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The Solubility and Stability of Amino Acids in Biocompatible Ionic Liquids

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Abstract: In recent years, ionic liquids (ILs) represent a new class of biocompatible co-solvents for biomolecules. In this work, we report the apparent transfer free energies ($\Delta G'_{tr}$) for six amino acids (AA) from water to aqueous solutions of six ammonium based ILs (diethylammonium acetate (DEAA), diethylammonium sulfate (DEAS), triethyl ammonium acetate (TEAA), triethylammonium sulfate (TEAS), triethylammonium dihydrogen phosphate (TEAP), and trimethylammonium acetate (TMAA)) through solubility measurements, as a function of IL concentration at 298.15 K under atmospheric pressure. Salting-out effect was found for AA in aqueous IL solutions with increasing IL concentrations. In addition, we observed positive values of $\Delta G'_{tr}$ for AA from water to ILs, indicating that the interactions between ILs and AA are unfavorable. From the obtained results, we found that the selected ammonium based ILs act as stabilizers for the structure of AA.

Keywords: Ionic liquid, amino acid, solubility, biomolecular interactions, apparent transfer free energy.

INTRODUCTION

Amino Acids (AA) are very important biocomponents. The nature and the arrangement of the AA side-chain along with the protein backbone are responsible for the individual characteristics of the biomolecule, and it has been recognized that all information pertaining to protein is contained in the AA sequence [1, 2]. Therefore, observing the individual physical properties of these AA in various solvent media helps us to characterize the features of protein surface and its interactions with the co-solvents [3-6]. The collapse of the protein backbone into a dense globule and the burial of hydrophobic AA side chains in the protein's core are both driven by the hydrophobic effect and each phenomenon are crucial to the stability of a folded state [7, 8]. Hence, it is very much important to have a clear idea on the solubility, stability and their related thermodynamic properties of these AA in aqueous solution and in different solvent media [9, 10].

It is generally accepted that hydrophobic interactions are the major forces involved in initializing protein folding and stabilizing the three-dimensional structures of protein which directly depends on the hydrophobicity of AA [11]. Both hydrophobicity and the packing of hydrophobes in the hydrophobic core of a protein affect protein stability [12, 13]. Thus, it is of prime importance to determine the relative contribution of these effects in the hydrophobic interiors of proteins in different solvent media. The notion that hydrophobicity is an energetic determinant in protein folding has led attempts to characterize the hydrophobic interactions with water and co-solvents through solubility measurements and the apparent transfer free energy ($\Delta G'_{tr}$) values.

To broaden the possibility of applications of co-solvents in combination with complex biomolecules in the field of protein stabilities, a better understanding of the mechanism of the interaction with fundamental units, AA, is essential. In 1959, Kauzmann elucidated the interactions contributing to the protein stability that the hydrophobic effect, as manifested in the burial of non-polar groups in the native protein [14]. Later, a more quantitative analysis was exploited by Tanford, who accepted the paradigm of transfer between two phases and estimated the hydrophobic contributions to the stability of biomolecules [15-17]. Knowledge of the contribution of the individual AA to the electrostatic field and energetics of proteins is of considerable value in designing enzymes with enhanced or altered function and stability.

In recent years, ionic liquids (ILs) are emerging as greener solvents that can be contentedly adopted in numerous applications of thermodynamics and bio-thermodynamics [18]. ILs are salts that are entirely composed of ions having designable properties and applications [19-21]. Several attempts to quantify the bimolecular interactions of biomolecules with ILs have been accounted in open literature by our research group [22-29]. To document the hydrophobic interactions of ILs and of proteins, there is a need to obtain thermodynamic results to explain the interactions occurring in between protein surface and the ILs. In this context, interactions between AA and ILs were identified as the main driving forces for the stability of proteins [25-29]. Dreyer and Kragl, stated that the salting-out effect and the electrostatic interactions are responsible for protein extraction and stability into the IL-containing phase [30]. Therefore, the thermodynamic contribution of AA from water to IL solutions provides information about solute-solvent interactions,

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which are important in understanding the stability of protein's structure and function. To have a better understanding of the effect of ILs on proteins and to reveal the molecular interactions between ILs and protein functional groups, more detailed thermodynamic studies are still needed.

