Biosolvation Nature of Ionic Liquids: Molecular Dynamics Simulation of Methylated Nucleobases in Hydrated 1-Ethyl-3-methylimidazolium Acetate

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ABSTRACT: Solvation free energies of methylated nucleobases were calculated in pure and hydrated 1-ethyl-3-methylimidazolium acetate, [Emim][Ac], ionic liquid, and pure water using classical molecular dynamics simulations using multistate Bennett’s acceptance ratio method. The calculated solvation free energies in pure water were compared with the previous experimental and theoretical findings and found to be in agreement. We observe that the solvation free energy of methylated nucleobases is more in the pure ionic liquid compared to that in the pure water and on changing the mole fraction of water in the ionic liquid, the solvation free energy decreases gradually. Comparing the Coulombic and van der Waals contribution to the solvation free energy, electrostatic contribution is more compared to that of the latter for all nucleobases. To obtain the atomistic details and explain the solvation mechanism, we calculated radial distribution functions (RDFs), spatial distribution functions (SDFs), and stacking angle distribution of cations to the nucleobases. From RDFs and SDFs, we find that the acetate anions of the ionic liquid are forming strong hydrogen bonds with the amine hydrogen atoms of the nucleobases. These hydrogen bonds contribute to the major part of the Coulombic contribution to the solvation free energy. Stacking of cations to the nucleobases is primarily due to the van der Waals contribution to the solvation free energy.

INTRODUCTION

Nucleobases are the nitrogen-containing heteroaromatic biomolecules, which are the main constituents of DNA. They are involved in a variety of nonbonded interactions, like electrostatic, van der Waals, hydrophobic, and π−π stacking. They are used in many applications, such as molecular therapeutics, biomimetics, as coordination polymers, in electronics, and photonics. They are also utilized in the agricultural and chemical industries as well. Uracil and its derivatives are used as anticancer and antiviral drugs. Even though their applications are vast, they are poorly soluble in water. On the other hand, an alternate solvent like ionic liquids, though their applications are vast, they are poorly soluble in water. An increased solubility of the hard-to-solvent can be understood by investigating molecular level phenomena. The cosolvents alter the intermolecular interactions, the resultant mixtures decrease significantly. In many applications, the cosolvents alter the intermolecular interactions that affect the solubility of sparingly soluble compounds. The solvation nature of the solvent toward a particular solute can be understood by assessing a thermodynamic quantity, solvation free energy. The link between solvation free energy and solubilizing nature of a particular solvent can be understood by investigating molecular level phenomena due to the exceptional solvation abilities. These consist of both electrostatic, van der Waals, hydrophobic, and π−π stacking. From RDFs and SDFs, we find that the acetate anions of the ionic liquid are forming strong hydrogen bonds with the amine hydrogen atoms of the nucleobases. These hydrogen bonds contribute to the major part of the Coulombic contribution to the solvation free energy. Stacking of cations to the nucleobases is primarily due to the van der Waals contribution to the solvation free energy.
interactions that can happen during the solvation process. Several studies reported the solvation free energies of pharmaceutical drugs,12−16 amino acid analogues,17−21 nucleic acid bases,22−30 and other organic molecules33,31−34 in various solvents using different analytical methods. These methods include free-energy perturbation,35 thermodynamic integration,36 Bennett and multistate Bennett’s acceptance ratio (MBAR),37,38 and quantum mechanics (QM)-based39,40 and classical models.41 The nucleobases are involved in a variety of interactions contributing to the solvation stability of DNA in hydrated ionic liquid. Along with structural properties, information about the stacking of cation with nucleobases are calculated, which provide the probability distributions of the stacking angle of Emim with nucleobases in pure and hydrated ionic liquids to improve and tune their solubility.

