PCCP

Accepted Manuscript

Volume 1 | Number 1 | Jan 2013 | Pages 1–100

PCCP

Physical Chemistry Chemical Physics

www.rsc.org/pccp

ROYAL SOCIETY OF CHEMISTRY

www.rsc.org/pccp

Journal Name

ARTICLE

Is seven the minimum number of water molecules per ion pair for assured biological activity in ionic liquid/water mixtures?

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

www.rsc.org/

Hiroyuki Ohno,[a,b,c] * Kyoko Fujita,[a,b,c] Yuki Kohno,[a,b]

Ionic liquids (ILs) containing small amount of water are called hydrated ILs and they show diverse physico-chemical properties that are strongly dependent on their water content. Some properties of hydrated ILs, such as biological activity and phase transition behaviour, were found to change non-linearly, with an inflection at a water molecule to ion pair ratio of around 7:1. This critical hydration number of ILs has been discussed in this paper with respect to the state of solvated water molecules.

1. Introduction

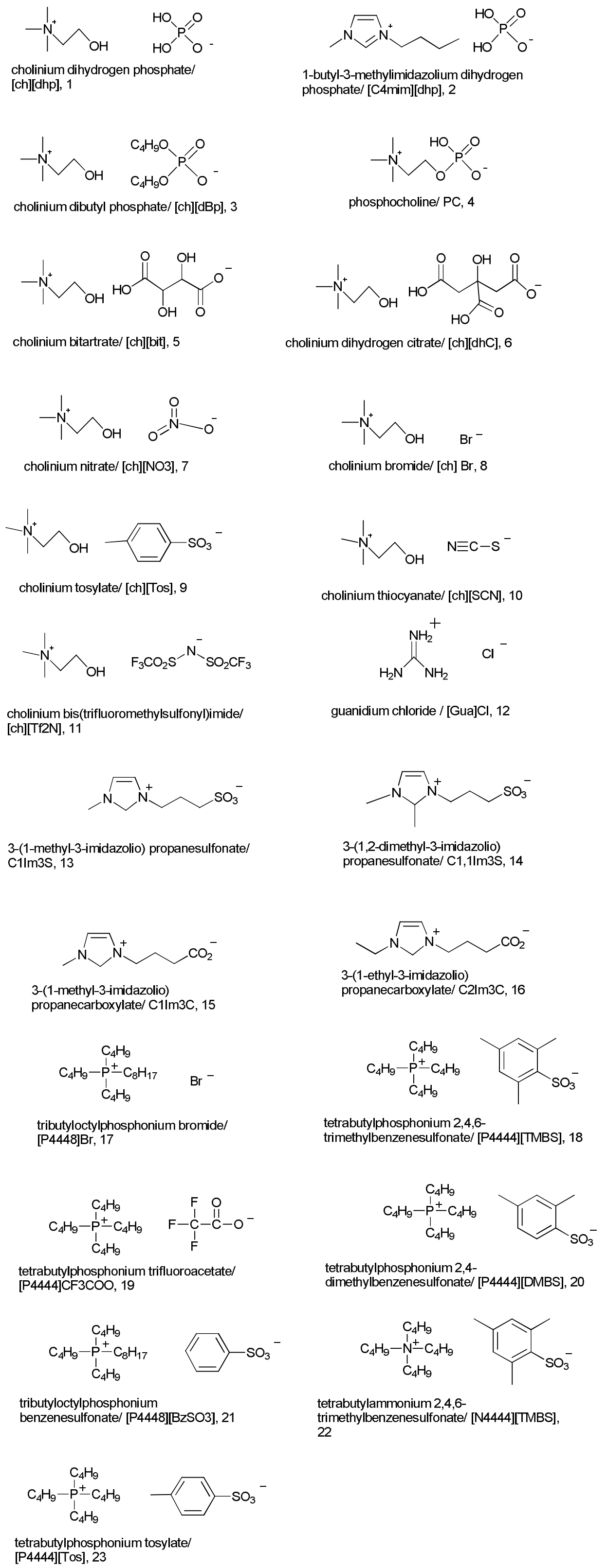
Ionic liquids (ILs) have potential applications as solvents in a variety of areas.[1,2] The properties of ILs can be controlled to some extent by appropriate structural design of their component ions. For example, polymers with poor solubility such as cellulose,[3] or biopolymers that are easily denatured such as proteins,[4] have been successfully dissolved in some ILs by suitably designing the structure of the component ions. In the case of protein dissolution, the component ions need to be designed such that higher ordered structures of proteins are retained while the proteins are dissolved. However, it is quite difficult to fine-tune the physico-chemical properties of ILs by modifying the structure of the component ions alone, which discontinuously change the properties of the ILs. An alternate method for tailoring the properties of ILs that has been gaining attention is the mixing of two different ILs. In this method, the properties of the ILs could possibly be altered by simply changing the mixing ratio of the component ILs.[5] An additional method for tuning the properties of ILs is by mixing them with molecular liquids, especially water. IL/water mixtures have been studied in the context of bio-related applications[6,7] including liquid/liquid biphasic systems such as biocatalytic reactions[8] and as extraction media.[9] Although there are many reports on aqueous solutions containing a small amount of ILs, we believe that the unique properties of ILs cannot be sufficiently harnessed in such dilute solutions and similar results could easily be achieved in aqueous solutions of ordinary salts. To exploit the remarkable properties of ILs, water must be added only in small amounts, so as to retain the basic properties of the ILs.

