV}\Delta/\partial x_{1P}). \qquad (3)$$

This quantity, a third derivative of G, shows the effect of 1P on the entropy–volume cross fluctuation in aqueous solution. It thus reflects how the peculiarity of H_2O is modified as the concentration of 1P increases. We showed that the x_{1P}-dependence pattern of H^E_{1P-1P} matches exactly with that of ${}^{SV}\Delta_{1P}$ on scaling the ordinate.[18] Hence, we concluded that the 1P–1P enthalpic interaction and the effect of 1P on the cross fluctuation share the same cause and the interaction between 1P molecules is mediated via the bulk H_2O which is somewhat modified overall yet its integrity retained.

Using these third derivative quantities, Eq. 1 and its entropy and volume analogues and Eq. 3 together with other fluctuation functions, we studied a complex mixture, the 1P–H_2O system. Generally for aqueous non-electrolytes including 1P–H_2O, we showed[3–5,19] that there are three distinctively different mixing schemes operating depending on the composition. We call them Mixing Scheme I, II, and III, starting from the H_2O-rich side. In Mixing Scheme I, H_2O protects its integrity against the invading solute and the hydrogen-bond network is retained. The manner in which H_2O does this depends on the characteristics of the solute. A hydrophobic solute such as 1P enhances the hydrogen-bond network of H_2O in its immediate vicinity ("iceberg formation"), but the hydrogen-bond probability of bulk H_2O away from the "icebergs" is reduced.[3–5,18,19] However, the long-range hydrogen-bond connectivity is still intact. As the composition of solute increases and the bulk hydrogen-bond probability decreases to a threshold value, the hydrogen-bond percolation is broken and this integrity of H_2O is lost. Thereupon Mixing Scheme II pertinent to the intermediate composition region sets in. The solution then consists of two kinds of clusters, one rich in H_2O and the other in 1P. The process of reaching this cross over point is depicted by the increase in H^E_{1P-1P} and ${}^{SV}\Delta_{1P}$ as sketched in Fig. 1 (the raw data are shown in Fig. 2 for the zero salt mole fraction). Namely, the process of 1P's modifying H_2O is shown in the initial increase in H^E_{1P-1P} up to point X. Or alternatively, the negative contribution due to the uniqueness of H_2O in ${}^{SV}\Delta_{1P}$, Eq. 3, decreases and hence the net value of ${}^{SV}\Delta_{1P}$ increases to point X. We discussed earlier at some

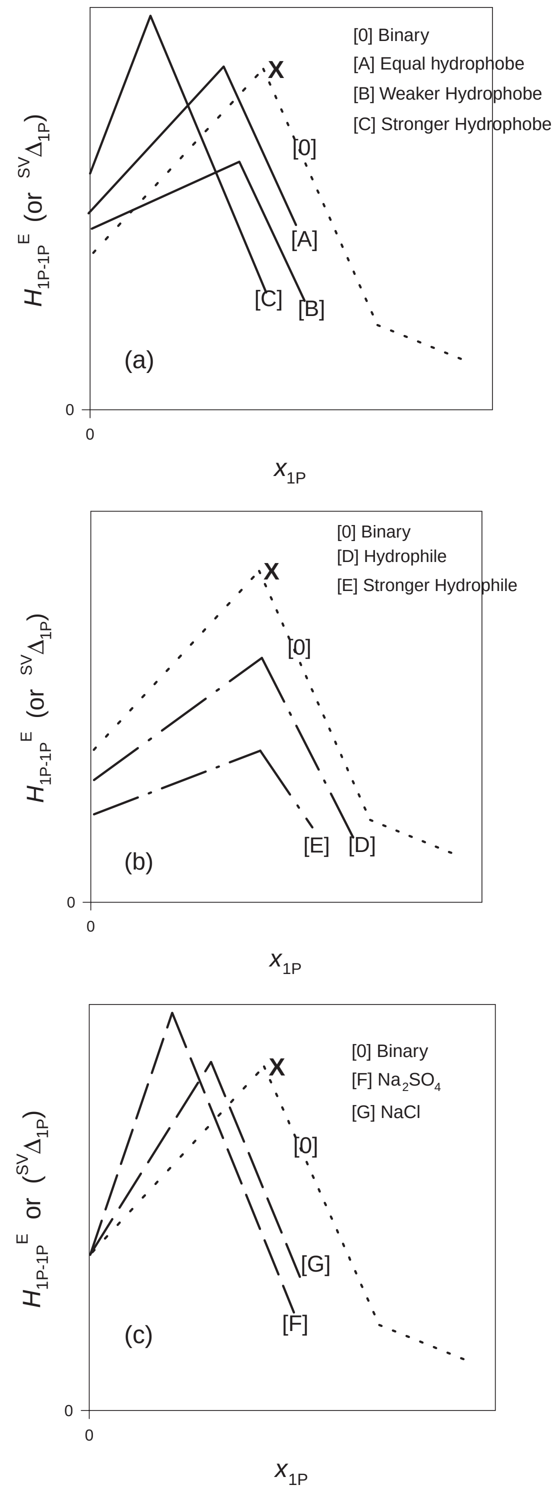

Fig. 1. Sketch of the x_{1P}-dependence of H^E_{1P-1P} (or ${}^{SV}\Delta_{1P}$) for binary 1P–H_2O [0], and ternary 1P–solute–H_2O. (a) For hydrophobic solutes, (b) for hydrophilic solutes, and (c) for hydration centers.