In this study, we report the solvation free energies of methylated nucleobases in pure and hydrated ionic liquids of [Emim][Ac] with changing mole fractions of water using classical molecular dynamics simulations. Experimental results for solubility of nucleobases are available in this ionic liquid with changing mole fraction of water. The polar bases prefer to solvate by short alkyl chains of ILs, and the less polar bases show better solubility in ILs with long alkyl chains. The stability of DNA duplex increases in hydrated ionic liquids compared to that in the aqueous solution. The direct chemical modification to the DNA is called DNA methylation. This methylation process can affect dynamics of many biorelevant processes. The methylated nucleobases can be simple models to study the solvation dynamics of chemically modified biomolecules. So far, there is no study on solvation free energy of methylated nucleobases in pure and hydrated liquid ions. It is essential to understand the thermodynamics and solvation mechanism of methylated nucleobases in pure and hydrated ionic liquids to improve and tune their solubility.

| mole fraction of water (x_w) | number of cations | number of anions | number of water molecules |
|-----------------------------|------------------|-----------------|--------------------------|
| 0.2                         | 100              | 100             | 25                       |
| 0.4                         | 100              | 100             | 67                       |
| 0.6                         | 86               | 86              | 125                      |
| 0.8                         | 83               | 83              | 320                      |
| 1.0                         | 0                | 0               | 1000                     |

**RESULTS AND DISCUSSION**

Solvation free energy is a thermodynamic quantity, which can describe the solubility of a solute in a particular solvent. We calculated this quantity for methylated nucleobases in pure water, pure IL, and various mole fractions of hydrated ILs. The obtained solvation free-energy values of methylated nucleobases in pure water are tabulated in Table 2. To check the reliability of our results from the methods we used, the results from the previous methods and experiments are also listed for comparison including those from similar models. Experimental values for solvation free energies are available for comparison only for methylated adenine and methylated thymine. For both bases, the calculated solvation free energies in our work are overestimated by ~1 kcal mol⁻¹, as compared to the experimental values. Our reported solvation free-energy values are in close agreement with previously calculated values using AM1/SM2 method except for methylated adenine. The results are qualitatively in agreement with previously calculated solvation free energies using QM/molecular mechanics (MM) method except for the same base. Among all nucleobases, m-guanine has more negative solvation free energy (~22.6 kcal mol⁻¹), whereas m-thymine and m-uracil have less and almost similar values (~13.4 and ~13.9 kcal mol⁻¹). The results show the following trend in solvation free energy: m-guanine > m-cytosine > m-adenine > m-uracil > m-thymine. This trend indicates that m-guanine is more soluble in pure water when compared to other nucleobases and m-thymine is less soluble. From the previous study, less polar bases had lower solubility³⁰ as compared with others. The trend in solvation free energy for pure bases in water found in the early study was: guanine > cytosine > thymine > uracil > adenine.³⁰ Overall the bases, guanine, cytosine, and uracil, follow a similar trend in solvation free energy, whereas the pattern is changed for methylation of adenine and thymine (Figure 1).

The solvation free energies of methylated nucleobases in pure as well as hydrated ionic liquids with changing mole fraction of water and in pure water are shown in Figure 2. The convergence of solvation free-energy values is shown in the Supporting Information, Figure S1. The standard deviations for solvation free energies are within 1 kcal mol⁻¹ for all
Table 2. Solvation Free Energy (kcal mol\(^{-1}\)) of Methylated Nucleobases in Pure Water Compared with Previous Works

| s. no. | FFs/method   | m-adenine   | m-thymine   | m-guanine   | m-cytosine   | m-uracil   |
|-------|--------------|-------------|-------------|-------------|-------------|------------|
| 1     | our work     | \(-14.9 \pm 0.08\) | \(-13.4 \pm 0.08\) | \(-22.6 \pm 0.09\) | \(-19.6 \pm 0.06\) | \(-13.9 \pm 0.06\) |
| 2     | experimental\(^{28}\) | \(-13.6\) | \(-9.1\) to \(-12.7\) | \- | \- | \- |
| 3     | AM1/SM2\(^{21}\) | \(-20.9\) | \(-13.3\) | \(-24.3\) | \(-18.7\) | \(-14.8\) |
| 4     | QM/MM\(^{51}\) | \(-5.1\) | \(-8.5\) | \(-13.5\) | \(-16.3\) | \(-9.9\) |

Figure 1. Models of methylated nucleobases and ionic liquid ions. (A) m-Adenine, (B) m-guanine, (C) m-thymine, (D) m-cytosine, (E) m-uracil, (F) EMIM cation, and (G) acetate anion. Color schemes are as follows: white-hydrogen, cyan-carbon, blue-nitrogen, red-oxygen.

