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ARTICLE

Effect of Water on Solvation and Structure of Lipase in Deep Eutectic Solvents Containing Protein Destabilizer and Stabilizer

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Aqueous deep eutectic solvent (DES) solutions emerge as new media for biocatalysis. The large number of DESs provides a space for designing solutions with desired features. One challenge for this design is to understand the fundamental relationship between the water effect on biocatalysis and the DES compositions. We investigate the solvation and structure of a lipase protein in two DESs containing protein destabilizer (choline: urea (1:2)) and stabilizer (choline: glycerol (1:2)) and their 1:1 aqueous solution using molecular dynamics simulations. The lipase protein in the pure aqueous solution is simulated as the reference. The lipase protein remains folded in both DESs and their aqueous solutions. In both DESs, water molecules weaken the solvation shell of the lipase protein by reducing the protein-DES hydrogen bond lifetimes. However, the water molecules change the surface area and conformation of the active site on the lipase protein differently in the two DESs. Our simulations indicate that the impact on active sites plays an important role in differentiating the effect of water on biocatalysis in aqueous DESs.

1. Introduction

Aqueous deep eutectic solvent (DES) solutions emerge as promising liquid media for biocatalysis.[1-4] One critical issue for industrial applications of biocatalysis is to search for suitable liquid solvents other than pure water since many reagents and reactants cannot dissolve well in water.[2, 3] DESs are composed of hydrogen bond donors (HBDs) and acceptors (HBAs). The ability to form hydrogen bonds makes DESs an attractive ingredient for liquid media of biocatalysis because hydrogen bonds could help control protein solvation and functions. Experiments have shown the promising role of aqueous DES solutions for biocatalysis.[5-10] The large number of DESs provides a space for designing suitable candidates. However, rationalizing such design needs us to understand better the mechanisms behind the solvation effect on biocatalysis and the relationship between the effect and the solvent composition.

One question is how the effect of water molecules on biocatalysis relates to DES compositions. Adding water is a practical approach reported in many experiments investigating biocatalysis in DES. These experiments have reported changes in biocatalysis with adding water.[6, 10-14] The variation of these experimental observations indicates that the effect of water on function of enzymes shall have a close relationship with the DES composition. This question is not trivial if we consider the large and rapidly growing DES library.

We plan to seek the answer to this question by investigating the solvation, structure and dynamics of a model enzyme in selected DESs and their aqueous solutions. The overall performance of an enzyme depends on its ability to bind to the substrate and to catalyse the reactions. The two abilities rely on the solvation, structure and flexibility of the whole protein and particularly the active site. The DES molecules shall form a solvation shell around the protein and make the protein possess a certain structure and flexibility. The presence of water molecules may change the composition and structure of the molecules in the solvation shell and affect the structure and flexibility of the whole protein or its active site. The DES composition determines how the presence of water molecules may change the original behaviour of the protein in pure DES. Revealing this mechanism could help better understand the effect of water molecules on biocatalysis in individual DESs and develop principles for designing aqueous DES solutions for that can regulate the biocatalysis as desired.

We will use two DESs: choline Cl (ChCl): urea (1:2) and ChCl: glycerol (1:2) as the models. Among aqueous DES solutions for biocatalysis, it is particularly interesting to notice that some systems contain protein stabilizers while some contain protein destabilizers. For instance, urea can destabilize proteins while glycerol can stabilize proteins.[15-18] Both urea and glycerol can form DES and their aqueous solutions have been used for biocatalysis.[2, 19-21] Lindberg et al.[22] found that DESs contain choline (HBAs), urea and glycerol (HBDs) could be used as cosolvents to increase the enzyme-catalysed epoxide hydrolysis. Zhao et al.[23] reported that choline acetate: glycerol increases the enzymatic production of biodiesel. Maugeri et al.[6] performed whole-cell biocatalysis in DES containing choline chloride and glycerol. They found that the whole cells could stay stable in choline chloride: glycerol with up to 20

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vol% water. The structure of an enzyme plays a vital role in its function. The different role of urea and glycerol regarding the protein structure makes the DESs containing them a great pair for investigating how the water effect relates to the DES composition.