length and concluded that point X in the figure marks the onset of the transition of mixing scheme from I to II.[3–5,18,19]

We stress that in Mixing Scheme I the solute–solute interaction is bulk H_2O mediated.[3–5,18,19] It is the somewhat modified but still hydrogen-bond percolated H_2O that dictates the solute–solute interaction. This is true between different solute molecules for multi-component aqueous solutions[20,21] as well as the same solutes. These findings contribute to the basis of the present methodology. Namely, the changes induced in the pattern of H^E_{1P-1P} vs x_{1P} on addition of a salt reflect what the added salt does to H_2O, and are used as a probe to elucidate its effects on H_2O, as long as the system remains within Mixing Scheme I.

We have used this methodology in clarifying the effects of mono-ols,[9,14,22] poly-ols,[7,9,14] polyethylene glycol,[9] salts,[1,8] and urea.[6] From the comparative studies of these results, we summarize qualitatively the effects of a hydrophobe, a hydrophile, and a hydration center on the pattern of H^E_{1P-1P}, which in turn are the key information in elucidating their influences on the molecular organization of H_2O. Figure 1 shows such a sketch. When a small enough amount of a hydrophobe to keep the entire system still in Mixing Scheme I is added, the original H^E_{1P-1P} pattern for binary 1P–H_2O, [0] in the figure, shifts to the left, to a smaller value of x_{1P}, as shown as [A], [B], or [C]. For an equally hydrophobic mono-ol as the probing 1P, i.e. 2-propanol, the value of H^E_{1P-1P} at the peak remains the same,[22] [A]. For a weaker (methanol),[14] or a stronger (*tert*-butanol) hydrophobe,[9] the value of H^E_{1P-1P} at the peak is lower or higher, respectively, as shown as case [B] or [C]. We note that the value of H^E_{1P-1P} at the start, $x_{1P} = 0$, increases necessarily due to the shift to the left. This behavior was attributed to the fact that the third component hydrophobe has modified H_2O in a similar manner as 1P would have, and hence 1P is left to modify H_2O for the rest of the way to point X at a smaller value of x_{1P}.[9,14,22] For a hydrophile, Fig. 1b, the main effect is to reduce the value of H^E_{1P-1P} without changing the x_{1P}–locus of point X, as typified by urea.[6] This pattern change was attributed to forming hydrogen bonds directly to the network of H_2O and reducing the degree of fluctuation inherent in liquid H_2O.[6] The effects of amphiphiles were found to work in an additive manner of hydrophobic and hydrophilic moieties.[9] We showed that the hydrophilicity of the ether –O– and the hydroxy –OH give the same effect on the H^E_{1P-1P} pattern change. For hydrophobic moiety, the number of carbon atom in the alkyl chain is the main factor, except for that of the methyl group that provides a stronger hydrophobicity.[9] Figure 1c shows the effect that was observed for NaCl,[8] and Na_2SO_4.[1] For Na_2SO_4, case [F], we discussed at some length in paper I[1] as a special case of a hydration center. Here, we pay attention to NaCl, case [G], for its relevance to the present study. The behavior of the H^E_{1P-1P} pattern of case [G] in Fig. 1c was attributed to the hydration by Na^+ and Cl^-. The hydrated H_2O molecules are no longer available for 1P to interact and point X is reached at a smaller value of x_{1P}. From the proportionality of the decrease in x_{1P} at point X, the hydration number can be estimated.[1,8] We note that the values of H^E_{1P-1P} at the start ($x_{1P} = 0$) and at point X remain the same. This suggests that the bulk H_2O away from the hydration shell is not affected by the presence of Na^+ and Cl^-. Thus, we use behavior [G] in Fig. 1c as a signature for a hydration center that leaves the bulk H_2O outside of the hydration shell unperturbed, within the present methodology. In the same token, the wordings we use, "hydrophobic" and "hydrophilic" pertain to the behaviors of H^E_{1P-1P} sketched in Figs. 1a and 1b, respectively.

In the following, we use the same methodology for elucidating the effects of $CaCl_2$, NH_4Cl, and $(CH_3)_4NCl$ (TMAC). We compare the resulting pattern changes in H^E_{1P-1P} with Fig. 1, and make an attempt at elucidating the effects of these chlorides on the molecular organization of H_2O.