Figure 2. Solvation free energy of methylated nucleobases with changing mole fraction of water.

nucleobases throughout all mole fractions and are shown in the Supporting Information (Table S2). All methylated nucleobases show more negative solvation free energies in pure ionic liquid when compared to water; this is in agreement with the previous study on solvation free energies of parent nucleobases in pure imidazolium-based ionic liquids.\(^{50}\) The nucleobases are polar and more soluble in ionic liquids whose polarity is more than water. The solvation free energy trend for the nucleobases in the pure ionic liquid is similar to the values of bases in the pure water: m-guanine > m-cytosine > m-adenine > m-uracil > m-thymine. The difference between free energies of nucleobases in pure ionic liquid and pure water also follows...
the same trend. This trend is based on the number of polar sites available on methylated nucleobases. m-Guanine has more number of polar sites among all nucleobases: each of NH₂, NH, and C=O groups. Cytosine contains one each of NH₂ and C=O groups; adenine has one NH₂ group; thymine and uracil have each one NH and two C=O groups. Even though m-adenine has only one NH₂ group, it is more polar than m-uracil and m-thymine, because it also has three unprotonated nitrogen ring atoms, which can form polar interactions. m-Guanine and m-cytosine also have two and one unprotonated nitrogen ring atom, respectively. Although m-thymine and m-uracil are having the same number and type of polar groups, m-uracil has slightly more negative solvation free energy. The reason for this is that a methyl group in m-thymine is attached close to the C=O group, which prevents interaction of C=O group with the solvent molecules, whereas there is no methyl group nearby C=O group in m-uracil that can freely interact with the solvent molecules. The acetate anions of the ionic liquids, which are good hydrogen bond acceptors compared with the water, are the primary interacting entities with the nucleobases. They form hydrogen bonds with the amino groups of the nucleobases. Emim cation has acidic hydrogen atoms that can form hydrogen bond with C=O groups and unprotonated nitrogen ring atoms of nucleobases. The interaction of these polar sites with the solvent molecules is discussed explicitly in the following paragraphs. As we go from the pure ionic liquid to the pure water with changing mole fraction of water, the solvation free energies increase for all nucleobases. In the case of m-guanine, a steady increase of solvation free energy is observed with changing mole fraction. For remaining nucleobases, the increase in solvation free energy is found up to \( X_w = 0.4 \); however, from \( X_w = 0.4 \) to 0.8 the change in solvation free energy is less significant, followed by a sudden change in values after \( X_w = 0.8 \).
The values of relative solubility of nucleobases in pure ionic liquid and hydrated ionic liquids as compared to those in pure water are shown in Figure 3. The solubility values of methylated nucleobases in pure and hydrated ionic liquids are presented in this figure considering the solubility in pure water as the reference. This property can be used to compare the solubility of nucleobases with experimental values. The relative solubility is high for methylated nucleobases in pure ionic liquid, and it decreases with increasing mole fraction of water. This fact is in agreement with the experimental solubility measurements of parent nucleobases in pure and hydrated ionic liquids.11 We also calculated the Coulombic and van der Waals contribution to the solvation free energy, and the values are shown in Figure 4. van der Waals contribution to the solvation free energy of nucleobases in pure water is insignificant, and electrostatic interactions play a dominating role. Nucleobases are soluble in pure water due to hydrogen bonding interactions between polar sites of nucleobases and solvent. In the case of pure and hydrated ionic liquids, the contribution from Coulombic interactions is more compared to van der Waals contribution; the hydrogen bonding plays a significant role in solubilizing the nucleobases. van der Waals

Figure 5. Radial distribution functions with number integrals of COM of methylated nucleobase and COM of Emim cation with changing mole fractions of water.

