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Organic solvents aggregating and shaping structural folding of protein, a case study of the protease enzyme

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ABSTRACT

Low solubility of reactants or products in aqueous solutions can result in the enzymatic catalytic reactions that can occur in non-aqueous solutions. In current study we investigated aqueous solutions containing different organic solvents / deep eutectic solvents (DESs) that can influence the protease enzyme's activity, structural, and thermal stabilities. Retroviral aspartic protease enzyme is responsible for the cleavage of the polypeptide precursors into mature viral components, a very crucial step for virus life cycle. In molecular dynamic simulations (MDS), the complex of the protease enzyme with Darunavirwas found highly stable in urea aqueous solution compared to when with the ethylene glycol (EG) or glycerol solvents. Particularly, in different organic solvents the presence of Darunavir induced protein-protein interactions within the protease homodimer. For the systems with EG or glycerol solvents, the flap domains of the enzyme formed an "open" conformation which lead to a weak binding affinity with the drug. Conserved D25 and G27 residues among this family of the aspartic protease enzymes made a stable binding with Darunavir in the urea systems. Unfolding of the protease dimer was initiated due to self-aggregation for the EG or glycerol organic solvents, which formed an "open" conformation for the flap domains. On the contrary lack of such clustering in urea solvent, the protease showed conventional structural folding in the presence or absence of the drug molecule. These novel findings may help to better understand the protease enzymes, which could be controlled by deep eutectic solvents.

1. Introduction

Solvents (in particular water), play an important role in structure, dynamics, and function of biological systems. The structure and dynamics of water molecules in the vicinity of the proteins absolutely depend on the nature of proteins such as hydrophilicity or hydrophobicity, which is the main factor of thermodynamic stability of folded proteins in aqueous solutions [1]. A deeper understanding of the solvation phenomena of biomolecules in aqueous and non-aqueous solutions is crucial. Since water is a native solvent of biomolecules and it can influence the enzymatic catalysis activity by many ways such as providing a solvation shell for reactants, products, and transition state complexes [2,3]. Though, majority of enzymatic catalytic reactions take place in aqueous solutions, some can happen in non-aqueous solutions of organic solvents (OS), ionic liquids (ILs) or deep eutectic solvents (DESs) due to low solubility of reactants or products in aqueous solutions. Additionally, changing the micro-solvation environment of the enzymes or by other solvents than water enzymatic activity, bio-catalysis selectivity or enzymatic stability can be altered [4–10]. In our current study we investigated effects of the aqueous solutions of organic solvents that can influence the enzyme's activity, structural, and thermal stabilities. These effects can happen to HIV-1 (human immunodeficiency virus type 1) protease enzyme by OS and ILs by changing the dielectric constant of mixture, as the organic solvents in general have lower dielectric constants than water.

The protease enzyme is a retroviral aspartic protease responsible for the cleavage of the polypeptide precursors into mature viral enzymes and structural proteins [11,12]. This is a critical processing step in the late phase of HIV life cycle, and it has been shown that budded immature viral particles containing catalytically inactive protease cannot undergo maturation to an infective form. The necessity as well as importance of this protease enzyme in the virus life cycle, makes it a promising target to investigate [11,12]. HIV-1 protease functions as a homodimer, where each 99 amino acid monomer contains an extended β-sheet region and a

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glycine rich loop known as the 'flap'. The active site lies between the identical subunits of protease and has the characteristic Asp-Thr-Gly (D25, T26, and G27) sequence which is conserved among the aspartic protease enzymes [13,14]. The 'flap' constitutes in part the substrate-binding site and plays an essential role in binding the substrate, and one of the two catalytic aspartyl residues (D25 and D25') which lie on the bottom of the cavity [13,15]. Jaskólski et al. have proposed the mechanism for protease enzyme cleavage, in which the water molecules act as a nucleophile and simultaneous conjunction with well-placed catalytic aspartates to hydrolyse the scissile peptide bond [16,17]. For the active protease homodimer, there are two molecular 'flaps' which move up to 7 Å of distance when the enzyme becomes associated with a substrate [18].