Experimental

Tetramethylammonium chloride (TMAC), (Wako. Special grade, 98.0%) and NH_4Cl (Wako, Special grade, 99.0%) were used as supplied. Stock solutions were prepared by dissolving the samples from freshly opened bottles. They were diluted as required. $CaCl_2{\cdot}2H_2O$ (Analar, Analytical reagent, BDH) was used from an old bottle and its stock solution was subjected to a standard EDTA titration to determine its concentration. 1-Propanol (1P) (ACROS Organic, >99.5% or Wako, Special grade, >99.5%) was used in a dry nitrogen atmosphere throughout the calorimetric measurement.

The excess partial molar enthalpy of 1P, H^E_{1P}, was determined by a home-made titration calorimeter of a similar design to an LKB Bromma 8700.[23] The uncertainty is estimated as ±0.03 kJ mol^{-1}. The liquid–liquid phase boundary was determined by conventional turbidity titration for 1P–$CaCl_2$–H_2O. For this determination, $CaCl_2{\cdot}2H_2O$ (Sigma-Aldrich, 99+%, ACS Reagent) was used from a freshly opened bottle. For the other two ternary systems of TMAC and NH_4Cl, a novel detection method of phase separation, developed by some of us,[24] was used.

Results and Discussion

The measured values of the excess partial molar enthalpy of 1P, H^E_{1P}, are plotted in Fig. 2 against the mole fraction of 1P, x_{1P}. The arrows in the figure indicate the liquid–liquid phase separation. By comparison with equivalent plots in paper I[1] in which the third components studied were Na-salts of $SO_4{}^{2-}$, OAc^-, $ClO_4{}^-$, and SCN^-, the differences in the effect among the present cation chlorides is less conspicuous. In order to identify the subtle differences among Fig. 2a to Fig. 2c we calculate the enthalpic interaction, H^E_{1P-1P}, by Eq. 1. We draw a smooth curve through all the data points by means of a flexible ruler, and read the values of H^E_{1P} off the smooth curve drawn at the interval of $\delta x_{1P} = 0.004$. We then make the approximation,

$$\left(\frac{\partial H^E_{1P}}{\partial x_{1P}}\right) \approx \left(\frac{\delta H^E_{1P}}{\delta x_{1P}}\right), \tag{4}$$

with $\delta x_{1P} = 0.008$. The reliability of this approximation has been checked earlier and proved to be satisfactory within an uncertainty of ±10 kJ mol^{-1}.[7] Alternatively, we could use a computer graphics technique, the cubic B-spline method, for this purpose. However, we did not do so for the present study for the reasons discussed in the Appendix.

The results are plotted in Fig. 3. In view of the uncertainty in H^E_{1P-1P}, the maximum, point X in the figure was determined as the intersect of two straight lines extended from both sides of the H^E_{1P-1P} peak. The H^E_{1P-1P} plots for NaCl are revisited from paper I with typographical errors corrected, in Fig. 3d. It is now clear that the effect of TMAC, Fig. 3a is qualitatively