Extensive experimental efforts have been conducted to investigate the properties of the protein in DESs or aqueous DES solutions.[12, 15, 22, 24-26] Esquembre et al.[27] investigated the stability of egg white lysozymes in ChCl: urea and ChCl: glycerol solutions. They found that lysozyme partially folds in the ChCl: glycerol and this process is reversible with the presence of water. Sanchez-Fernandez et al.[28] studied the folding and stability of bovine serum albumin and lysozyme in DESs that contain choline chloride, urea and glycerol. Their result shows that protein forms a more stable structure in DESs. Mamashli et al.[29] investigated the stability of bovine ribonuclease A (RNase A) in DESs containing choline chloride, urea, 1,3-dimethylurea, glycerol and ethylene glycol. They reported that RNase A shows higher thermodynamic stability in ChCl: glycerol than in ChCl: urea mixtures. Gunny et al.[30] explored cellulase activity in DESs composing of choline chloride, glycerol, ethylene glycol, malonic acid. Their result shows that the cellulase remains 90% of its activity in the DESs, and DESs improve the saccharification processes. These experimental results indicate the importance of investigating the water effect on protein structure and functions in DESs.

Molecular simulations have been conducted to investigate the properties of proteins in specific DES or aqueous DES solutions. Most of the simulations focus on a particular DES and its aqueous DES solutions. Monhemi et al.[16] investigated the structure and stability of lipase in ChCl: urea (9M urea), water and 8M urea using molecular dynamics (MD) simulations. Their result shows that lipase remains stable in the DESs, whereas denatured in the 8M urea solution. They also discussed that the protein-DES hydrogen bonds could help stabilize the lipase. Shehata et al.[31] investigated the stability of lipase in ChCl: urea DES and aqueous DES aqueous solution via MD simulations and experiments. They found that water has little effect on lipase stability, whereas it can increase lipase activity compared with pure DES. Kumari et al.[32] studied the effect of water on the conformation and stability of lysozyme in ChCl: urea via MD simulations. Their result shows that adding water destabilizes both the active site and the secondary structure of the lysozyme in DES. Pal et al.[33] investigated the thermal stability of Trp-cage mini-protein in ChCl: glycerol and pure aqueous solution at different temperatures using MD simulations. Their simulations show that DES can better protect the protein structure than water. These simulation studies have shown the molecular-level effect of DES on protein properties and the impact of water molecules in particular DES. These simulations highlight the molecular-level effect of DES and aqueous DES on the properties of proteins. Their distinct observations also highlight the importance of revealing the connection between water impact and the original DES compositions.

This work aims to investigate the mechanism that connects the DES composition and the water effect on protein functions in an aqueous DES solution. This work will use two DESs: choline: urea (1:2) and choline: glycerol (1:2) and their aqueous solution as the model as discussed in the above paragraph. The lipase protein will be used as the model enzyme because it has been well studied in enzyme simulations. Lipase also plays an important role in biocatalysis applications. We will use the lipase in pure aqueous solution as the reference to analyze if the protein may possess any features in DES and aqueous DES solutions. The rest of the paper will be organized as follows: section 2 will present the detail of the model and simulation, section 3 will present the result and discussion and section 4 will present a conclusion.

2. Molecular model and simulation detail

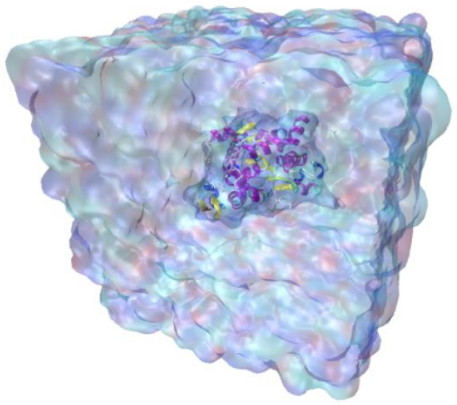


Figure 1. Snapshot of the box containing one lipase protein in 1512 choline molecules, 1500 Cl^- and 3000 urea molecules. The extra 12 choline molecules are used to compensate the net charge of the lipase protein. The lipase protein: new-cartoon model, colored based on the secondary structure. The choline molecules, Cl^- and urea molecules are shown in the QuickSurf model, colored based on the name of the atoms.

The simulation systems are created by placing a lipase protein from Candida Antarctica (PDB ID: 1tca) in a cubic box and filling the box with specific numbers of solvent molecules and ions (water, choline, urea, glycerol and Cl^-). All-atom models are used to describe the lipase protein, urea, choline and glycerol (Gly) molecules. Table 1 lists the numbers of solution molecules in the five simulation boxes. Figure 1 shows a snapshot of a lipase protein in a box of ChCl-Urea solution.