The protease inhibitors specifically bind to the active site and essentially become stuck, which disables the enzyme. This disabling of protease enzymes results in the production of immature proteins that cannot assemble into infectious virions [19]. In recent time several protease inhibitors have been licensed for antiretroviral therapy. However, the high mutation rates of retroviruses change a few amino acid residues within HIV-1 protease and render it much less visible to an inhibitor. The active site of this protease enzyme can change rapidly when under the selective pressure of replication-inhibiting drugs [20,21]. One of the most common HIV-1 protease inhibitors is Darunavir (DRV; Fig. 1A) and considered in this work, is the first to be shown to block the dimerization and approved by the US Food and Drug Administration (FDA) [22–24]. Moreover, a combination of four amino acid substitutions in the proximity of the active site; V32I, L33F, I54M, and I84V emerged in protease when HIV-1 infected individuals were heavily treated with multiple inhibitors. However, such treatment failed and when HIV-1 was selected in vitro in the presence of increasing concentration of Darunavir [25], it has been shown to decrease the dimerization inhibition activity of this drug molecule [23,26,27].

There have been several studies performed investigating the dynamics and drug resistance-related events linked to the structural conformations of protease enzyme [28–30]. In addition, the protease enzyme flexibility and tracing binding over the surfaces were traced by mixed-solvent (acetonitrile-water, isopropanol-water, and pyrimidine-water) [27,31]. Through a mixed solvent approach, well-defined probe occupancies have been analysed in the catalytic site and allosteric sites [31–33]. Structural dynamics have revealed the protease flap domains are crucial for the ligand binding and catalytic activity [17]. In our study, we focused on measuring changes in the active site of HIV-1 protease, as due to lack of water molecules to solvate the active site when enzymes are placed in highly concentrated solutions of OS and ILs. The role of water molecules in the structure, dynamics, and function of biomolecular systems is important especially in low water content solutions, hence, we investigated how much water content is needed in enzymatic catalytic reactions in aqueous solutions of organic media. Different system constructs were built for the protease enzyme in the presence or absence of Darunavir, as well as by a range of molecular dynamics simulation (MDS) where deep eutectic solvents (DESs) differ by organic solvents or concentration. The following four different organic solvents; choline chloride (CHL), ethylene glycol (EG), glycerol, and urea (Fig. 1B) were considered with a focus on behaviour of protease active site and changes in ligand bindings when present in different solvent environments.

2. Materials and methods

The structure of HIV-1 protease enzyme with the Darunavir molecule was retrieved from the protein data bank (pdb id.: 4ll3) [27,34]. Internal ions and water molecules present in the crystal structure [27] were removed, and the protein-ligand complex was used to generate different systems as listed in Fig. 1C and D. BIOVIA Discovery Studio visualizer package (Dassault Systèmes, BIOVIA Corp., San Diego, CA, USA), was used to visualize different structures. To build configurations of organic solvents for molecular dynamics simulations, the PACKMOL package [35] was used, in which the protein-ligand complexes were solvated in a box containing water or the mixtures of other solvents (Fig. 1D).