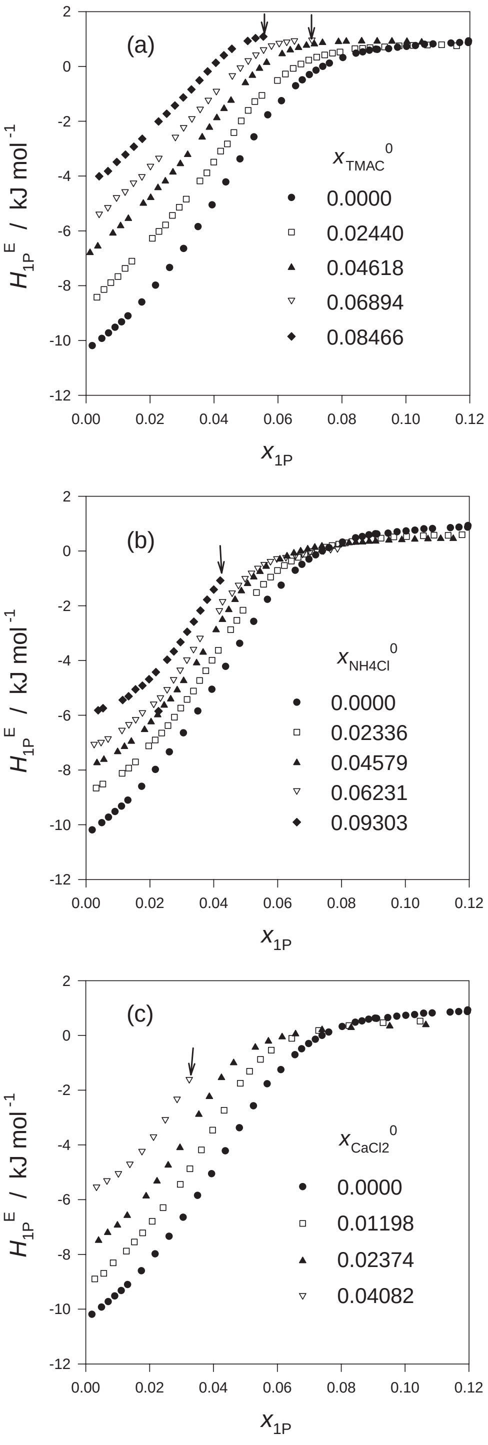

Fig. 2. Excess partial molar enthalpy of 1P, H_{1P}^{E}, against mole fraction of 1P, x_{1P}. (a) For 1P–TMAC–H_2O, (b) 1P–NH_4Cl–H_2O, and (c) 1P–$CaCl_2$–H_2O at 25 °C. $x_{salt}{}^0$ is the initial mole fraction of salt before addition of 1P.

different from those of NH_4Cl, Fig. 3b, and $CaCl_2$, Fig. 3c. The induced changes in the pattern of $H_{1P\text{–}1P}^{E}$ for the latter two salts are similar to that for NaCl,[1,8] Fig. 3d, which is sketched as case [G] in Fig. 1c. For NH_4Cl, Fig. 3b, the value of $H_{1P\text{–}1P}^{E}$ at the start, at $x_{1P} = 0$, might have a systematic decrease. The total deviation, however, is about the size of the uncertainty, and it would be safe to conclude that the value of $H_{1P\text{–}1P}^{E}$ at the start remains the same in comparison with $CaCl_2$, Fig. 3c, and NaCl, Fig. 3d. Namely, NH_4Cl acts as a hydration center together with NaCl and $CaCl_2$, as depicted as [G] in Fig. 1c.

In Fig. 4 are plotted the initial mole fraction of salt, $x_{salt}{}^0$, and the x_{1P} locus of point X in Fig. 3 together with the liquid–liquid phase boundary for a ternary 1P–salt–H_2O. The uncertainty in x_{1P} of the locus of point X is estimated as ±0.005, and that for the phase boundary is better being experimentally determined, ±0.0005. We note in Fig. 4 that the mixing scheme boundaries between Mixing Schemes I and II are straight lines, which assures proportionality between $x_{salt}{}^0$ and the amount hydrated. From this proportionality, the hydration number, n_H, can be estimated. Or alternatively, the $x_{salt}{}^0$ intercept of the Mixing Scheme boundary would be the value of $x_{salt}{}^0$ at which the entire bulk of H_2O were to be consumed for hydration. It follows that the $x_{salt}{}^0$ intercept is equal to $1/(1 + n_H)$. Considering the error bars in the figure, the hydration numbers are estimated as 3.5 ± 0.4, and 11.0 ± 1.6 for NH_4Cl and $CaCl_2$, respectively. For NaCl, when revisited using Fig. 4d, the uncertainty is estimated as ±0.6; hence n_H for NaCl is 7.5 ± 0.6. If we take the hydration number of Na^+ to be 5.2,[15] as our standard, then that for Cl^- would be 2.3 ± 0.6 within the present method of hydration number estimation. The results are listed in Table 1. There are a great many estimates by various methods under various assumptions in the literature. For example, the hydration number for Na^+ varies from 3.9[16] to 0.2,[17] Ca^{2+} 12[16] to 2[17] and $NH_4{}^+$ 1.8[16] to 0.[17] We stress here that the hydration numbers in Table 1 are estimates by the present methodology taking a theoretical estimate for Na^+ to be 5.2 as a reference standard. This should suffice for the purpose of the present comparative study.

There has been a great deal of controversy regarding the effect of tetraalkylammonium ion on H_2O. In the following discussion, we use for describing the effect of a solute on H_2O the terminology, "hydrophobic" and "hydrophilic" throughout. The former means loosely what an apolar moiety does to H_2O and includes "structure making" or "kosmotropic (or salting out)" used in the literature, while the latter describes a function of polar moiety, and the terms like "structure breaking," or "chaotropic (or salting in)" could be included. From our previous studies,[3–5] by "hydrophobic solute" we mean a solute that enhances the hydrogen-bond network of H_2O in the immediate vicinity of the solute with a concomitant reduction of the hydrogen-bond probability of the bulk H_2O away from the solute.[3–5] A "hydrophilic solute," on the other hand, forms hydrogen bonds to the existing network of H_2O. However, the degree of fluctuation inherent in H_2O is reduced by breaking the H donor/acceptor symmetry of H_2O in the hydrogen-bond network of pure H_2O.[5,7,14,25] Within the present 1P-probing methodology, the hydrophilicity manifests itself in the $H_{1P\text{–}1P}^{E}$ behavior shown in Fig. 1c. Following this terminology, heat capacity measurements[26–28] indicate that TMA^+ appears to work as a hydrophobic solute. The partial molar volume (apparent molar volume at very dilute solution) also points to the same conclusion.[28] Neutron scattering studies, on the other hand, provided mixed conclusions.[29,30] A molecular dynamic simulation did not give a clear conclusion whether TMA^+ is "hydrophobic" or "hydrophilic."[31] For ternary tetrapropyl hal-