Table 1. Detail of the solution molecules in the five simulation systems

System	Solution	Choline	Cl^-	Urea/Gly	Water
Ref	Water	-	-	-	18683
ChCl-Urea	ChCl: urea (1:2)	1512	1500	3000	-
ChCl-Gly	ChCl: glycerol (1:2)	1512	1500	3000	-
ChCl-Urea-sol	ChCl: urea (1:2) + water	1512	1500	3000	2000
ChCl-Gly-sol	ChCl: glycerol (1:2) + water	1512	1500	3000	2000

This work deploys the OPLSAA/M force field[34, 35] to describe bonded and nonbonded interactions in the systems. The OPLSAA/M force field has been widely used for simulating

small molecules and biomolecules. The DES molecules were described by the OPLS-derived force field developed for DES molecules.[36] The non-boned interactions are a sum of short-range Lennard-Jones 12-6 potential and long-range coulombic potential, as shown in Equation 1. The bonded interactions are a sum of the bond, angle, and dihedral potentials, as described in the force field.

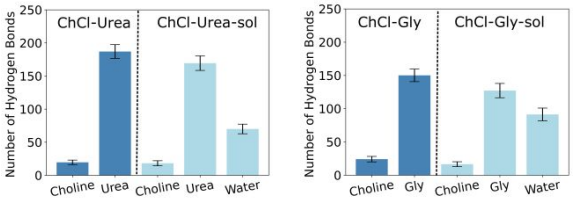
$$E_{ij}(r_{ij}) = 4\varepsilon_{ij}\left(\left(\frac{\sigma_{ij}}{r_{ij}}\right)^{12} - \left(\frac{\sigma_{ij}}{r_{ij}}\right)^{6}\right) + \frac{e_i e_j}{4\pi\varepsilon_0 r_{ij}} \qquad (1)$$

where E_{ij} is the potential energy due to the nonbonded interactions between atoms i and j, r_{ij} is the distance between atoms i and j, ε_{ij} is the energetic parameter, σ_{ij} is the geometric parameter and e_i is the partial charge of atom i. The Jorgensen mixing rule is applied to obtain ε_{ij} and σ_{ij} for atoms belonging to different types.

A three-step simulation process is conducted for every simulation system. First, energy minimization was conducted to remove any too-close contacts between atoms. Second, a 200-ns isobaric-isothermal (NPT, T=300 K, P= 100 KPa) ensemble MD simulation (integral step = 2 fs) was conducted to let the system reach thermodynamic equilibrium. Third, a 500-ns canonical (NVT, T=300 K) ensemble MD simulation (integral step = 2 fs) was conducted to collect the trajectory at a frequency of 50 ps. The Berendsen method[37] is used to control the temperature and pressure of the system in the second step because it allows the system to reach the desired pressure and temperature at a fast pace. The velocity-rescaling method[38] is used to control the temperature of the system in the third step. The short-range van der Waals interactions use a 1.2-nm cut-off, and the long-range electrostatic interactions were calculated using the particle mesh Ewald sum.[39] All bonds involving H atoms were constrained during the simulations. The energy minimization and MD simulations for all the systems were conducted using Gromacs-2020.3.[40]

3. Results and discussion

3.1 Solvation of lipase protein



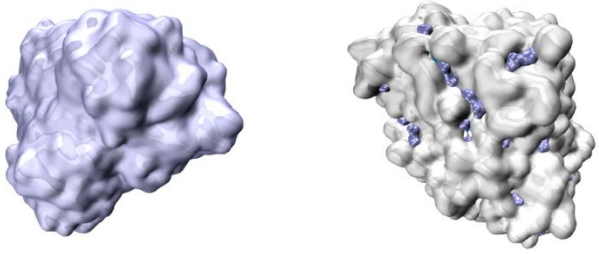
(a) (b)

Figure 2. Number of protein-solvent hydrogen bonds in the two DESs and their aqueous solutions. (a) ChCl-Urea and ChCl-Urea-sol and (b) ChCl-Gly and ChCl-Gly-sol.

We characterize the solvation of the lipase protein using protein-DES and protein-water hydrogen bonds because the hydrogen bonds play an important role in protein-solvent interactions. A hydrogen bond is defined by the criteria proposed by the Chandler group[41]. Figure 2 shows the average number of protein-solvent hydrogen bonds (N_{HB}) in the four DES and aqueous DES solutions.

The DES solvation shell possesses much fewer protein-solvent hydrogen bonds comparing with the water-only solvation shell. Our calculation shows that N_{HB} is 499.99 for the lipase protein in the pure aqueous solution. This number decreases to 206.54 and 174.10 in the ChCl-Urea, and ChCl-Gly solutions, only <40% of that in the pure aqueous solution. This deduction of the hydrogen bond number may be due to the bigger molecular size of DES components. Since the hydrogen bond is a vital part of protein-solvent interactions, this decrease indicates that the connection between protein and the nearby solvent molecules becomes weaker in the DES solutions.