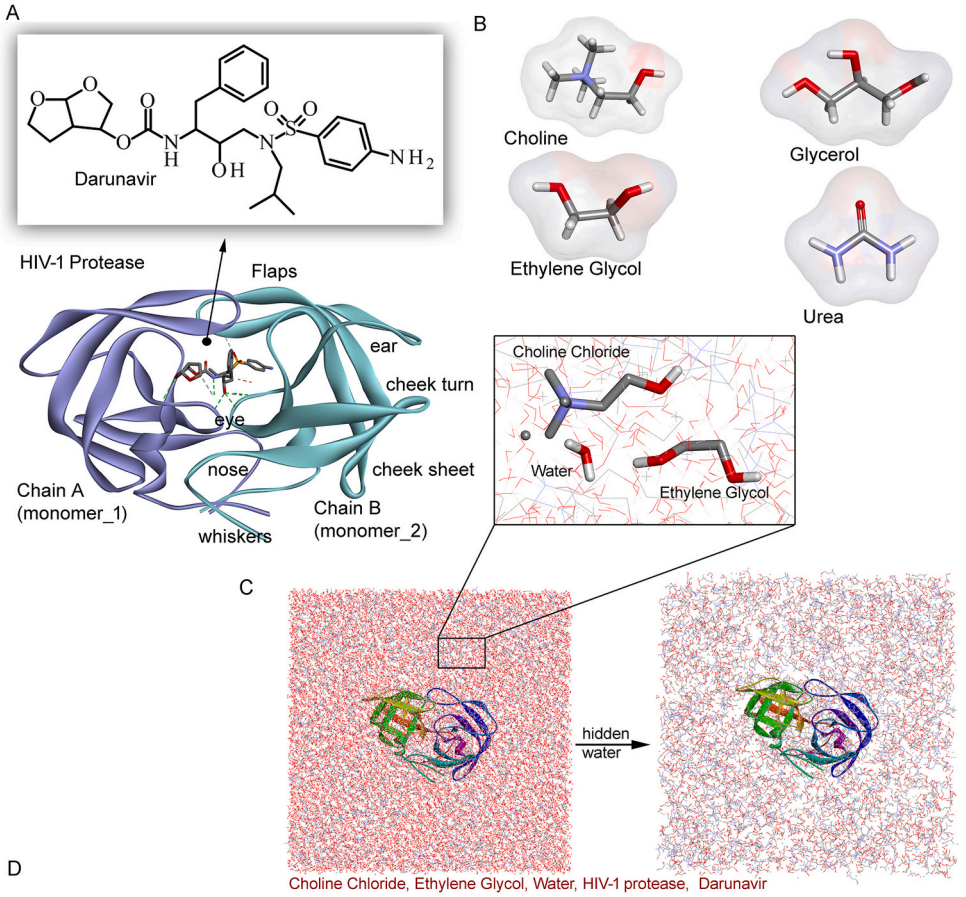
Implementing the modules from PACKMOL [35], the molecules were packed within spatial regions with the desired characteristics, in such a way that atoms from different molecules keep a safe pairwise distance (Fig. 1C). The distance tolerance dtol was set to 2 Å. The prepared systems of organic solvents with the protease enzyme were further processed through molecular dynamics simulations.The MD simulations were performed on different systems using GROMACS package [36], and the structures of the protease enzyme were parameterized by Gromos96 43a1 forcefield [37]. Each designed 12 systems (Table S1) with different organic solvents were equilibrated in GROMACS. Equilibration was performed using the NPT (isobaric-isothermal) ensemble simulation for 1000 ps / till the local minima was gained for a particular system. The following parameters were set for the equilibration; temperature (300 K; V-rescale thermostat [38]) and pressure (1 bar; Parrinello–Rahman barostat [39]).

The PRODRG [40] server was used to generate topologies or GROMACS compatible simulation parameters for the Darunavir molecule (Fig. 1C). The flexible simple point-charge water model (SPC) was used for the water molecules, and the simulation box was defined as a dodecahedron. Periodic boundary conditions (PBC) were applied in all directions, and the solvated system was minimized by 50,000 steps of steepest descents. The minimized systems were then equilibrated for 1000 ps (1 ns) under isothermal-isobaric conditions, NPT ensemble. The particle mesh Ewald method [41] for the long-range electrostatic interactions and LINCS algorithm [42] for the constraints (all bond lengths), were applied. V-rescale thermostat [38] and Parrinello-Rahman barostat [39] were used to maintain constant temperature (300*K*) and pressure (1 bar), respectively. The production run was carried out for 500 ns (Fig. 1D) using leapfrog integrator [43] and the coordinates were saved every 10 ps. The resultant MD trajectories for different simulated systems were visualized using the following packages: VMD (Visual Molecular Dynamics) tool [44], and Molecular Operating Environment (MOE; Chemical Computing Group Inc., Montreal, QC, Canada), BIOVIA Discovery Studio (Dassault Systèmes, BIOVIA Corp., San Diego, CA, USA).

3. Results and discussion

The molecular dynamics simulations on different protease model systems, guided to understand effects of the aqueous solutions of organic solvents on the protease enzyme's structural activities. These happening effects are very crucial as the organic solvents in general have lower dielectric constants than water, which could influence overall activity of the catalytic enzyme. Firstly, over the simulated protease enzyme in different conditions and organic solvents, we measured the stability of individual monomers of protease homodimer with the Darunavir molecule (Figs. 1 and 2). The root-mean-square deviation (RMSD; excluding hydrogen atoms) for the protease revealed a significant difference when placed with different organic solvents (choline chloride / ethylene glycol / glycerol / urea). In the aqueous solution of choline chloride + ethylene glycol or choline chloride + glycerol, the protease enzyme has similar trends in terms of stability, i.e., either of the monomers from homodimer have higher flexibility compared to others (Fig. 1E and F). Particularly, the protease enzyme in the choline chloride + urea aqueous solution has shown greater stability, compared when in presence of other studied solvents. The protease enzyme with the Darunavir, appeared to be a stable structure compared to the apo-form (CHL + urea; Fig. 1G). A similar behaviour was observed for Darunavir, i.e., ligand is less flexible in the CHL + urea, compared to other studied aqueous solutions (Fig. 1F and H).