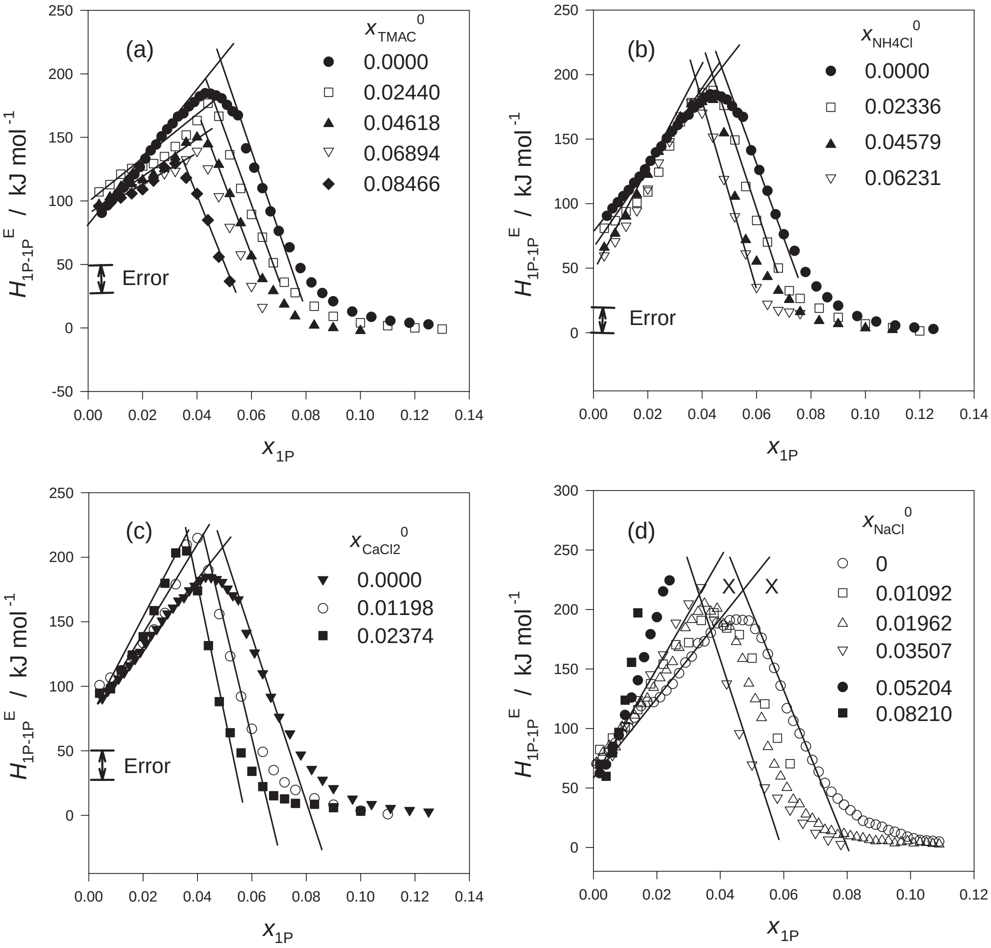

Fig. 3. Enthalpic interaction function, H^E_{1P-1P}. (a) For 1P–TMAC–H_2O, (b) 1P–NH_4Cl–H_2O, and (c) 1P–$CaCl_2$–H_2O. (d) 1P–NaCl–H_2O at 25 °C, reproduced from Ref. 1 correcting for typographical errors in the original.

ides–alcohol–H_2O systems, which are relevant to the present study, vapor–liquid equilibrium measurements indicated that tetrapropyl bromide works as a hydrophilic solute for the range $x_{1P} < 0.4$, but as a hydrophobic one above this mole fraction.[32,33] In the present work, which is limited to the range $x_{1P} < 0.15$, TMA^+ should be hydrophilic, since a methyl group is less hydrophobic than a propyl group. For ethanol–TMA–halides–H_2O, conductance and viscosity measurements suggested that TMA^+ is a hydrophilic solute.[34]