The protein prefers to form hydrogen bonds with the hydrogen bond donors (urea and Gly) in the DESs. As shown in Figure 2, the average number of protein-choline hydrogen bonds is only 10% and 26% of that between the protein and the other components (urea or Gly). This ratio is much less than the molar ratio between choline and the other compounds (1:2). The big size of choline molecules may contribute to this preference. Such disproportional distributions of hydrogen bond numbers may indicate that the urea or Gly accumulate around the lipase protein. This accumulation could be interpreted from their role as protein stabilizer and destabilizer. Urea molecules have been reported to accumulate around a protein.[42-44] For instance, Stumpe et al.[42] investigated the interaction between urea and 22 tripeptides using MD simulations. They found that urea molecules prefer to contact the protein backbone of all amino acids. Vagenende et al.[45] found that glycerol could form multiple hydrogen bonds with lysozyme in their MD simulations. The other simulation of protein in DES also observes this phenomenon. The urea and Gly may play a more direct role in enzyme functions in the DESs than choline.



(a) pure aqueous solution (b) ChCl-Urea-sol solution

Figure 3. Schematic of solvation shell of the lipase protein in (a) pure aqueous solution and (b) ChCl-Urea-sol solution. Iceblue surface: water molecules, sliver surface: choline molecules and urea molecules. The schematic in (b) shows the spot of water molecules among choline and urea molecules.

The presence of water molecules enhances the wetting of the lipase protein without reducing the number of protein-DES hydrogen bonds in a large amount. As shown in Figure 2a, the value of protein-solvent N_{HB} increases by almost 25% when adding water to ChCl: urea DES. A similar phenomenon can also be observed for the ChCl: Gly system. The significant increase of protein-solvent N_{HB} is mainly contributed by the increase of protein-water hydrogen bonds. The value of protein-DES NHB decreases by 9.16%. Such a small decrease indicates that the water molecules do not replace many DES molecules from the solvation shell of protein. Instead, these

water molecules may prefer to fill the gap between big DES molecules. Our simulation implies that one lipase could be fully surrounded by around 500 water molecules, much less than the 2000 water molecules in the aqueous DES solutions. However, the water molecules do not replace these DES molecules. The snapshot in Figure 3 supports this scenario. Figure 3 shows the solvation shells of the lipase protein in the pure aqueous solution and the ChCl-Urea aqueous solution. As shown in Figure 3b, the lipase is mainly surrounded by DES molecules and water molecules only formed discretely small domains around the lipase protein.

We then investigate protein-solvent hydrogen bonds lifetime in the DES and aqueous DES solutions. Table 2 shows the lifetime (τ) of protein-DES and protein-water hydrogen bonds in the four DES and aqueous DES solutions. The hydrogen bond lifetime is calculated in two steps: (1) calculate the hydrogen bond autocorrelation function C(t) and (2) calculate the τ by the numerical integration of the C(t) curve. The calculation of C(t) is shown in equation 2:

$$C(t) = \frac{\langle N_{HB}(t)\rangle}{\langle N_{HB}(0)\rangle} \qquad (2)$$

where $\langle N_{HB}(0)\rangle$ is the ensemble average of the number of hydrogen bonds at time=0, and $\langle N_{HB}(t)\rangle$ is the ensemble average of the number of remaining hydrogen bonds at time=t. Based on Rappaport's definition[46], the hydrogen bonds are counted even if they were broken intermittently. Then the τ is calculated by numerically integrating the C(t) curves.

Table 2. Lifetime of hydrogen bonds between protein and solvate molecules

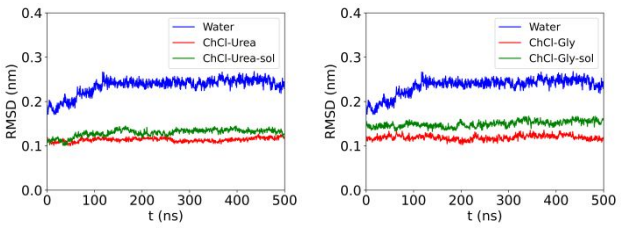
	ChCl-Urea	ChCl-Urea-sol
	τ (ns)	
Choline ($HB_{p\text{-}Ch}$)	17.67	12.91
Urea ($HB_{p\text{-}U}$)	20.04	14.43
Water ($HB_{p\text{-}w}$)	-	23.13
	ChCl-Gly	ChCl-Gly-sol
	τ (ns)	
Choline ($HB_{p\text{-}Ch}$)	12.28	9.47
Gly ($HB_{p\text{-}G}$)	13.23	7.82
Water ($HB_{p\text{-}w}$)	-	17.75

The protein-DES hydrogen bonds present distinct lifetimes in the two DES solutions. Table 2 shows that the lifetime of $HB_{p\text{-}Ch}$ and $HB_{p\text{-}U}$ is 17.67 and 20.04 ns in ChCl-Urea, about 43.89% and 51.47% longer than those (12.28 and 13.23 ns) in the ChCl-Gly solutions. The lipase solvation shell in the ChCl-Urea could be more stable and harder to penetrate than the shell in the ChCl-Gly solution.