From this point of view, we have been investigating hydrated ILs, which are prepared by mixing ILs with small amounts of water, as potential solvents for proteins. In hydrated ILs, the ions are strongly hydrated and no “free” water molecules exist.[10,11] Hydrated ILs maintain most of the inherent properties of neat ILs, and in some cases, their physico-chemical properties including viscosities are improved as a result of mixing with a small amount of water. Most proteins are, however, difficult to dissolve in typical ILs, even after adding water. It has been shown that the solubility and structure of proteins in ILs containing water are affected by the kosmotropicity of the component ions.[10] Keeping this in view, we have carefully selected component ions that have good compatibility with biomolecules and have mixed the synthesised ILs with small amounts of water to prepare hydrated ILs. Excellent stability of the proteins was achieved in some of the hydrated ILs. In addition, the proteins were found to retain their functionality even after being left mixed with the hydrated ILs at room temperature for over a year.[10,11] It has been suggested that the hydration state of ions, including the number of water molecules per ion pair, strength of interactions between the water molecules and ions, and the physico-chemical properties of the component ions is an important factor for controlling the protein activity. However, the hydration state of most ions is difficult to analyse, especially at very low water contents, because the salts remain in the solid state and are inhomogeneously hydrated. Examples of such ions include potassium and cholide. On the other hand, ILs exist as homogeneous liquids even at low water contents. Therefore, the hydration state of ions can be analysed easily in ILs containing very low water contents, i.e., in the absence of free water molecules.

In the basic studies involving mixtures of water and a series of cholinium-based ILs, we found that the properties of hydrated ILs were strongly dependent on their water content.

---

[a] *Department of Biotechnology, Tokyo University of Agriculture and Technology, 2-24-16 Nakacho, Koganei, Tokyo 184-8588, Japan.*
[b] *Functional Ionic Liquid Laboratories (FILL), Graduate School of Engineering, Tokyo University of Agriculture and Technology.*
[c] *Japan Science and Technology Agency (JST), Core Research for Evolutional Science and Technology (CREST), Chiyoda, Tokyo 102-0076, Japan.*
E-mail:ohnoh@cc.tuat.ac.jp