Turning back to our results for TMAC, Figure 3a indicates that TMA^+ is indeed a hydrophilic ion, as in Fig. 1b, if Cl^- is a hydration center that is responsible for shifting point X to the left (As shown below, it is indeed the case that Cl^- shifts Point X to the left with the same hydration number, 2.3.). Similar to urea[6] and glycerol,[7] the induced changes in the pattern of H^E_{1P-1P}, Fig. 3a, suggest that TMA^+ retards the degree of fluctuation. While the positive charge on the N atom may be a cause of hydrophilic behavior, this is still puzzling in view of the four methyl groups, hydrophobic moieties. We recall our previous studies for the binary tetramethylurea (TMU)–H_2O[35] and the ternary 1P–TMU–H_2O systems.[36] The H^E_{1P-1P} pattern in the ternary 1P–TMU–H_2O[36] was similar to case [B] at low concentration of TMU, but turned to case [D] in Fig. 1b on increasing the amount of TMU, in spite of the four methyl groups. Thus, we conclude that TMA^+ is basically a hydrophilic species. However, whether a solute is hydrophobic or hydrophilic could be a matter of relativity. Methanol (ME) is known to work as a hydrophobic solute, though the weakest among mono-ols from our earlier extensive studies on aqueous alcohols.[3–5] The H^E_{1P-1P} in ternary 1P–ME–H_2O[14] is sketched as case [B] in Fig. 1a, which also shows the decrease in the value of H^E_{1P-1P} at point X. These observations may hint that it is a competition between a hydrophobic and a hydrophilic moieties for interaction with H_2O that dictates the overall results. Accordingly, case [B] in Fig. 1a is simply a manifestation of the fact that ME is more hydrophilic, or less hydrophobic, than the probing 1P. In a similar fashion, Fig. 3a might suggest that TMA^+ is less hydrophobic, or more hydrophilic than 1P.

Another point to note in Fig. 3a is that in addition to the reduction of the values of H^E_{1P-1P}, there is a trend of the shift to the left of point X as TMAC is added. Is this due to the hydration of Cl^-? If so, the Mixing Scheme boundary should be a straight line and the intercept should give the hydration number 2.3 for Cl^-. Indeed, as shown in Fig. 4a, the boundary could be regarded as a straight line and that the value of intercept is estimated as 0.325 ± 0.038, which translates to the hydration number of 2.1 ± 0.4 for Cl^-. Thus, we recover the

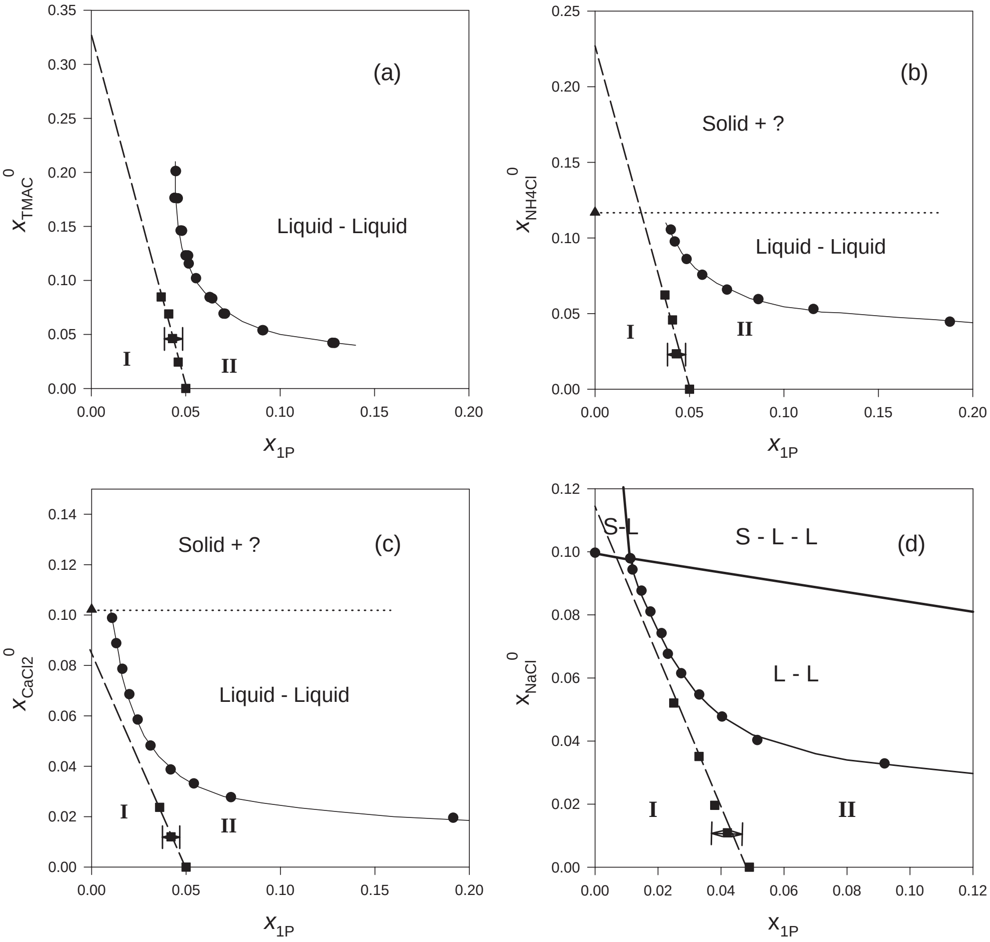

Fig. 4. Phase and mixing scheme diagram. Filled circles indicate phase boundary, filled square symbols mixing scheme boundary, and triangle symbols precipitation. (a) For 1P–TMAC–H_2O, (b) 1P–NH_4Cl–H_2O, (c) 1P–$CaCl_2$–H_2O, and (d) 1P–NaCl–H_2O at 25 °C.