The free energy change (ΔG) of transporting a solute molecule between different solvent environments provides the thermodynamic framework that is needed to explain the protein denaturation, and the hydrophobic effect [31, 32]. In continuation of our earlier experimental works [25-29], we present here some results on the thermodynamic contribution of the $\Delta G'_{tr}$ of AA in a series of ILs aqueous solution. We have studied the $\Delta G'_{tr}$ of transfer of alanine (Ala), valine (Val), leucine (Leu), histidine (His), tryptophan (Trp), and tyrosine (Tyr) from water to diethyl ammonium acetate ($[Et_2NH][CH_3COO]$, DEAA), diethyl ammonium sulfate ($[Et_2NH][HSO_4]$, DEAS), triethyl ammonium acetate ($[Et_3NH][CH_3COO]$, TEAA), triethyl ammonium sulfate ($[Et_3NH][HSO_4]$, TEAS), triethyl ammonium phosphate ($[Et_3NH][H_2PO_4]$, TEAP) and trimethyl ammonium acetate ($[Me_3NH][CH_3COO]$, TMAA). Current evidence suggests that the $\Delta G'_{tr}$ of AA in ILs are dependent of the nature of the ILs as well as on the structural properties of AA. We have demonstrated conclusively, ILs influence on the hydrophobic $\Delta G'_{tr}$ of AA from water to ILs. We observed positive values of $\Delta G'_{tr}$ for the AA from water to ILs, indicating that the interactions between ILs and AA surface are unfavorable. The results demonstrate that the effect of IL concentration on AA may be due to their influence on the water structure around the AA thereby bringing about a change in the thermodynamic properties. Finally, the effects of anions and alkyl chain length are discussed to identify the factors that determine the stabilizing performance of ammonium ILs.

MATERIALS AND METHODS

Materials

Amino acids having 99% purity were purchased from Across Organics (USA). All chemicals were used as received. NANO pure water was used for preparing the aqueous solution of ILs. The distilled and de-ionized water with a resistance of 18.3 Ω cm was used for the sample preparation. All the solutions were prepared using a Mettler Toledo balance with a precision of ± 0.0001 g.

ILs used in this work, namely, DEAA, DEAS, TEAA, TEAS, TEAP and TMAA of general type [amine][anion] were synthesized in our laboratory and the detailed procedures have been elucidated elsewhere [29, 33, 34].

Solubility Measurements

The approach used for solubility measurements were similar to that described elsewhere [15-17]. Solubility of the AA_s in aqueous ILs is measured through a solid-disappearance method. The related procedure includes each sample vials containing a fixed amount of solvent (aqueous ILs) that was added to a weighed amount of AA. Several samples were made in increasing amount of AA by weight in order to make a series of mixtures with increasing composition of AA in the fixed amount of solvent. Each sample mixture is prepared by weighing pure compounds to ±0.1 mg. The samples are sealed in a small glass vial and placed in a cold bath to crystallize the liquid sample. The solidified sample is then immersed in a visual thermostatic bath and shaken vigorously for observation of solid disappearance at a fixed temperature. The bath temperature is elevated by a tiny increment each time, if the solid in the vial still existed after about 30-min observation. The increment must be as small as 0.1 K, when the temperature is near that of solid disappearance.

In the case of Trp, to prevent air oxidation during the equilibration, the contents of the vials were flushed with nitrogen. Known amounts of solvent and AA were placed in glass vials with screw caps. Care was taken that the amount of AA in each vial was more than the maximum solubility under the conditions employed. The solubility of AA was measured using density measurements, which is similar to the method of Nozaki and Tanford [15]. The related procedure includes each sample vials containing a fixed amount of solvent (aqueous or aqueous IL solutions) that was added to a weighed amount of AA. Similarly, a series of samples with increasing weight of AA was prepared in order to make a series of sample mixtures with increasing composition of AA. The vials were completely immersed in a low temperature shaker equipped with a water bath (Metrex, New Delhi, India). The bath temperature was controlled to (298.15 ± 0.01) K and the bath was housed in a constant room temperature maintained at nearly 298.15 K.

After shaking for 36–48 h, the solution was left overnight so that the undissolved solid particles settle at the bottom of the sample vial. The supernatant of each solution was removed with the help of a syringe and filtered through a 0.47 μm disposal filter (Millipore, Millex-GS) before performing the density measurements. The density measurements (ρ) were performed with an Anton-Paar DMA 4500 M (Austria) vibrating-tube densimeter, equipped with a built-in solid-state thermostat and a resident program with accuracy of ± 0.03 K. The ρ values of the samples were measured at 298.15 K under ambient pressure with a precision of ± 0.00005 g cm^{-3}. Proper calibration at each temperature was achieved with doubly distilled, deionized water and with air as standards. The uncertainty of the solubility limit is lower than ± 1.2%.

RESULTS AND DISCUSSION

Solubility Measurements

The solubility limit of the amino acids (AA) was obtained at the point of intersection of the two fitted lines such as unsaturated and saturated for each system. The solubility limits of each of the AA (Ala, Val, Leu, His, Trp, and Tyr) in the absence and in the presence of all six ILs (DEAA, DEAS, TEAA, TEAS, TEAP and TMAA) aqueous solutions, expressed as grams of AA per 100 g of co-solvent, and the densities (ρ*) of the AA at the solubility limit are summarized in Table **1**. The solubility limit of AA at 25 ^{0}C under atmospheric pressure was plotted as a function of the concentration of ammonium based IL, in the six panels of Fig. **1**.