cholinium dihydrogen phosphate/ [ch][dhp], 1

1-butyl-3-methylimidazolium dihydrogen phosphate/ [C4mim][dhp], 2

cholinium dibutyl phosphate/ [ch][dBp], 3

phosphocholine/ PC, 4

cholinium bitartrate/ [ch][bit], 5

cholinium dihydrogen citrate/ [ch][dhC], 6

cholinium nitrate/ [ch][NO3], 7

cholinium bromide/ [ch] Br, 8

cholinium tosylate/ [ch][Tos], 9

cholinium thiocyanate/ [ch][SCN], 10

cholinium bis(trifluoromethylsulfonyl)imide/ [ch][Tf2N], 11

guanidium chloride / [Gua]Cl, 12

3-(1-methyl-3-imidazolio) propanesulfonate/ C1Im3S, 13

3-(1,2-dimethyl-3-imidazolio) propanesulfonate/ C1,1Im3S, 14

3-(1-methyl-3-imidazolio) propanecarboxylate/ C1Im3C, 15

3-(1-ethyl-3-imidazolio) propanecarboxylate/ C2Im3C, 16

tributyloctylphosphonium bromide/ [P4448]Br, 17

tetrabutylphosphonium 2,4,6-trimethylbenzenesulfonate/ [P4444][TMBS], 18

tetrabutylphosphonium trifluoroacetate/ [P4444]CF3COO, 19

tetrabutylphosphonium 2,4-dimethylbenzenesulfonate/ [P4444][DMBS], 20

tributyloctylphosphonium benzenesulfonate/ [P4448][BzSO3], 21

tetrabutylammonium 2,4,6-trimethylbenzenesulfonate/ [N4444][TMBS], 22

tetrabutylphosphonium tosylate/ [P4444][Tos], 23

Fig. 1. Structure of the ILs examined in this paper

The properties of the hydrated ILs exhibited a non-linear dependence on water content, suggesting the existence of a threshold water content where the properties of hydrated ILs would abruptly change. A considerable difference in the properties of the hydrated ILs above and below the threshold was observed. In the present study, we report the effect of water content on the properties of hydrated ILs from different viewpoints such as enzymatic activity and phase transition behaviour. Hereafter, the water content in the ILs is expressed in terms of the "hydration number", which is the number of water molecules per ion pair.

2. Ionic liquids used in this study

We have previously reported that a mixture of water and cholinium dihydrogen phosphate ([ch][dhp]) exhibited an excellent ability to sustain the biological activity of some proteins.[11] In this paper, we have studied a series of ILs having ionic structures similar to [ch][dhp]. As mentioned previously, there are many literature reports discussing the properties of mixtures of ILs and large amounts of water. It should be re-emphasised that the unique and inherent properties of ILs are unlikely to manifest in such diluted solutions, owing to the large excess of free water. In contrast, hydrated ILs, (i.e., highly concentrated IL solutions) may be expected to show completely different properties compared to very dilute aqueous salt or IL solutions.

The ILs and organic salts used in this study have been synthesised according to the modified methods reported previously.[12,13] The ILs and salts were identified by ^{1}H NMR, differential scanning calorimetry (DSC), and electrospray mass spectrometry. The names, abbreviations, and numbers assigned to the various ILs and organic salts investigated in this study are as follows: cholinium dihydrogen phosphate ([ch][dhp]: **1**), 1-butyl-3-methyl-imidazolium dihydrogen phosphate ([C4mim][dhp]: **2**), cholinium dibutyl phosphate ([ch][dBp]: **3**, phosphocholine (PC: **4**), cholinium bitartrate ([ch][bit]: **5**), cholinium dihydrogen citrate ([ch][dhC]: **6**), cholinium nitrate ([ch][NO$_3$]: **7**), cholinium bromide ([ch]Br: **8**), cholinium tosylate ([ch][Tos]: **9**), cholinium thiocyanate ([ch][SCN]: **10**), cholinium bis(trifluoromethylsulfonyl)imide ([ch][Tf$_2$N]: **11**), guanidium chloride ([Gua]Cl: **12**), 3-(1-methyl-3-imidazolio) propanesulfonate (C1Im3S: **13**), 3-(1,2-dimethyl-3-imidazolio) propanesulfonate (C1,1Im3S: **14**), 3-(1-methyl-3-imidazolio) propanecarboxylate (C1Im3C: **15**), 3-(1-ethyl-3-imidazolio) propanecarboxylate (C2Im3C: **16**), tributyloctylphosphonium bromide ([P$_{4448}$]Br: **17**), tetrabutylphosphonium 2,4,6-trimethylbenzenesulfonate ([P$_{4444}$][TMBS]: **18**), tetrabutylphosphonium trifluoroacetate ([P$_{4444}$]CF$_3$COO: **19**), tetrabutylphosphonium 2,4-dimethylbenzenesulfonate ([P$_{4444}$][DMBS]: **20**), tributyloctylphosphonium benzenesulfonate ([P$_{4448}$][BzSO$_3$]: **21**), tetrabutylammonium 2,4,6-trimethylbenznenesulfonate ([N$_{4444}$][TMBS]: **22**), and tetrabutylphosphonium tosylate ([P$_{4444}$][Tos]: **23**). Their structures are provided in Fig. 1. The salts and ILs were mixed with different amounts of water to prepare the hydrated salts for analyses.

3. Effect of hydration number on biological activity in IL/water mixtures

In the case of dilute solutions of simple inorganic ions, the hydration states of the ions (e.g., 1st hydration layer, 2nd hydration layer, and so on) may be analysed in a straightforward manner. However, it is difficult to analyse the hydration states of most inorganic ions in concentrated aqueous solutions with very small amounts of water, because the electrostatic interactions between the ions are too strong to allow an independent ionic state. Therefore, the hydration states of inorganic salts are typically analysed in the presence of an excess amount of water (i.e., dilute solutions). Fortunately, ILs are in the liquid state on their own. Hydrophilic ILs, in particular, are miscible with any amount of water. Therefore, it is possible to analyse the solution properties of hydrated ions in IL/water mixtures with a wide range of water contents, including very small amounts of water, i.e., hydrated ILs even in the absence of free water.