Figure 6. COM–COM spatial distribution functions of cation, anion, and water around methylated nucleobases for $X_w = 0.2$ (top) and 0.8 (bottom). (A) m-Guanine, (B) m-cytosine, (C) m-adenine, (D) m-uracil, and (E) m-thymine. Color schemes are as follows: magenta cation, green anion, orange water.
The interactions between nucleobase and solvent molecules are primarily governed by the hydrogen bonding interactions. There are two types of amine groups present in nucleobases: primary amine (exocyclic NH$_2$ group) and secondary amine (ring NH group). The primary amine group interacts with solvents differently compared with secondary amine. These amine groups can undergo hydrogen bonding with both water and acetate anion. The atom--atom RDFs between primary amine hydrogens and acetate oxygen atoms and primary amine hydrogens and water oxygen atoms for mA, mG, and mC nucleobases are shown in the Supporting Information (Figure S5). The first minima for all RDFs are found at around 0.25 nm. The peak height represents a strong hydrogen bonding interaction between primary amine and acetate groups. This height for RDFs involving acetate oxygen atoms decreases with increasing mole fraction of water. For mG, we do not see the well-defined first peak for $X_w = 0.2$ and 0.4 as for mA and mC; however, it appears from $X_w = 0.6$. Moreover, for mG, the peak height is less compared to that of mA and mC nucleobases due to the presence of a secondary amine group close to the primary amine group. The atom--atom RDFs between secondary amine hydrogens and acetate oxygen atoms and secondary amine hydrogens and water oxygen atoms for mT, mG, and mU nucleobases are shown in the Supporting Information (Figure S6). In this case, we also observe the first minima at around 0.25 nm. The peak heights are more when compared to that in the case of primary amine hydrogen atoms; the hydrogen bonding interaction is stronger with secondary amines compared with primary amine. The height of first peaks of RDFs for acetate oxygen atoms with secondary amine hydrogens decreases with increase in mole fractions of water. Overall, we could not establish any trend in interaction between secondary amine group of nucleobases and the water molecules due to competition for preferential hydrogen bonding with the acetate anion.

We also present atom--atom RDFs between oxygen atoms of nucleobases and water hydrogen atoms. The corresponding RDFs are shown in the Supporting Information (Figure S7). In this case, we observe the first minima at 0.25 nm except in a few cases. For all bases, the peak heights increase with increase in water mole fractions as compared with pure water. Atom--atom RDFs between oxygen atoms of methylated nucleobase and Emim acidic hydrogen atoms have been calculated and are also shown in the Supporting Information (Figure S8). The first minima, in this case, are very broad and can be found around 0.4 nm. The peak heights are less compared to those in the RDFs involving water hydrogen atoms, indicating very weak hydrogen bond interaction. The RDFs between ring nitrogen atoms of nucleobase and Emim acidic hydrogens and ring nitrogen atoms and water hydrogen atoms are shown in the Supporting Information (Figure S9). We observe first minima at 0.35 nm in the case of Emim acidic hydrogens, which shows a moderate hydrogen bonding between ring nitrogen atoms and Emim hydrogens. The peak height is not significant and vanishes with increasing mole fraction of water for mA and mG; however, the peak height is more for mC. The first minima are observed at 0.25 nm in the case of water hydrogens. In all cases, the peak height increases up to $X_w = 0.8$, contrary to that for pure water.