Table 1. The Hydration Numbers, n_H of Ions by Using the Present Methodology

Ion	Hydration number, n_H	Reference
Na^+	5.2 (reference standard)	15
Cl^-	2.3 ± 0.6	1, 8
Ca^{2+}	6.4 ± 1.6	This work
NH_4^+	1.2 ± 0.4	This work

same hydration number for Cl^-. We therefore conclude that within the present methodology, the effect of TMA^+ is not a hydration center, but is to reduce the value of $H^E_{1P\text{–}1P}$ in a similar manner as a hydrophilic moiety as urea or glycerol. The counter anion, Cl^- is to shift the locus of point X to the left by hydration of (2.1 ± 0.4) molecules of H_2O. Namely, Cl^- strips 2.3 molecules of H_2O out of the hydrogen-bond network of H_2O, while TMA^+ tacks onto the existing hydrogen-bond network H_2O and retards the degree of fluctuation inherent in H_2O.

At high concentrations regions of $x_{salt}{}^0$, the liquid–liquid phase separation occurs at $x_{1P} = 0.05$, 0.035, 0.02,[1] and 0.015 for TMAC, NH_4Cl, NaCl, and $CaCl_2$, respectively as shown in Fig. 4. Namely, the salting out effect on 1P ranks in the order of $Ca^{2+} > Na^+ > NH_4^+ > TMA^+$ assuming that the effect of Cl^- remains the same. This is consistent with the Hofmeister ranking scheme for cations summarized by some,[37,38] but not by others.[11,13] The hydration number for Ca^{2+}, Na^+, or NH_4^+ listed in Table 1 also gives a consistent ranking for the former Hofmeister series.[37,38] Namely, Ca^{2+} is hydrated by more H_2O than Na^+ which is in turn more hydrated than NH_4^+, and hence for the Ca^{2+} case the lesser H_2O is available for proteins to dissolve in.

Figure 5 indicates the loci of point X of the four cation chlorides. Crudely put, the hydrophobic signature shows up in the decrease in the value of x_{1P} at point X, while that of hydrophilicity appears as a decrease in the value of $H^E_{1P\text{–}1P}$ at point X. If any trend is extracted from Fig. 5 at all, it is in the order of $TMA^+ > NH_4^+ > Na^+ > Ca^{2+}$ that shows hydrophilicity, which is associated with the salting-in or chaotropic power, as urea is ranked at the chaotropic end in the Hofmeister series.[11–13] The hydrophilic behavior in the $H^E_{1P\text{–}1P}$ pattern was also apparent in paper I[1] for $ClO_4{}^-$ and SCN^- together with the typically hydrophilic urea which are ranked near to the chaotropic end of the anion series. Thus, within the present study, the ranking of cations from the kosmotrope to the chaotropes is $Ca^{2+} > Na^+ > NH_4^+ > TMA^+$.

We point out that the above ranking is only relative to 1-propanol whose thermodynamic behavior is used as a probe.

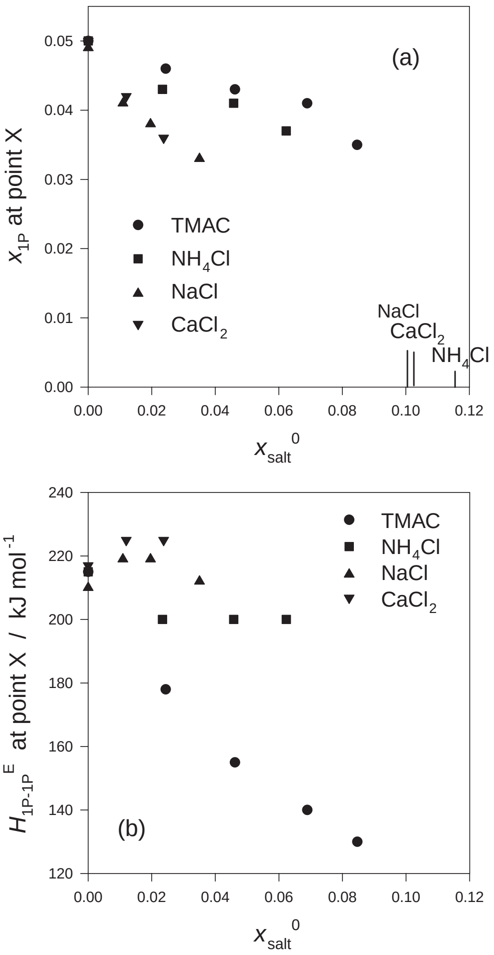

Fig. 5. Locus of point X against the initial mole fraction of the added salt. (a) In terms of x_{1P}, and (b) in terms of H^E_{1P-1P} The bars on the abscissa indicate the saturation point of each salt in H_2O at 25 °C.