3.1 Water contents and water activity

Water activity is a key parameter that must be considered when evaluating the contribution of water molecules to the (bio) chemical reactions, and this value is related to the amount of free water.[14] Therefore, the ratio of free water to bound water is significant in determining the biological activity in IL/water mixtures. Since strong interactions of water molecules with the solute ions reduce the vapour pressure, water activity (a_w) is defined as the ratio of the partial pressure of aqueous salt solutions (p) to that of pure water (p_0) (i.e., $a_w = p/p_0$).[14] In this study, water activity was measured using Pawkit (Decagon Inc.) and Aqua Lab (Decagon Inc.).

It has been reported that some proteins are stable in hydrated [ch][dhp] solutions.[11,15] Therefore, the water activities of hydrated [ch][dhp] and its analogues were compared with those of other salts (Fig. 2). As shown in Fig. 2, a_w increased rapidly with hydration number at low hydration numbers, but the slope decreased as the hydration number was further increased. Further, the magnitude of a_w for a given hydration number appeared to depend on the kosmotropicity of the component ions. The a_w values of kosmotropic salts, such as [ch][dhp] (**1**) and [C4mim][dhp] (**2**), were lower than those of chaotropic salts such as [Gua]Cl (**12**). Thus, when the same amount of water was added to the salts (i.e., for a given hydration number), water molecules interacted more strongly with kosmotropic salts than with chaotropic salts.

From the biological viewpoint, microbes need free water to grow,[16] and typically, an a_w value of over 0.98 is needed to allow microbes to breed. In the case of halophilic microorganisms, growth has been reported at a_w values of over 0.75.[16] As shown in Fig. 2, the hydration number corresponding to an a_w value of 0.75 was around seven (between six and nine), except in a few cases such as [ch][SCN] (**10**), [ch][Tf_2N] (**11**), and [Gua]Cl (**12**), which are chaotropic salts or hydrophobic ILs. Below an a_w value of 0.75, generally, microbial growth has not been observed.[16] While there are many other factors such as toxicity and osmotic pressure that are involved in keeping microbes alive, our intent in this paper is to merely emphasise that aw, which is an important factor for sustaining microbial activity, is certainly related to the hydration number.

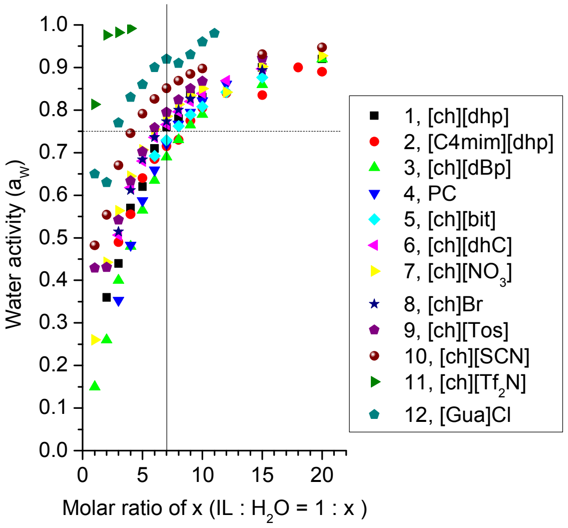


Fig. 2. Water activity of hydrated ILs with different water contents.

3.2 Cold crystallisation of water molecules in ILs

DSC is an excellent technique for detecting water molecules under different hydration states. DSC measurements have showed that some water-saturated polymers exhibit cold crystallisation (CC) behaviour.[17] The effect of the hydration state of some polymers on their compatibility with proteins has been previously studied.[17] Hydrated polymer systems showing CC behaviour have also been reported to exhibit biocompatibility.[17] This CC behaviour has been generally considered to be a phase transition based on the growth of crystal domains containing bound water. Similar CC behaviour has also been observed in some hydrated ILs.[12] Generally, CC behaviour is not observed in mixtures of most ILs and water in a wide range of water content.