Table 1. Solubility of amino acids (S_{AA}) in water (or) in aqueous ILs and densities (ρ*) at solubility limits at 298.15 K.

Co-solvent	S_{AA}(g/100 g of solvent)				ρ^*_{AA} (g/cm^3)			
	0%	30%	50%	70%	0%	30%	50%	70%
Ala								
water	16.62				1.04289			
DEAA		9.61	7.43	4.88		1.02730	1.02746	1.02912
DEAS		6.36	4.15	2.16		1.10249	1.10693	1.10760
TEAA		8.51	6.35	4.02		1.02521	1.02574	1.02589
TEAS		5.45	3.33	1.30		1.07737	1.09212	1.10288
TEAP		7.43	5.16	2.96		1.09008	1.14368	1.15009
TMAA		10.55	8.18	5.71		1.05099	1.05228	1.05301
Val								
water	5.75				1.00945			
DEAA		3.52	2.74	1.92		1.01379	1.01845	1.01927
DEAS		2.38	1.59	0.89		1.05290	1.07525	1.10253
TEAA		3.13	2.26	1.52		1.01187	1.01772	1.01816
TEAS		2.02	1.25	0.59		1.06349	1.08676	1.10643
TEAP		2.73	1.92	1.16		1.07488	1.10279	1.14854
TMAA		3.81	3.02	2.25		1.02681	1.03583	1.04338
Leu								
water	2.15				1.00091			
DEAA		1.32	0.81	0.37		1.00130	1.01054	1.01772
DEAS		1.42	0.95	0.52		1.00414	1.04703	1.07630
TEAA		1.53	1.09	0.67		1.00098	1.01042	1.01732
TEAS		1.64	1.23	0.83		1.00444	1.05502	1.07566
TEAP		1.75	1.38	1.02		1.00804	1.06552	1.10436
TMAA		1.86	1.48	1.16		1.03450	1.04006	1.05732
His								
water	4.30							
DEAA		3.35	2.47	1.6		1.07117	1.08115	1.08818
DEAS		3.76	3.03	2.2		1.06346	1.09780	1.12675
TEAA		3.20	2.26	1.4		1.03485	1.04378	1.05127
TEAS		2.92	2.06	1.2		1.08151	1.10856	1.12603
TEAP		3.57	2.78	1.93		1.09198	1.12336	1.14899
TMAA		3.91	3.33	2.43		1.05250	1.09320	1.12630
Trp								
water	1.33				0.99705			
DEAA		1.21	1.09	1.00		1.07117	1.08115	1.08818
DEAS		1.13	1.00	0.89		1.04546	1.07780	1.10675

(Table 1) Contd….

Co-solvent	S_{AA}(g/100 g of solvent)				ρ^*_{AA} (g/cm^3)			
	0%	30%	50%	70%	0%	30%	50%	70%
TEAA		1.18	1.05	0.96		1.03485	1.04378	1.05127
TEAS		1.06	0.94	0.83		1.08151	1.10856	1.12603
TEAP		1.03	0.90	0.79		1.09198	1.12336	1.14899
TMAA		1.23	1.11	1.02		1.0005	1.0065	1.01960
Tyr								
water	0.05				0.99705			
DEAA		0.04	0.04	0.03		1.07117	1.08115	1.01195
DEAS		0.04	0.03	0.03		1.06346	1.09780	1.12675
TEAA		0.04	0.04	0.03		1.03485	1.04378	1.05127
TEAS		0.03	0.03	0.02		1.08151	1.10856	1.12603
TEAP		0.03	0.03	0.02		1.09198	1.12336	1.14899
TMAA		0.04	0.04	0.03		1.04981	1.09940	1.10003

[a]The uncertainty in solubility limit is lower than ±1.2%.

[b]S_{AA} for amino acids-water is compared with Ref. [1]

The solubility of AA in water decreases in the order as Ala > Val > His > Leu > Trp > Tyr. The order appears to be grouped according to the increasing hydrophobicity from the aliphatic (Ala) to aromatic (Tyr) residues. The decrease in the solubility from Ala to Tyr is due to the presence of the aromatic groups in structure of AA when moving from Ala to Tyr in the series above. Moreover, these hydrophobic groups, being nonpolar, cannot form hydrogen bonds with the water molecules in their vicinity. However, in presence of nonpolar solute the water molecules adopt a more specific orientation depending on the geometry of the hydrophobic group. Therefore, the variation in the solubility is due to the formation of a cage like structure or ''clathrate'' when long chain residues are in an aqueous medium which restricts the motion and the number of possible arrangements of the water molecules, thereby lowering their solubility. Due to this, the AA is only partially surrounded by water molecules. A polarity scale based on the tendencies of AA to leave water and accumulate at the surface has been developed and free energy changes based on the surface tension of aqueous solutions of AA has been evaluated by Maheshwari and Dhathathreyan [35]. As a result, the structure of the long chain amino acid leads to lower values of solubility in water. For these reasons, Ala and Val are very soluble, His and Leu are moderately, and Trp and Tyr are weakly soluble in water.