The presence of water molecules weakens the protein-DES hydrogen bonds for both ChCl-Urea and ChCl-Gly. As shown in Table 2, the lifetime of $HB_{p\text{-}Ch}$ and $HB_{p\text{-}U}$ decreases to 12.91 and 14.43 ns in ChCl-Urea-sol solution, around 73.06% and 72.01% of that in ChCl-Urea. ChCl-Gly and ChCl-Gly-sol solutions show the same tendency. The lifetime of $HB_{p\text{-}Ch}$ and $HB_{p\text{-}G}$ decreases to 9.47 and 7.82 ns in ChCl-Gly-sol solution, around 77.12% and 59.11% of that in ChCl-Gly solution. Such a decrease implies that the presence of water molecules weakens the stability of protein-DES hydrogen bonds. Since the DES molecules dominate the solvation shell of the lipase protein, such a decrease of protein-DES protein hydrogen bonds may make ligands easier to approach the lipase.

It is worth noting that the lifetime of protein-water hydrogen bonds is much longer in the aqueous DES solution than in the pure aqueous solution. The latter is 0.3875 ns, only ~2% of the that in the aqueous DES solutions. This 50-fold increase could be due to the low mobility of DES molecules. Our result agrees with other simulations. Stumpe et al.[43] also reported that the protein−urea hydrogen bonds are much weaker than protein−water hydrogen bonds in their MD simulations of 22 tripeptides.

3.2 Root mean square deviation of the whole lipase



(a) (b)

Figure 4. RMSD of C_α atoms on the lipase protein in DES and aqueous DES solutions. (a) ChCl-Urea, and ChCl-Urea-sol, (b) ChCl-Gly, and ChCl-Gly-sol. The RMSD of C_α atoms on the lipase protein in water (Ref) is shown in both figures as a reference.

The lipase protein remains folded in the four DES and aqueous DES solutions. Figure 4 shows the root mean square deviation (RMSD) of C_α atoms on the lipase protein in the five solutions. RMSD quantifies the difference between a protein structure and a reference. The original crystal structure of the lipase protein in the PDB is used as the reference. The RMSD value will increase to >1 nm if the lipase protein unfolds. As shown in Figure 4, the lipase protein presents RMSD around 0.2-0.3 nm in the pure aqueous solution and around 0.1-0.15 nm in the DES and DES aqueous solutions. These small RMSD values imply that the lipase protein keeps folded in the four DES solutions and the DES aqueous solutions. The folded lipase protein should be able to perform its catalytic function in these solutions, as observed in the experiment.

The lipase protein in the DES and aqueous DES solutions may present a conformation different from that in the pure aqueous solution. As shown in Figure 4, the RMSD curves for the lipase protein are around 0.1 to 0.15 nm in the DES and aqueous DES solutions, while the RMSD curve for the lipase protein is > 0.2 nm in the pure aqueous solution. This difference in RMSD indicates that the structure of the lipase protein in the DES and aqueous DES solutions should differ from that in the aqueous solution. Such differences could be due to the changes in secondary structure. We will analyze the secondary structure of the whole lipase protein and its active site later.

The presence of water molecules may further change the conformation of the lipase protein in the DES solutions. As shown in Figure 4, the RMSD curves for the lipase protein in the aqueous DES solutions present a small deviation from those in the same DES solution. This small deviation implies the changes in the lipase protein conformation when adding

water to the DES solutions. Such conformation changes may only occur on several amino acid residues instead of the overall protein. However, such local changes could impact the function of the lipase protein if it occurs on or near the active site.