The thermal behaviour of [ch][dhp] and its analogues was investigated using DSC measurements in mixtures with different water contents. Some ILs such as PC (**4**), [ch][dhC] (**6**), and [C4mim][dhp] (**2**) exhibited exothermic peaks attributed to CC behaviour, at appropriate water contents, as shown in the second row in Table 1. The CC behaviour observed in the case of hydrated [ch][dhp] occurred at a water content corresponding to the hydration state of seven water molecules per ion pair, as shown in Fig. 3.[12]

Physical analysis of mixture of ILs and water molecules is important to understand the original properties, e.g., molecular geometries of the ILs and interaction among ions and water molecules. Some papers have reported the formation of clusters of water in ILs[18] and the evolution of hydrogen bonding network of water molecules.[19] We have also tried

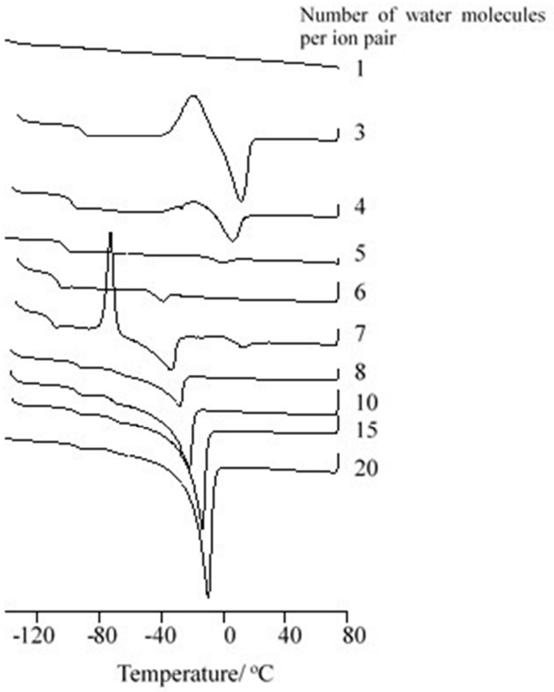


Fig. 3. DSC heating curves of hydrated [ch][dhp] (2nd heating run). Heating rate: 10 °C min^{-1}. Cited from ref. 12.

Table 1. Water to IL molar ratio at which CC behaviour was observed in the DSC measurements.

	W/IL*	Coordination state of heme in cytochrome c
#**1**: [ch][dhp]	7:1	6CLS
#**4**: PC	8:1	6CLS
#**6**: [ch][dhC]	7:1	6CLS
#**2**: [C4mim][dhp]	12:1	6CLS
#**10**: [ch][SCN]	-	D
#**3**: [ch][dBp]	-	D
#**12**: [Gua]Cl	-	D

* Molar ratio of water molecules to IL when CC was observed.
-: No CC was observed.
6CLS: six-coordinated low spin state.
D: Soret band showed blue shift and the disappearance of the charge transfer band (695 nm) in the UV-Vis spectrum. RR spectra showed coordination states different from those generally detected.

molecular simulation analysis to investigate the molecular geometries and physical properties of ILs-water mixtures used in this study. However, no critical change was presented by varying water content. Other experimental investigations are under way.

Next, the effect of the hydration state of ILs on the coordination state of heme in cytochrome c was also investigated. The spin state and axial coordination were analysed using resonance Raman (RR) spectroscopy (JASCO NRS-1000 spectrometer). The coordination state of heme of cytochrome c is shown in Table 1. Generally, heme in cytochrome c exhibits a six-coordinated low spin state (6CLS) in a buffered aqueous solution. The coordination state in hydrated ILs was investigated as a function of water content. As shown in Table 1, there was no significant change in the active site of cytochrome c, when it was dissolved in hydrated ILs 1, 2, 4, and 6, indicating CC behaviour.[12] On the other hand, the active sites changed significantly in hydrated ILs 3, 10, and 12, which did not exhibit CC behaviour. Although the details are summarised in the next section, it is worth noting here that the coordination states of heme of cytochrome c were different depending on whether the IL species show CC behaviour, under the hydrated state.

3.3 Hydration number and enzymatic reactions

Next, we investigated the minimum hydration number of a series of ILs at which protein activity was confirmed. We have reported that various biomolecules exhibit excellent solubility and protein activity in hydrated [ch][dhp],[11,15,20] when the hydration number is greater than or equal to seven. The water content corresponding to this specific hydration state (seven water molecules per ion pair on average) was considered to be the threshold for supporting enzymatic activity. Considering hydrated ILs containing more than seven water molecules per ion pair and those containing less than seven water molecules per ion pair, the observed results suggest that while the former support enzymatic activity, the latter do not support enzymatic activity. Therefore, it may be difficult to realise ionic activity in IL/water mixtures with hydration numbers less than seven.