Analysis of the solubility of AA in ILs in Fig. **1** reveal that AA values decrease monotonically with increasing concentrations of ILs, which indicates that the salting-out effect is dominant. The salting-out of the AA reflects an increase in stability of the native structure of proteins in the presence of ILs [36]. The magnitude of this salting-out effect depends on the nature of ILs and generally follows the physical interface of AA structure. The salting-out effect mainly indicates that ILs increase the stability of AA. To the best of our knowledge, no solubility data of AA in ammonium ILs have been reported in the literature, thereby, our results cannot be compared with the literature values.

The interpretation of the solubility behavior of AA based on the specific individual effect of cation and anion of IL is difficult to predict, since a balance between competitive interaction of the cation, anion and water exist with the AA. The combinations of these complex interactions determines the preferential interaction as well as solvation of the IL with AA which is helpful in predicting the differences in the solubility limits of the AA in the presence of varying concentrations of the ILs in aqueous solution. Moreover, Tome *et al*. [37, 38] proposed through experimental as well as computational techniques that salting-in and salting-out phenomena possess a common basis which is the competition between water-AA side chain, IL-AA side chain, and water-IL interactions. They concluded that the delicate balance between these interactions is dependent on the relative affinities of the biomolecules to water or to IL cation and anion and the magnitude of the solubilities. As presented, in Table **1** the water affinity of the AA decreases in the order ranging from Ala to Tyr, reflecting an increasing hydrophobicity as the side chain increases. The solubility data from Table **1**, predicts that TMAA ILs acted as the good solvent for the all AA, whereas these AA were having lower solubility in TEAS. This trend in solubility of each of the AA is observed even at higher concentrations of the ILs too. Therefore seems that at each IL concentration the electrostatic forces are dominant based on the effect of ions of ILs depending on their ability to screen effective charges on the AA surface. Additionally, it was found that the performance of AA extraction in IL water systems is sensitive to the hydrophobic character and chemical structures of ions in the ILs [38-41]. The polarity of the AA provokes an increase interaction with