A correlating flexibility in individual amino acids was observed between RMSD and root mean square fluctuation (RMSF) over the C-alpha atoms (Figs. 1 and 2). Monomer_1 (Chain A) in absence of Darunavir,



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No.	Ratio	Deep eutectic solvents (DESs)	Other components
1	1:2	Choline chloride + Ethylene Glycol (20%)	+ Water (80%) + enzyme + ligand
2	1:2	Choline chloride + Ethylene Glycol (20%)	+ Water (80%) + enzyme (without ligand)
3	1:2	Choline chloride + Glycerol (20%)	+ water (80%) + enzyme + ligand
4	1:2	Choline chloride + Glycerol (20%)	+ water (80%) + enzyme (without ligand)
5	1:2	Choline chloride + Urea (20%)	+ water (80%) + enzyme + ligand
6	1:2	Choline chloride + Urea (20%)	+ water (80%) + enzyme (without ligand)

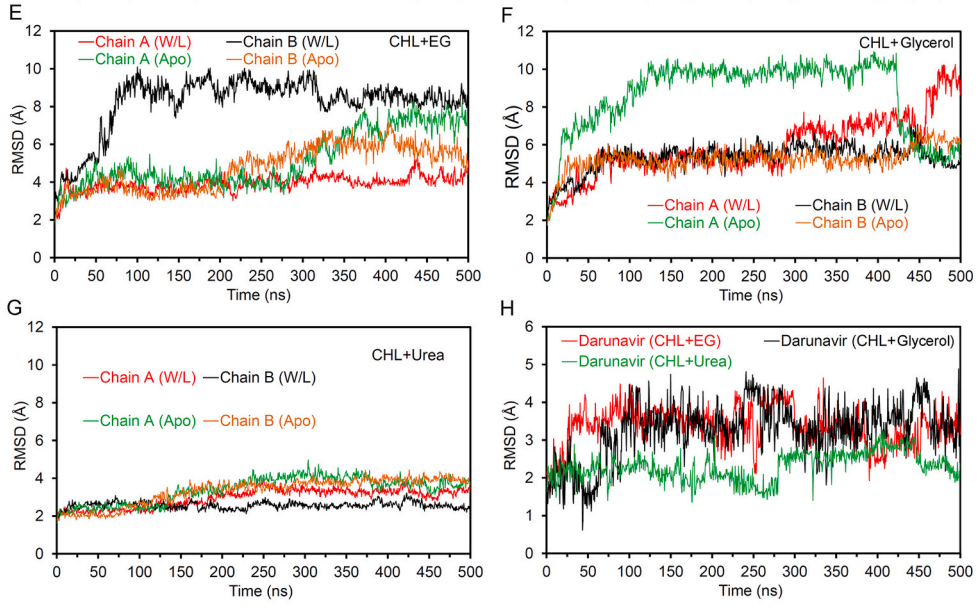

Fig. 1. Components building up systems for protease with different organics solvents. (A) The protease (pdb id.: 4ll3 [27]) enzyme with the Darunavir (Chain A, monomer_1; and Chain B, monomer_2). (B) Structures of different organic solvents: choline chloride (CHL), ethylene glycol (EG), glycerol, and urea with their charge distribution represented as surface. (C) Molecular dynamics simulation (MDS) in periodic boundary conditions (PBC) for the system containing the following components: choline chloride, ethylene glycol, water, protease, and Darunavir. The top right panel represents MDS in PBC with hidden water molecules. (D) Parameterization of the simulated systems containing the protease enzyme and presence/absence of the Darunavir compound consisting of different organics solvents. For each constructed modelled system, 500 ns MD simulations were performed. (E) The RMSDs (root-mean-square deviation; excluding hydrogen atoms) of protease in presence or absence of Darunavir in the system containing choline chloride and ethylene glycol. (F) The protease enzyme in the presence or absence of Darunavir ligand in the system consists of CHL and glycerol. (G) The systems containing choline chloride and urea with HIV-1 protease (with or without ligand). (H) RMSDs for the Darunavir molecule complexed with the protease enzyme in different solvents. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