Thus, the chaotropic anions studied in paper I[1] should be said to be more hydrophilic than 1P. Similarly, TMA^+ is more hydrophilic than 1P, in spite of the presence of three methyl groups. In the present study, together with paper I,[1] we did not control pH. Furthermore, the salt concentrations are in the range from about 0.5 to 6 M. Whether the cation tendency we found here is altered when the above conditions are changed and the ranking becomes different is yet to be studied. After all, biopolymers are better regarded as colloidal systems,[39] and their surfaces are covered with hydrophilic and hydrophobic moieties including local charges.[40] No doubt that each part interacts with mixed aqueous solvents in a qualitatively different manner. It would thus be interesting if a typical hydrophilic substance is used as a probe instead of 1-propanol. Such a project is now in progress.

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Supporting Information

The H^E_{1P} data as Table S1. This material is available free of charge on the web at http://www.csj.jp/journals/bcsj/.

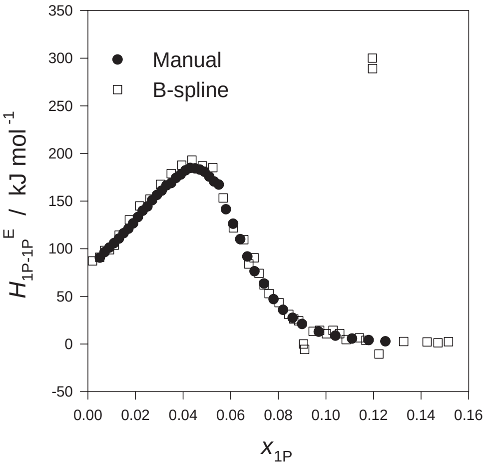

Fig. 6. Cubic B-spline vs manual graphing. Satisfactory case. However, the two bad points at $x_{1P} \approx 0.12$ indicates a weakness of B-spline method.

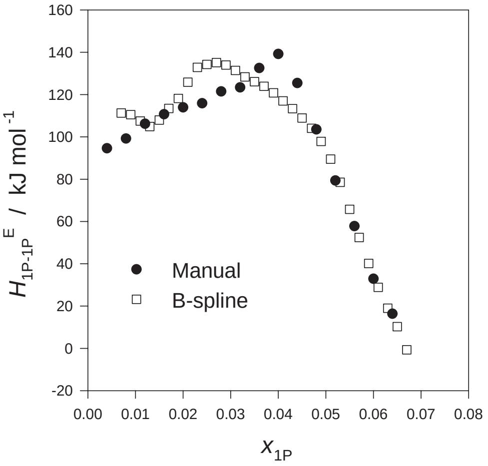

Fig. 7. Cubic B-spline vs manual graphing. Unsatisfactory case.

Appendix

Graphical Differentiation by Means of B-Spline. In computer graphics, it is common to draw a smooth curve through data points using the B-spline method. We have been doing manual graphing using a flexible ruler followed by reading the data off the smooth curve drawn at a given interval. These data are used to approximate the slope of the mid point. The reliability of such an approximation is discussed elsewhere at some length.[7,41] The advantage of manual graphing is that human judgment in the quality of each data point is readily applicable. Figure 6 shows the H^E_{1P-1P} values for the best data set to our knowledge. As is evident the standard cubic B-spline and our manual graphing provide the same H^E_{1P-1P} plots within an acceptable uncertainty. It is apparent from the figure, however, in the region where the slopes are close to zero, i.e. $x_{1P} > 0.08$, the resulting slopes by B-spline are unstable. Note the two bad points at about $x_{1P} \approx 0.12$. Another data set, in which a number of data points were known to be taken at a temperature higher than the set point by an unknown degree, were subjected to the cubic B-spline procedure. The results are shown in Fig. 7 together with the manual graphing method applying human judgment as to how much to correct for these data points.

There are more recent advancements in B-spline,[42] which allow uncertainties in both abscissa and ordinate and also flexible boundaries. However, for this newest method to work the curve to be interpolated must not contain an inflection point.[42] This is not suitable for the present purpose, since we seek an inflection point in our data. Further developments in a more robust computer interpolation technique are awaited, in view of the tediousness of manual graphing and subsequent reading the data off the smooth curve drawn.

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