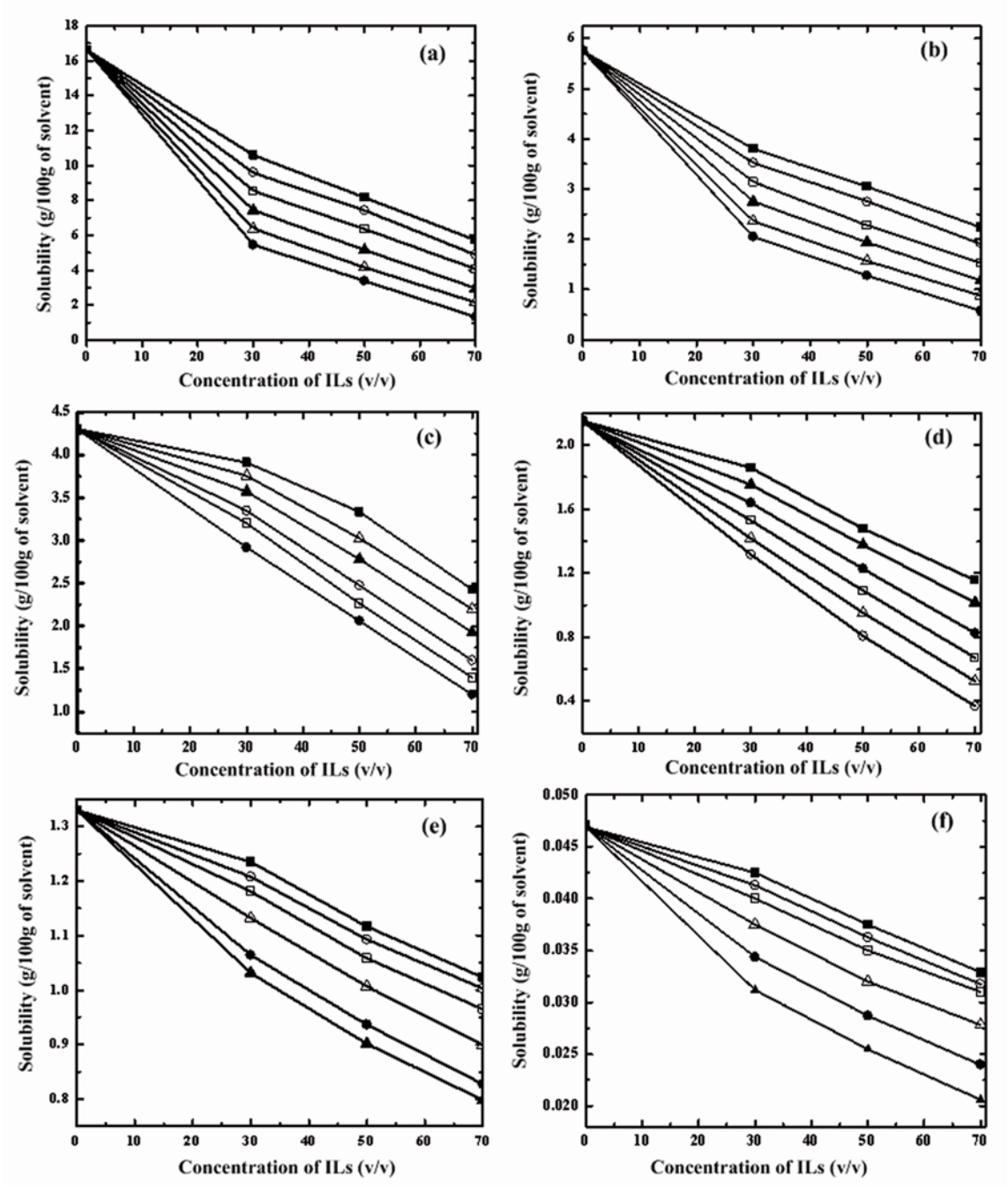

Figure 1. Plot of solubility against concentration to illustrate the solubility limits (S_{AA}) for (**a**) Ala, (**b**) Val, (**c**) His, (**d**) Leu, (**e**) Trp and (**f**) Tyr in an aqueous solution of ILs: TEAS (●), DEAS (Δ), TEAP (▲), TEAA (□), DEAA (○) and TMAA (■) at 298.15 K. Solid lines show the smoothness of the solubility points.

the more polar ILs. The more polar the AA, the stronger the interaction with the IL, consequently the magnitude of the interaction of IL with AA is expected to vary as compared to the solubility of the AA in ILs aqueous solution.

Apparent Transfer Free Energy ($\Delta G'_{tr}$) of AA from Water to ILs

Protein stability and associations depend on the electrostatic, hydrophobic, van der Waals, and steric interactions with itself and with solvent molecules. Virtually, the net stability of a protein is defined as the difference in the free energy (ΔG) of the native (folded) and denatured (unfolded) states. The protein surface constitutes of AA with varying individual properties that primarily interacts with the surrounding solvent and as a whole contributes to folding/unfolding. Evidently, the unfavorable interactions of solvents with proteins (salting-out effect) account for the positive contribution of the $\Delta G'_{tr}$ and that the solubility decreases with increasing the concentration of co-solvents indicating protein is in a stable state. In contrast, the salting-in effects leads to the favorable interactions of the solvents with the

protein surface that leads to protein denaturation. Traditionally, the evaluation of $\Delta G'_{tr}$ is based on measuring the differential solubility of a model compounds in a water/co-solvent mixture. The $\Delta G'_{tr}$ measurements have played a key role in understanding the effect of stabilization or destabilization of proteins and their functional groups in various co-solvents. To predict the protein folding/unfolding, the objective of the present work is to obtain the apparent transfer free energy ($\Delta G'_{tr}$) data AA in stabilizing or destabilizing the native and unfolded forms in ILs.

The solubility data were used to obtain the $\Delta G'_{tr}$ of AA from water to ILs at 298.15 K and the detailed procedure for obtaining the $\Delta G'_{tr}$ of this work has been depicted in our previous article [26-29]. At the solubility limit of the model compounds, solid and liquid phases are at equilibrium, and thus the fugacities of the component (or) the model compounds in the solid and the liquid phases should be equal [5]. Assuming that the solid phase is pure compound *i*, the fugacity equality becomes

$$f_{i(pure\ solid)} = \hat{f}_{i(solute\ i\ in\ liquid\ solution)} \quad (1)$$ or

$$f_{i(pure\ solid)} = x_i \gamma_i f_i^o \quad (2)$$

where x_i is the solubility of component *i* in water or in the aqueous solutions, γ_i is the activity coefficient of component *i* in the liquid phase, and f_i^o is the standard-state fugacity to which γ_i refers. As a consequence, the transfer free energy of model compounds from water to the aqueous IL solutions, ΔG_{tr}, can be calculated from the following equation:

$$\Delta G_{tr} = \bar{G}^{\infty}_{mc,ws} - \bar{G}^{\infty}_{mc,w} = \mu^{\infty}_{mc,ws} - \mu^{\infty}_{mc,w}$$
$$= RT \ln\left(\frac{f^{\infty}_{mc,ws}}{f^{\infty}_{mc,w}}\right) = RT \ln\left(\frac{x_{mc,w}\gamma^{*}_{mc,w}}{x_{mc,ws}\gamma^{*}_{mc,ws}}\right) = RT \ln\left(\frac{x_{mc,w}}{x_{mc,ws}}\right) + RT \ln\left(\frac{\gamma^{*}_{mc,w}}{\gamma^{*}_{mc,ws}}\right) \quad (3)$$

where the subscript w refers to the aqueous, i.e., the IL-free systems and ws refers to the aqueous IL solutions and mc denoted model compound. Eq 3 can also be expressed in terms of molar concentration,

$$\Delta G_{tr} = RT \ln\left(\frac{C_{mc,w}}{C_{mc,ws}}\right) + RT \ln\left(\frac{\gamma^{\#}_{mc,w}}{\gamma^{\#}_{mc,ws}}\right) \quad (4)$$

In 1960-1970, Nozaki and Tan ford [15-17], noted that the activity coefficient term is a self – interaction, coefficient term and found that the ratio of the activity coefficient term makes only a small contribution of $\Delta G'_{tr}$, thereby; this effect of the simplification is negligible and also much greater than the experimental uncertainty. Therefore, we have ignored the activity coefficient term on the right hand side of equation (4) for determining transfer free energies of various solutions, because rigorously obtaining the activity coefficients of model compounds in multi component liquid mixtures from phase equilibrium data is extremely problematic and difficult [1, 5, 15-17], when the activity coefficient term was neglected, ΔG_{tr} is better denoted as apparent transfer free energies ($\Delta G'_{tr}$). The uncertainty of $\Delta G'_{tr}$ in the present study is lower than ±1.5%. The $\Delta G'_{tr}$ values are listed in Table **2** and graphically displayed in Fig. **2** as a function of IL concentrations at 298.15 K. Fig. **2** illustrates that the addition of IL strongly increases the $\Delta G'_{tr}$ values of AA with increasing the concentration of IL. The increase in $\Delta G'_{tr}$ values of AA indicates that there is unfavorable interaction between ILs and AA that leads to stabilize the structure of AA. The positive contribution shows that these ILs are biocompatible co-solvents for AA and stabilize the structure of AA. Upon comparison in the set of ILs, TEAS shows the highest $\Delta G'_{tr}$ values for Ala, Val, Leu and His. This IL is a strong stabilizer for the AA. Whereas, TEAP is found to be biocompatible for Trp and Tyr, since we observed highest $\Delta G'_{tr}$ values.

However, the difference in the stabilization behavior of the AA in ILs can be attributed to the variation of the alkyl chain at the substituted ammonium cations that led to variation in the efficacy of the ILs as stabilizers for the AA. Greaves and Drummond [42] significantly reviewed the properties of protic ionic liquids (PILs) and concluded that for the PILs with alkylammonium cations, the viscosity increases with increasing alkyl chain length and significantly increased with methyl substitution on the alkyl chain. Therefore, it is noteworthy to compare the stabilizing ability between alkyl chains at the substituted ammonium cations of the ILs. For the sake of comparison, we consider the case of triethylammonium cation (TEA^+) and the diethylammonium cation (DEA^+); TEA^+ and DEA^+ behaved as stabilizers for all AA. This difference in the behavior of water-soluble ILs is attributed to an increase in the alkyl chain length of their cations that may lead to increase in the viscosity of the ILs. Apparently, TEAA has higher viscosity (24.12 mPa s) than DEAA (16.36 mPa s) thereby resulting in higher stabilizing ability in TEAA than DEAA for the structure of AA [29]. The possible explanation is that the high viscosity of ILs slows down the conformational changes of biomolecules, allowing a more compact structure of AA. Moreover, because of the strong interaction of the ions of IL with water competes effectively for the water molecules associated with the AA surface, thus the strongly solvated IL is excluded from the AA surface. This encourages the AA surface to be primarily and preferentially solvated by the water molecules.

The role of anion in predicting AA stability in ILs is a complex phenomenon and has not been completely explored yet. For the sake of comparison the $\Delta G'_{tr}$ values from Table **2**, for Ala, Val, Leu, His, Trp and Tyr in 30 % of TEAS was 2433.78, 2374.94, 228.45, 456.74, 595. 48 J mol^{-1}; and $\Delta G'_{tr}$ values for the same AA in 30 % of DEAS was 2015.16, 2001.92, 155.52, 49.42, 314.99 and 427.66 J mol^{-1}. Obviously, these values are higher than the $\Delta G'_{tr}$ values of 1241.63, 1523.03, 1681.06, 975.89 J mol^{-1} for Ala; 1153.04, 1439.49, 1619.05, 932.09 J mol^{-1} for Val; 67.74, 91.88, 98.04, -59.14 J mol^{-1} for Leu; 283.15, 505.56, 106.20, -168.77 J mol^{-1} for His; 106.03, 232.71, 505.39, -46.51 J mol^{-1} for Trp; 155.38, 302.08, 800.51, 11.55 J mol^{-1} for Tyr; in 30 % of DEAA, TEAA, TEAP and TMAA, respectively. From these results we observed that the HSO_4^- anion acted as the best stabilizer for the AA whereas, $H_2PO_4^-$ and CH_3OO^- anions acted as moderate and weak stabilizers and obeys the Hofmeister order as $HSO_4^- > H_2PO_4^- > CH_3OO^-$. Moreover, the $\Delta G'_{tr}$ values corresponding to each of the AA