Cytochrome c dissolved in hydrated ILs maintained the original spin state of heme in the active sites at water contents where CC behaviour was observed. For example, phosphocholine (**4**), which is the head-group of typical phospholipids contained in cell membranes, showed an exothermic peak corresponding to CC behaviour when the water to IL ratio was 8 to 1. The phosphocholine structure is considered to be zwitterionic, with the cholinium cation tethered to the phosphate anion ([ch][dhp] (**1**)). An interesting example was seen in the case of [C4mim][dhp] (**2**). The data in Table 1 show that the phosphate anion is quite important for controlling the hydration state of the water molecules. On the other hand, the active site of cytochrome c was changed in other hydrated ILs that did not show CC behaviour regardless of the water content.

3.4 Effect of the hydration states of ILs on other phenomena

Some other ILs have been reported to exhibit a threshold when the hydration number is seven. For example, phosphonium-type zwitterions mixed with equimolar HTf2N yielded lyotropic hexagonal columnar liquid crystalline phases, after the addition of around seven water molecules per ion pair or more.21 As another example, the esterification reaction in Brønsted acidic ILs proceeded most effectively when the hydration number of sulfonic acid was approximately seven.22 All of the above data suggest that the properties of IL/water mixtures undergo a transformation at a hydration number of around seven.

4. Effect of hydration number of ILs on the phase transition behaviour of moderately hydrophobic ILs

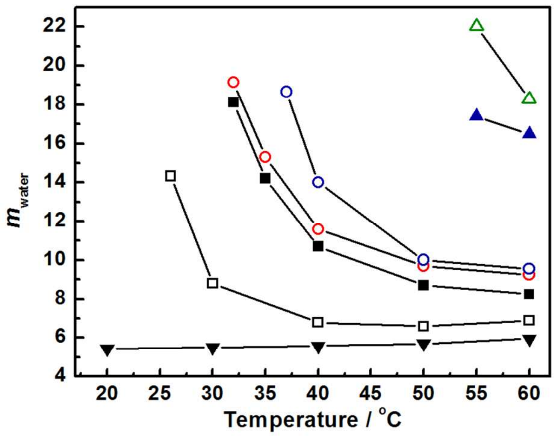


Fig. 4. Effect of temperature on the number of water molecules per ion pair (*m*) for a series of ILs. ▼: #21 [P_{4448}][$BzSO_3$], □: #17 [P_{4448}]Br, ■: #18 [P_{4444}][TMBS], ○: #19 [P_{4444}]CF_3COO, ○: #20 [P_{4444}][DMBS], ▲: #22 [N_{4444}][TMBS], △: #23 [P_{4444}][Tos]

Table 2. Hydration number of several ILs at 60 °C after mixing with an equal amount of water (50 wt %).

No. [a]	IL	Hydration number	Phase state
	[P_{5555}][Tf_2N]	0.16±0.01	Biphasic
	[P_{4448}][Tf_2N]	0.21±0.03	
	[P_{5555}]CF_3SO_3	0.45±0.05	
	[P_{4448}]BF_4	0.61±0.03	
	[P_{4448}]CF_3SO_3	0.71±0.02	
	[P_{5555}]NO_3	2.1±0.2	
	[P_{4448}]NO_3	2.9±0.1	
	[P_{5555}][TMBS]	3.2±0.1	
	[P_{5555}] [DMBS]	3.6±0.1	
	[P_{5555}] CF_3COO	3.6±0.1	
	[P_{5555}][Tos]	3.9±0.1	
	[P_{5555}][$BzSO_3$]	4.0±0.4	
	[P_{4448}][TMBS]	4.1±0.1	
	[P_{5555}]Br	4.3±0.4	
	[P_{4448}][DMBS]	4.4±0.5	
	[P_{4448}]CF_3COO	4.6±0.1	
	[P_{4448}][Tos]	4.7±0.2	
	[P_{4448}][$BzSO_3$]	5.0±0.2	
	[P_{5555}]Cl	5.3±0.6	
17	[P_{4448}]Br	6.7±0.3	LCST
18	[P_{4444}][TMBS]	8.1±0.3	
20	[P_{4444}][DMBS]	8.9±0.4	
19	[P_{4444}]CF_3COO	9.0±0.6	
22	[N_{4444}][TMBS]	15.9±0.2	
23	[P_{4444}][Tos]	18.8±0.7	

[a] The sample numbers shown in the column correspond to the numbers assigned for the ILs, as shown in Fig. 1.