3.3 Flexibility of the whole lipase protein

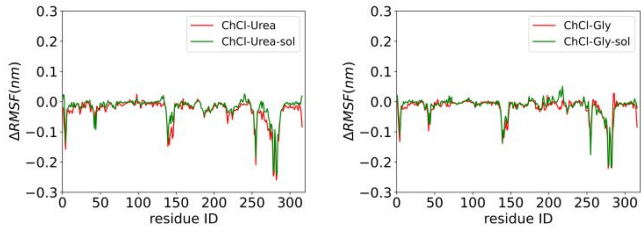


Figure 5. ΔRMSF of the C_α atoms on the lipase protein in the DES and aqueous DES solutions. (a) ChCl-Urea, and ChCl-Urea-sol, (b) ChCl-Gly, and ChCl-Gly-sol. The RMSF values of the C_α atoms on the lipase protein in the pure aqueous solution are used as the reference.

The solvation could affect the protein function by altering the dynamics of amino acid residues on it. We characterize the variation of residue dynamics using ΔRMSF of the C_α atoms on the protein calculated as $RMSF_i^S - RMSF_i^w$, where $RMSF_i^S$ is the root mean square fluctuation (RMSF) of C_α atom on amino acid residue *i* in solution *S*, and $RMSF_i^w$ is the RMSF of C_α atoms on amino acid residue *i* in the pure aqueous solution. Figure 5 shows the values of ΔRMSF for C_α atoms on the lipase protein in the four DES and aqueous DES solutions.

Only a few amino acid residues reduce their flexibility in the ChCl-Urea DES. As shown in Figure 5a, most of the amino acid residues present ΔRMSF close to zero, indicating that they remain the flexibility in the pure aqueous solutions. Only amino acid residues in several regions present ΔRMSF < 0, and no amino acid residues present significantly positive ΔRMSF. Table 3 lists the numbers of the amino acid residues with ΔRMSF ≤ -0.1 nm and their IDs. Figure 6 highlights these amino acid residues with ΔRMSF <-0.1 nm on the lipase protein. Figure 6 also shows the three amino acid residues (SER105, ASP187 and HIS224) in the active site.[47] These amino acid residues mainly locate in the α-helix regions.

Table 3. The amino acid residues changed their RMSF< -0.1 nm in the two DES solution

System	# of Res	Res ID
ChCl-Urea	23	3,4,139-146, 253-255, 274-285
ChCl-Gly	11	4, 139-141, 277-283

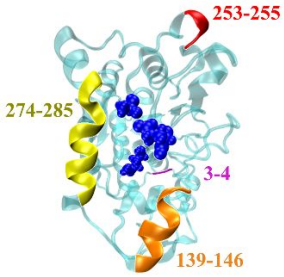


Figure 6. The location of the four regions with ΔRMSF <-0.1 nm on the lipase protein. The lipase protein: new-cartoon model, and the active site of the lipase: VDW model. Cyan: lipase protein, purple: RES3-4, red: RES253-255, yellow: RES274-285, orange: RES139-146, blue: activate site of SER105, ASP187 and HIS224, respectively.

Less amino acid residues on the lipase protein reduce their flexibility in the ChCl-Gly DES. As shown in Table 3, the numbers of amino acid residues with ΔRMSF < -0.1 nm are only 11 in the ChCl-Gly, only 50% of the ChCl-Urea DES. These amino acid residues mainly locate on the α-helix region. The amino acid residues 274-285 and 139-146 are close to the active site. The variation of these amino acid residues may impact the activity of the enzyme and affect the chance for the approach of a ligand.

The presence of water molecules has a minute effect on the dynamics of amino acid residues on the lipase protein in DES solutions. As shown in Figure 5, the ΔRMSF curve for the lipase protein in the aqueous DES solution is similar to that in the corresponding DES solution. This similarity indicates that adding water molecules does not change the dynamics of the lipase protein in the two DES solutions. This similarity in ΔRMSF is consistent with that the solvation shell of the lipase protein is dominated by DES molecules instead of water molecules, as shown in Figure 3b. Kumari et al.[32] found that lysozyme shows distinct flexibility in pure ChCl: urea DES and DES aqueous solutions.