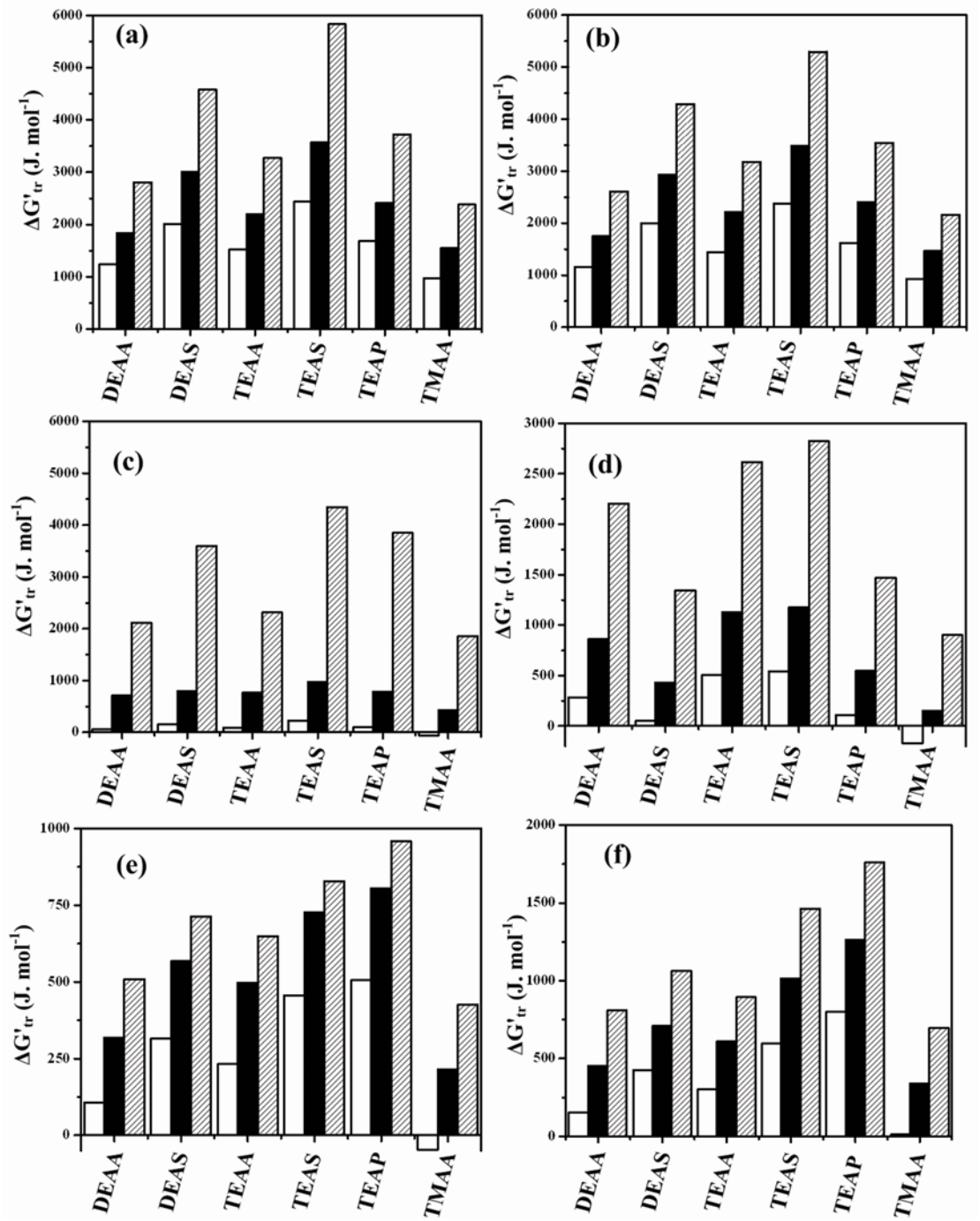

Figure 2. Plot of apparent transfer energy, $\Delta G'_{tr}$ against concentration for (**a**) Ala, (**b**) Val, (**c**) Leu, (**d**) His, (**e**) Trp and (**f**) Tyr in 30% (colorless column), 50% (black column) and 70% (striped column) aqueous solution of ILs: TEAS, DEAS, TEAP, TEAA, DEAA and TMAA at 298.15 K.

in various ILs at different concentrations follow the same order (Table **2**). The trend in the $\Delta G'_{tr}$ values of the AA in ILs are consisting with the results of distinguishing kosmotropic ions and chaotropic ions based on the Gibbs free energies of hydration (ΔG) [43]**.** It is seen that the kosmotropic ions have larger negative Gibbs free energies of hydration (ΔG) than chaotropic ions. Thus, it can be expected that the larger the ΔG, the larger the strength of hydration.