Seven water molecules per ion pair in IL/water mixture has been shown to be a key number from the point of view of biological activity. Besides biological systems, there are other examples of IL/water mixtures where the hydration number of seven is of significance. We have already reported that some moderately hydrophobic ILs show thermoresponsive behaviour and reversible phase transitions between the homogeneous state and the phase-separated state when mixed with water. This phase change is known as lower critical solution temperature (LCST)-type behaviour.[23,24] Generally, LCST behaviour is scarcely observed in mixtures of ILs and water. Among the hydrophobic ILs, only moderately-hydrophobic ILs have been found to exhibit the LCST behaviour.[25] The solubility of ILs in water depends on the hydrophobicity of the component ions. We have also previously reported that a considerable change in the hydration number of the separated ILs was detected around the phase transition temperature. The hydration number decreased with an increase in the temperature, resulting in the formation of two separate phases. While the effect of temperature on the hydration number is insignificant at higher temperatures compared to the effect at the phase transition temperature, the hydration number always stays above seven (Fig. 4). It is clear that ILs showing LCST-type phase transitions always have a hydration number greater than seven, even after phase separation.[25] In contrast, the hydration number of hydrophobic ILs that show stable phase separations, irrespective of the temperature, was always found to be less than six (Fig. 4,▼). Table 2 summarises the hydration numbers of various ILs at 60 °C. ILs showing LCST-type phase transitions are highlighted in red. The hydration numbers of the ILs in mixtures containing equal amounts of water and ILs at 60 °C are also shown in this table.[25] As shown in Table 2, the phase behaviour of IL/water mixtures may be classified into two distinct types, based on whether the hydration number is above or below seven. In the case of hydrophobic ILs that show phase separation regardless of the temperature, the hydration number was always below six. On the other hand, the hydration number is always larger than seven in the separated IL phase when they show the LCST-type phase behaviour.

5. Conclusions: Hydration states governing biological and physico-chemical interactions in the hydrated ILs are different

IL/water mixtures have been gaining attention since they have a variety of potential applications in many different research areas. We have been studying these mixtures as solvents for proteins, enzymes, nucleic acids, and cells. In addition, some IL/water mixtures also exhibit temperature-driven phase changes. Different hydration states that are dependent on the hydration number and independent of the ion structure have been observed in ILs.

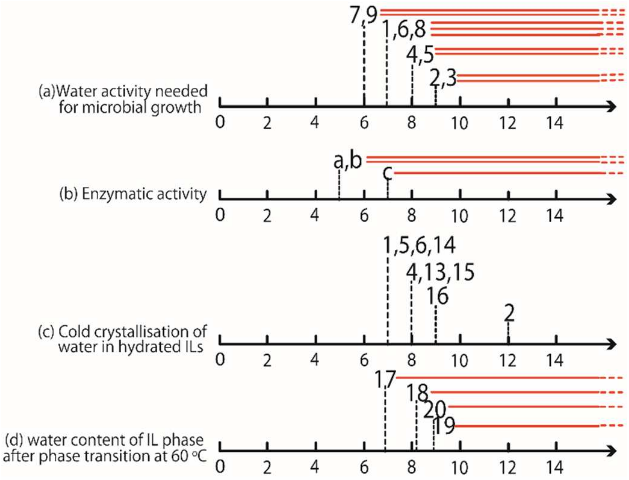


Fig. 5. The minimum hydration number required for several (bio)chemical phenomena. (a) water activity needed for microbial growth, (b) enzymatic activity,[11,15,20] (c) cold crystallisation[12] in the hydrated ILs, and (d) LCST phase transition at 60 °C. The comma-separated numbers specified in the figure indicate the ILs used in this study.

Analysing the hydration states of inorganic ions with small amounts of water is difficult, since a large excess of water is required to achieve a homogeneous state. On the other hand, by using ILs, it is possible to investigate the hydrated states of ions with small amounts of water. In this study, we have discovered a threshold hydration number at which the dynamics of IL/water mixtures and their effect on biomolecules undergo drastic changes, regardless of the IL species.[15,20] Fig. 5 summarises the minimum hydration number required for several biochemical phenomena such as microbial growth, enzymatic activity, CC of IL/water mixture, and for achieving LCST-type phase transitions in mixtures of some ILs and water. All of these parameters show non-linear relations with water content, strongly suggesting the existence of different hydration states above and below the threshold water content corresponding to the hydration number of around seven. As seen in Fig. 5(a), no microbial growth was found in systems with hydration numbers less than six. Similarly, enzymatic activity was not observed in systems with hydration numbers less than five. Further, a minimum hydration number of seven is required for both CC as well as LCST-type behaviour in moderately hydrophobic ILs. The results obtained suggest that all of these phenomena occur when the hydration number is greater than seven. In other words, the first six water molecules interact rather strongly with the ions compared to the additional water molecules in the ILs. It should be mentioned here that it is difficult to differentiate every hydration state of a few different levels of water molecules interacted with ions. As described in this paper, IL/water mixtures exhibit a threshold hydration state of seven water molecules per ion pair. This number appears to be independent from the ion species and is clearly an important number in the physical chemistry of IL/water mixtures.