As mentioned earlier, another important interaction between Emim cation and methylated nucleobase is the stacking interaction; the aromatic rings of cations can stack with the nucleobase aromatic rings. We calculated the probability...
distributions of the stacking angle of Emim cations with methylated nucleobases. The schematic representation of angles and COM–COM distance (R) between Emim cation and methylated nucleobase used in the calculation is shown in Figure 7. We define R as the distance between the center of masses of nucleobase ring atoms and cation ring atoms. $n_i$ and $n_j$ are the normal vectors to the planes of nucleobase and Emim cation ring, respectively. These vectors are the cross product of two vectors, which are lying in the plane of nucleobases and the cation ring. The vectors in the plane are defined from the center of mass of base and cation to any of the ring atoms. The representation of these vectors are shown in the Supporting Information (Figure S10). $\theta$ is the angle between the normal vector ($n_i$) to the plane of nucleobase and the vector $R$. $\alpha$ is the angle between normal vectors $n_i$ and $n_j$ that represents the stacking angle between nucleobase and cation. We calculated the distribution of angle $\alpha$ only when it satisfies two conditions: (a) $R < 0.45$ nm, which is the position of minimum of COM–COM RDF of cation and nucleobase; (b) the angle $\theta$ should be in between $0 < \theta < 60^\circ$ or $120 < \theta < 180^\circ$. If the angle $\theta$ is not in the range mentioned above, then it is considered that the cation is not in the proper stacking position. The distribution of angle $\alpha$ with a bin width of $5^\circ$ for all nucleobases with changing mole fractions of water is shown in Figure 8. We see the probability distribution of angle $\alpha$ is more at $\sim 15$ and $\sim 165^\circ$ for all nucleobases and all mole fractions. The distribution at $\sim 15^\circ$ represents the stacking of cation to the nucleobase from the top, whereas the distribution at $\sim 165^\circ$ represents the stacking of cation to the nucleobase from the bottom. We see the probability distribution of the stacking angle is more in the case of m-adenine and m-guanine as compared to that in remaining nucleobases. The reason is mA and mG have purine rings that consist of both six-membered and five-membered rings fused together, whereas other nucleobases have six-membered pyrimidine ring. The corresponding stacked structures indicated in Figure 8A–J are extracted from the trajectory and are shown in the Supporting Information (Figure S11). The excess and significant solvation free energy in the case of pure and hydrated ionic liquid is coming from van der Waals interaction that is the stacking of the Emim cations with the methylated nucleobases; cations play a major role in the solubility of methylated nucleobases in pure and hydrated ionic liquids.

Figure 7. Schematic representation of angles ($\theta$, $\alpha$) and COM–COM distance (R) between nucleobase and Emim cation.

Figure 8. Probability distribution of angle ($\alpha$) between methylated nucleobases and Emim cations with changing mole fractions of water. The structures corresponding to the labels (A)–(J) are shown in the Supporting Information (Figure S11).
CONCLUSIONS

In this study, we have calculated the solvation free energy of methylated nucleobases in pure ionic liquid [Emim][Ac] hydrated ionic liquids with changing mole fractions of water and in pure water. The solvation free energies in pure water are in good agreement with previous experimental and theoretical calculations. The free energies of nucleobases follow the trend: mG > mC > mA > mU > mT both in pure ionic liquid and in pure water. Relative solubility of methylated nucleobases in pure and hydrated ionic liquids with reference to pure water are also calculated, which is more for nucleobases in the pure ionic liquid as compared to that in hydrated ionic liquids. It decreases with increasing mole fractions of water. The order of solvation free energy in pure water changed only for adenine and thymine when we are going from pristine to methylated nucleobases. The methylation of nucleobases can cause changes in the electronic structure of the nucleobase, which reflects in the charges of atoms in the nucleobases due to electron-donating methyl group. However, the difference between solvation free energies of adenine, thymine, and uracil in pristine and methylated (current study) nucleobases is very small when compared to that of guanine and cytosine. A small structural modification can cause the change in the order of these three nucleobases. Coulombic contribution to the solvation free energies is more compared to the van der Waals contribution in pure and hydrated ionic liquids, whereas in pure water, the Coulombic contribution plays a major role than the negligible van der Waals contribution. Reported solvation free energies and relative solubility of methylated nucleobases may help in understanding the stability of DNA duplex in hydrated ionic liquids. The center of mass and nucleobases may help in understanding the stability of DNA duplex and in pure and hydrated ionic liquids, whereas in pure water, the Coulombic contribution plays a major role than the negligible van der Waals contribution. Reported solvation free energies and relative solubility of methylated nucleobases may help in understanding the stability of DNA duplex in hydrated ionic liquids. The center of mass and nucleobases may help in understanding the stability of DNA duplex in hydrated ionic liquids.