Tome *et al* [38] through simulation studies calculated that contrary to the hydrophobic species, AA with a more polar character do not interact with the IL cation and are less effectively bound to the hydrophobic moieties of the IL anion, showing thus a clear preference for polar environment. This can be considered as one of the mechanism by which AA prefers to be highly stabilized by the ILs. According to Yang and Pan [44] the stability through anions of ILs usually

Table 2. Apparent transfer free energies ($\Delta G'_{tr}$) of amino acids from water to aqueous ILs solutions at 298.15 K

Amino acids	ILs	$\Delta G'_{tr}$ (J mol^{-1})		
		30%	50%	70%
Ala	DEAA	1241.63	1829.23	2807.78
	DEAS	2015.16	3011.46	4580.90
	TEAA	1523.03	2197.71	3275.74
	TEAS	2433.78	3570.97	5829.20
	TEAP	1681.06	2414.44	3725.84
	TMAA	975.89	1548.91	2381.05
Val	DEAA	1153.04	1743.91	2603.65
	DEAS	2001.92	2930.71	4289.78
	TEAA	1439.49	2211.51	3175.72
	TEAS	2374.94	3492.28	5292.74
	TEAP	1619.05	2408.42	3538.64
	TMAA	932.09	1467.51	2160.53
Leu	DEAA	67.74	710.61	2114.38
	DEAS	155.52	797.71	3601.15
	TEAA	91.88	773.09	2316.70
	TEAS	228.45	967.63	4349.01
	TEAP	98.04	787.56	3851.67
	TMAA	-59.14	433.46	1859.50
His	DEAA	283.15	863.94	2206.03
	DEAS	49.42	430.78	1344.85
	TEAA	505.56	1130.02	2617.70
	TEAS	541.72	1174.34	2824.64
	TEAP	106.20	547.29	1467.81
	TMAA	-168.77	148.31	902.24
Trp	DEAA	106.03	318.68	509.13
	DEAS	314.99	566.98	713.87
	TEAA	232.71	497.58	649.17
	TEAS	456.74	726.76	828.47
	TEAP	505.39	805.60	958.42
	TMAA	-46.51	214.56	424.85
Tyr	DEAA	155.38	454.66	809.15
	DEAS	427.66	708.65	1065.18
	TEAA	302.08	611.67	894.69
	TEAS	595.48	1015.39	1464.14
	TEAP	800.51	1263.38	1760.49
	TMAA	11.55	340.45	697.10

follows two types of mechanism. Firstly, due to the lower hydrogen bond basicity of biocompatible anions of ILs, the tendency of the anions to interfere with the internal hydrogen bonds of the biomolecule is minimized [44]. Secondly, the enzyme-compatible anions exhibit lower nucleophilicity and thus would show lower tendency to change the enzyme's conformation by interacting with the positively charged sites in the enzyme structure [45]. Because of the difference in the cations and anion species, different ILs are solvated to different extent. Thereby, strongly solvated ILs stabilize AA and promote salting-out. The solvated ILs interact poorly with weakly solvated AA and are excluded from the surface of AA.

The explanation of IL induced protein model compounds stability is still not clearly understood however, this discrepancy may be primarily due to the weak hydration as well as an increase in hydrophobicity of the anion which has an indirect stabilizing effect on the structure of the proteins. Overall, the results of the anion and cation, demonstrated that the combination of sulfate and acetate anions with higher alkyl chain at the substituted ammonium cation, IL show essentially effective and strong stabilizer for the structure of AA.

CONCLUSION

Ammonium based ILs are a large class of non-volatile and also biocompatible solvents for all investigated AA. Our results show the decrease in the aqueous solubility (salting-out) of the AA with increasing the concentration of ILs indicate towards the unfavorable interaction of the ILs with the AA surface. In order to quantify the molecular interaction induced stability of the AA by ILs at 298.15 K, we have obtained $\Delta G'_{tr}$ from water to aqueous IL solution under atmospheric pressure. The results are informative which brings forward the influences of solvation behavior of ILs on AA. This detailed information, concludes that all ILs used in this experiment are biocompatible and can stabilize three dimensional protein structure through unfavorable interactions with the AA of the protein.

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