Acknowledgements

This study was supported by a Grant-in-Aid for Scientific Research to H.O. (No. 21225007) and K.F. (No. 26810066) from the Japan Society for the Promotion of Science. This research was also supported by JST through the CREST program. K.F. is grateful to the Funds from the Women's Future Development Organization of Tokyo University of Agriculture and Technology. Y.K. acknowledges the Japan Society for the Promotion of Science for financial support.

References

1. M. Armand, F. Endres, D. R. MacFarlane, H. Ohno and B. Scrosati, *Nat. Mater.*, 2009, **8**, 621.
2. P. Wasserscheid, T. Welton and Eds., Wiley-VCH: Weinheim, Germany, 2007.
3. Y. Fukaya, K. Hayashi, M. Wada and H. Ohno, *Green Chem.*, 2008, **10**, 44.
4. K. Tamura, N. Nakamura and H. Ohno, *Biotechnol. Bioeng.*, 2011, **109**, 729.
5. K. N. Marsh, J. A. Boxall and R. Lichtenthaler, *Fluid Phase Equilibr.*, 2004, **219**, 93.
6. K. Fujita, D. R. MacFarlane and M. Forsyth, *Chem. Commun.*, 2005, 4804.
7. N. Byrne, L.-M. Wang, J.-P. Belieres and C. A. Angell, *Chem. Commun.*, 2007, 2714.
8. U. Kragl, M. Eckstein and N. Kaftzik, *Curr. Opin. Biotech.*, 2002, **13**, 565.
9. K. Shimojo, K. Nakashima, N. Kamiya and M. Goto, *Biomacromolecules*, 2006, **7**, 2.
10. K. Fujita, D. R. MacFarlane, M. Forsyth, M. Yoshizawa-Fujita, K. Murata, N. Nakamura and H. Ohno, *Biomacromolecules*, 2007, **8**, 2080.
11. K. Fujita and H. Ohno, *Biopolymers*, 2010, **93**, 1093.
12. K. Fujita, Y. Nikawa and H. Ohno, *Chem. Commun.*, 2013, **49**, 3257.
13. Y. Ito, Y. Kohno, N. Nakamura and H. Ohno, *Chem. Commun.*, 2014, **48**, 11220.
14. S. P. Cauvain and L. S. Young, *Bakery food manufacture and quality; Water control and effects,* Blackwell Science, Oxford, 2000.
15. K. Fujita, N. Nakamura, K. Igarashi, M. Samejima, H. Ohno, *Green Chem.,* 2009, **11**, 351.
16. M. S. Rahman ed., *Handbook of food preservation,* CRC Press, 2007.
17. M. Tanaka, T. Motomura, N. Ishii, K. Shimura, M. Onishi, A. Mochizuki and T. Hatakeyama, *Polym. Int.*, 2000, **49**, 1709.
18. T. M-Morales, J. Carrete, O. Cabeza, L. J. Gallego, L. M. Varela, *J. Phys. Chem, B*, 2011, **115**, 6995.
19. L. Cammarata, S. G. Kazarian, P. A. Salter, T. Welton, *Phys. Chem. Chem. Phys.*, 2001, **3**, 5192.
20. K. Fujita, N. Nakamura, K. Murata, K. Igarashi, M. Samejima and H. Ohno, *Electrochim. Acta*, 2011, **56**, 7224.
21. S. Ueda, J. Kagimoto, T. Ichikawa, T. Kato and H. Ohno, *Adv. Mater.*, 2011, **23**, 3071.
22. D. C. Forbes and K. J. Weaver, *J. Mol. Catal. A-Chem.*, 2004, **214**, 129.
23. Y. Kohno, H. Arai and H. Ohno, *Chem. Commun.*, 2011, **47**, 4772.

24. Y. Kohno, N. Nakamura and H. Ohno, *Aust. J. Chem.*, 2012, **65**, 1548.
25. Y. Kohno and H. Ohno, *Phys. Chem. Chem. Phys.*, 2012, **14**, 5063.