Overall, the solubility of methylated nucleobases is more in pure and hydrated ionic liquids as compared to that of pure water, which is evident from the solvation free energies. More than 95% of the solvation free energy is coming from the Coulombic interactions in the case of pure water as solvent. However, van der Waals contribution is very small and negligible. Hydrogen bonding of acetate anion and water molecules with the amine groups of methylated nucleobases correspond to the Coulombic part of the solvation free energy, which is equivalent to the Coulombic contribution of the solvation free energy in pure water. The stacking of cation with nucleobases corresponds to the excess solvation free energy in the form of van der Waals interaction.

Computational Methodology

We performed all-atom molecular dynamics simulations, where all interactions were modeled using conventional potential energy function, the sum of bonded and nonbonded energy terms. Bonded energy is comprised of bond stretching, bending, and dihedral potentials. Nonbonded energy constitutes van der Waals interactions and electrostatic interactions, which are represented by the Lennard-Jones (LJ) potential and Coulomb potential, respectively.

\[
U = E_{\text{bonded}} + E_{\text{non-bonded}}
\]

\[
E_{\text{bonded}} = \sum_{\text{bonds}} k_i (r - r_i)^2 + \sum_{\text{angles}} k_i (\theta - \theta_i)^2 + \sum_{\text{dihedrals}} k_i (1 + \cos(n_i \phi - \delta_i))
\]

\[
E_{\text{non-bonded}} = \sum_{i<j} \left\{ 4\varepsilon_{ij} \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{12} - \left( \frac{\sigma_{ij}}{r_{ij}} \right)^{6} \right\} + \frac{q_i q_j}{r_{ij}}
\]

\(k_i, k_{\phi}, k_{\theta}, \) and \(k_{\rho}\) are coefficients for bond stretching, bending, and dihedrals, respectively; \(r_{ij},\phi_{ij},\) and \(\theta_{ij}\) are the site separation distances between atoms \(i\) and \(j\), well depth of the LJ potential energy profile, and distance at which LJ interaction is zero, respectively. \(q_i\) and \(q_j\) are the partial charges of atoms.

The methylated nucleobases, Emim cation, and acetate anion were optimized followed by the Merz–Kollman charge calculation using density functional theory method employing B3LYP exchange correlation functional. 6-31g(d) and 6-31+g(2d,p) basis sets were used for methylated nucleobases and ionic entities, respectively, using Gaussian 09 software package. The ball and stick models of the five bases and ions of IL with the atom names are shown in Figure 1. The charges for atoms were obtained using restrained electrostatic potential method and these were scaled to 0.8 for Emim and acetate ions; the scaling produced good density (1.078 g cm\(^{-3}\)) compared to that from experimental data (1.099 g cm\(^{-3}\)). Water was modeled with extended simple point charge model. IL and methylated nucleobases were modeled with generalized AMBER force field using Antechamber module of AMBERTools. The details of the number of ions and water molecules used in different mole fractions of systems are provided in Table 1.

The starting coordinates of all systems were prepared using packmol software package. The systems were first minimized using the steepest descent minimization algorithm for 2000 steps. Then, we heated the systems to 600 K within a span of 1 ns and cooled back to 300 K within the same span of