3.4 Secondary structure of the whole lipase

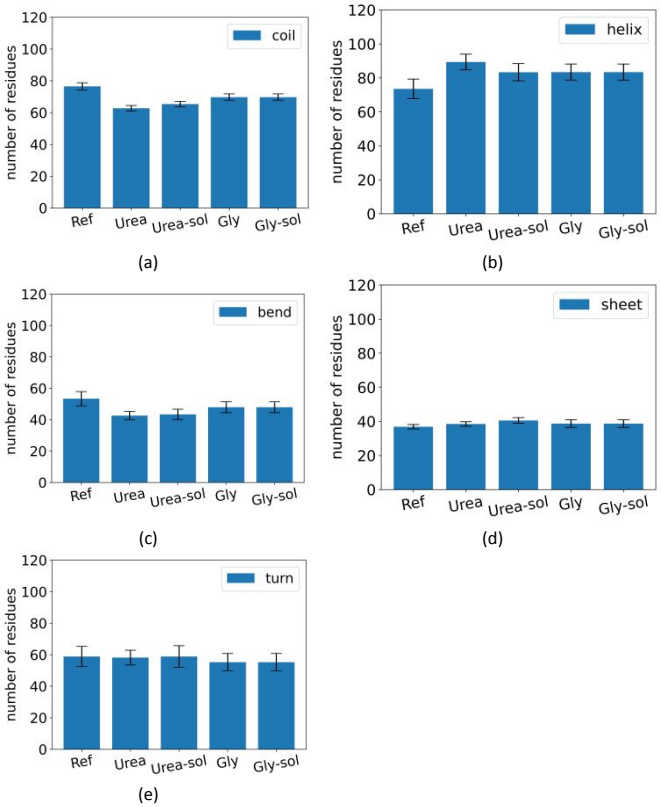


Figure 7. The number of amino acid residues in the (a) random coil, (b) α-helix, (c) β-sheet, (d) bend and (e) turn structures.

The lipase protein adapts more ordered structure in the DES solutions. Figure 7 shows the average numbers of amino acid residues in the random coil (N_c), α-helix (N_h), bend (N_b), β-sheet (N_s) and turn (N_t) structures in the pure aqueous solution and the four DES and aqueous DES solutions. As shown in

Figure 7a, the value of N_c is 76.51 in the pure aqueous solution and decreases to 62.75 and 69.73 in the ChCl-Urea and ChCl-Gly solutions. Around 13 and 7 amino acid residues on the lipase shift to ordered secondary structure in the ChCl-Urea and ChCl-Gly DESs. Kumari et al.[32] reported that the lysozyme becomes more disordered in ChCl: urea and ChCl: urea-water mixture. However, Esquembre et al.[27] found that the secondary and tertiary structures of lysozyme show little differences in buffer solutions and chloride-based neat eutectic at room temperature in experiment. Sanchez-Fernandez et al.[28] also reported that the secondary structure of the lysozyme and bovine serum albumin does not change much when the solvent varies from phosphate buffer to choline chloride-based DES based on the circular dichroism result. Such distinct observations indicate that the effect of DESs on the secondary structure of proteins may relate to the original folded structure of the proteins.

The DES solution results in slight changes in the number of amino acid residues in the four ordered secondary structures. Figure 7b shows that the number of amino acid residues in the α-helix structure increases by 10-16 in the two DES solutions compared to that in the pure aqueous solution. On the contrary, the number of the bend structure decreases by about 6-10 in the DES solutions compared with that in pure aqueous solution (53.35). The number of amino acid residues taking the β-sheet and turn structure changes slightly when the solution varies, as shown in Figures 7d and e.

The presence of water molecules restores the secondary structure of the lipase protein in the DES solutions toward that in the pure aqueous solution. Figure 7a shows that around three amino acid residues retake the random coil structure in the two aqueous DES solutions. The presence of water molecules also decreases N_h in the ChCl-Urea-sol solutions to a level closer to that in the pure aqueous solution.

3.6 Surface area and secondary structure of the active site

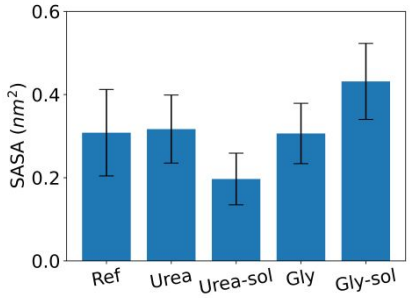
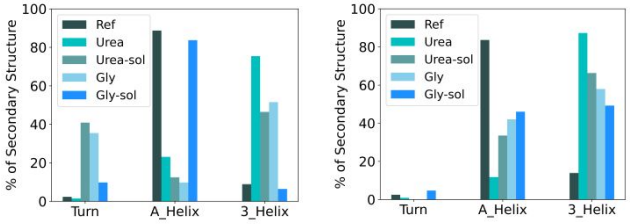


Figure 8. SASA of the active site on the lipase protein in the five solutions.

The active site of the lipase protein remains its surface area in the two pure DES solutions. We characterize the surface area of the active site using the solvent accessible surface area (SASA) of its amino acid residues (SER105, ASP187 and HIS224). Figure 8 shows the SASA of the active site on the lipase protein in the pure aqueous solution and the four DES and aqueous DES solutions. The original value of SASA for the pure aqueous solution is 0.30±0.18 nm^2. The corresponding values are 0.32±0.08 and 0.30±0.07 nm^2 in the ChCl-Urea and ChCl-Gly solutions. They overlap that in the pure aqueous solution very well, considering the deviation.