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8351
simulation time to get a homogeneous mixture of water and the ionic liquid. Then, the systems were equilibrated within NVT ensemble for 2 ns, followed by an NpT simulation for 5 ns. Again, the systems were equilibrated for another 20 ns in NVE ensemble using the equilibrated average box length obtained from NpT simulation. The average size of the simulation box lengths for all nucleobases with changing mole fractions of water is incorporated in the Supporting Information (Table S1). The final coordinates from NVE simulation were used for free-energy simulations. The time step used in each simulation was 2 fs. We used velocity rescale method for controlling temperature and the Berendson barostat for controlling pressure of the system, with coupling constants 0.1 and 1.0 ps, respectively, for the initial equilibration simulations. In the final free-energy simulations, we used stochastic dynamics using the Parrinello–Rahman barostat with a coupling constant of 1.0 ps. Electrostatic interactions were treated with particle mesh Ewald method with a cutoff of 1.2 nm, and van der Waals cutoff was taken to be 1.2 nm. All simulations were performed using Gromacs 5.0.4 simulation package. We employed multistage free-energy perturbation approach with the multistate Bennett’s acceptance ratio (MBAR) method. We used coupling parameter \( \lambda \) to control the LJ and the electrostatic interactions between solute and solvent. The coupling parameter for LJ interactions was changed from 0 to 1 in six stages (0, 0.2, 0.4, 0.6, 0.8, and 1.0), and electrostatic interactions were changed in four stages (0.25, 0.50, 0.75, and 1.0), keeping the LJ interaction to 1. Each window of \( \lambda \) was equilibrated for 16 ns under NpT ensemble. Relative solubility of methylated nucleobases in pure and hydrated ionic liquids with pure water was calculated using the following equation:

\[
\ln \left( \frac{c_{i,J}}{c_{i,\text{water}}} \right) = \beta \mu_{i, \text{water}}(T, P, N = 1, N_{\text{water}}) - \beta \mu_{i, \text{water}}(T, P, N = 1, N_{\text{i}})
\]

where \( c_{i,J} \) and \( c_{i,\text{water}} \) correspond to the molar concentration of methylated nucleobase in solvent \( i \) (pure and hydrated ionic liquid) and water, respectively. \( \mu_{i, \text{water}}^{\text{res,}0} \) is the residual chemical potential of nucleobase infinitely diluted in water, and \( \mu_{i, \text{water}}^{\text{res,}0} \) is the residual chemical potential of nucleobase infinitely diluted in pure and hydrated ionic liquid. The residual chemical potential is defined as the difference between the chemical potentials of solute in the solution phase and gas phase. In the case of the pure solvent, this difference is equal to the solvation free energy of the solute. In our case, we assumed the hydrated ionic liquid mixtures as pure solvents to calculate the relative solubility of nucleobases in hydrated ionic liquid with water.

\[
\beta \mu_{i,J}^{\text{res}} = \beta \mu_i^1 - \beta \mu_i^6
\]

\[
\beta \mu_{i,J}^{\text{res}} = \beta (\mu_i^1 - \mu_i^6)
\]

\[
\beta \Delta G_{i,\text{sol}}
\]

\( \beta \) is the reciprocal of \( k_B T \), \( k_B \) is the Boltzmann constant, and \( T \) is the temperature. \( \mu_i \) was computed using python implementation of MBAR (pyMBAR), https://github.com/choderalab/pymbar.

**ASSOCIATED CONTENT**

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acsomega.8b01231.

Table S1, averaged length of the simulation box; Table S2, solvation free energy with the standard deviation of methylated nucleobases; Table S3, number integrals of cation, anion, and water around methylated nucleobases; Figure S1, convergence plots for solvation free energy of methylated nucleobases; Figures S2 and S3, center of mass RDFs with number integrals for anion and water molecules; Figure S4, SDFs of cation, anion, and water around methylated nucleobases; Figures S5 and S6, atom–atom RDFs of primary and secondary amine hydrogen atoms, respectively; Figures S7 and S8, atom–atom RDFs of oxygen atoms of bases with water hydrogen atoms and Emim acidic hydrogens, respectively; Figure S9, atom–atom RDFs of ring nitrogen atoms and Emim acidic hydrogen atoms and water hydrogen atoms; Figure S10, vectors lying in the plane of base and Emim cation; Figure S11, corresponding stacked conformations of Emim cation with methylated nucleobase (PDF).

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**Notes**

The authors declare no competing financial interest.

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