The presence of water molecules decreases the surface area of the active site on the lipase protein in the DES solution containing urea. Such a reduction of the surface area could significantly downgrade the lipase protein performance. As shown in Figure 8, the SASA is 0.20±0.06 nm2 in the ChCl-Urea-sol aqueous solution, only around 66% of that in the pure DES solution.

On the contrary, the presence of water molecules increases the surface area of the active site on the lipase protein in the DES solution containing Gly. As shown in Figure 8, the SASA is 0.43±0.09 nm^2 in the ChCl-Gly-sol aqueous solution, 43% more than the SASA of the active sites in the ChCl-Gly solution. Such an increase of SASA may promote the possibility of protein-substrate attachment, which is critical for the catalytic function of the lipase protein.



(a) SER105 (b) HIS224

Figure 9. The percentage of secondary structure for the (a) SER105 and (b) HIS224 on the active site in the pure aqueous (Ref) and four DES and DES aqueous solutions.

The change of solvents also results in the variation of secondary structure for amino acid residues on the active site of the lipase protein. We focus our analysis on SER105 and HIS224 since ASP187 remains its secondary structure in all four DES and DES aqueous solutions comparing with that in the aqueous solution. Figure 9 shows the percentage of secondary structures including turn (P_t), bend (P_b), α-helix (P_h) and 3-helix (P_{3h}) for SER105 and HIS224 in the pure aqueous, DES and DES aqueous solutions.

The secondary structure of SER105 and HIS224 varies in two DES solutions compared to pure aqueous solutions. As shown in Figure 9a, the SER105 has its P_t decrease by 34.78% in ChCl-Urea whereas increases by 1441.74% in ChCl-Gly compared with that in pure aqueous solutions. The P_h decreases by 73.98% and 88.96% for ChCl-Urea and ChCl-Gly solutions compared with that in pure aqueous solutions. The P_{3h} increased by 748.37% (ChCl-Urea) and 479.87% (ChCl-Gly) compared with that in pure aqueous solutions. The P_t of HIS224 decreases by 85.90% (ChCl-Urea) and 49.70% (ChCl-Gly), whereas the P_h increased by 528.58% (ChCl-Urea) and 317.13% (ChCl-Gly) compared with those in pure aqueous solution.

The presence of water molecules partially restores the secondary structures of SER105 and HIS224. Adding water molecules readjust the secondary structure to a level closer to that in the pure aqueous solution for ChCl-Gly, as shown in Figure 9a. The P_t decreases to 9.79%, closer to the value of 2.30% in pure aqueous solutions than the value of 35.46% in

pure DES solution. The P_h increases to 83.72% (ChCl-Gly-sol), compared with 88.71% in pure aqueous solution. The P_{3h} decreases to 6.39% in DES aqueous solution, compared with 13.89% of that in pure aqueous solution. While adding water molecules does not change the secondary structure of SER105 to close to a level in the pure aqueous solution for ChCl-Urea. Figure 9a shows that all three types of secondary structures are far away from the reference level. While the secondary structures of HIS224 in DES aqueous solutions are still far from those in the aqueous solutions. The P_t of the two DES aqueous solutions is only 40~60% of that in the pure aqueous, while the P_h is around 300~500% compared with the pure aqueous solution.

4. Conclusion

We investigate the effect of water on the solvation and structure of a lipase protein in two DESs containing protein stabilizer and stabilizer using MD simulations. Our simulations indicate that water molecules may have two possible effects on the biocatalysis in DES solution. The first effect is the enzyme affinity. The protein-solvent hydrogen bonds show that water molecules do not replace the DES molecules around the lipase protein but weaken the protein-DES interactions. Such a weakening effect could destabilize the lipase solvation shell and make ligands easier to approach the enzyme. The second effect is the conformation of the active site. The secondary structure analysis indicates that the lipase presents a more ordered structure in both ChCl-Urea and ChCl-Gly DESs. Adding water molecules recover their structure toward that in the pure aqueous solution. Adding water molecules affects the surface area and secondary structure of the active site of the lipase distinctly in the ChCl-Urea and ChCl-Gly DESs. The surface area of the active site of the lipase in the ChCl-Urea decrease when adding water molecules. However, adding water molecules increases the surface area of the active site of the lipase in the ChCl-Gly. The SER105 and HIS224 on the active site also show distinct changes in their secondary structure when adding water molecules to the two DESs.

Conflicts of interest

There are no conflicts to declare.

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