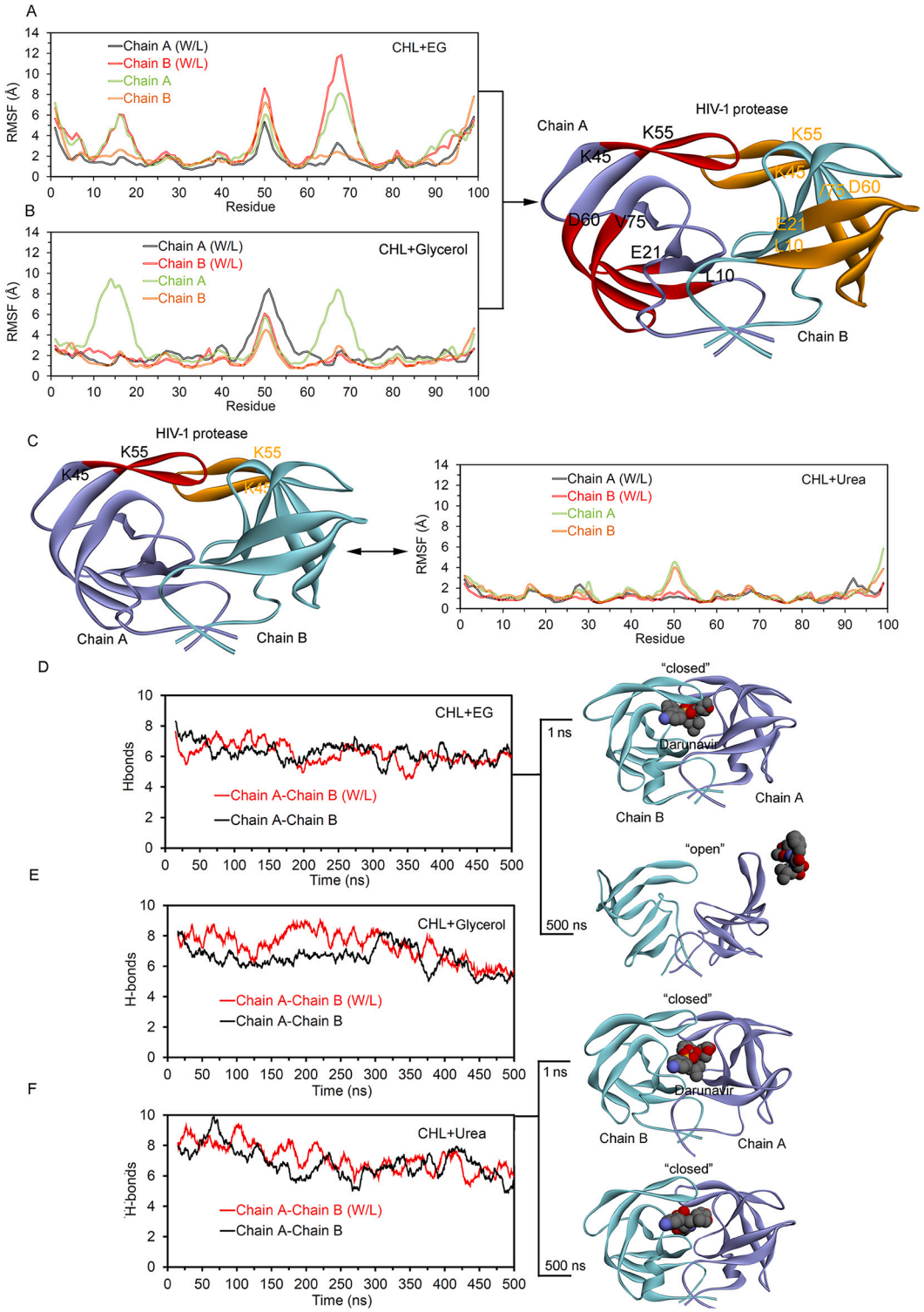

Fig. 2. The dynamics of individual protease amino acids measured by computing root mean square fluctuation (RMSF) based on the C-alpha atoms. (A) The protease enzyme in presence or absence of Darunavir in the system that consist of choline chloride and ethylene glycol. (B) The RMSFs of individual residues in presence or absence of Darunavir ligand in the system that consist of choline chloride and glycerol. Right panel represents residues showing higher flexibility over the enzyme structure (residue range: 10–21, 45–55, and 60–75). (C) The systems containing choline chloride and urea with protease (with or without ligand). Left panel represents residues showing higher flexibility over the enzyme structure (45–55 residue range). (D) Interactions between two protease monomers in the presence or absence of Darunavir for the system that consist of CHL and EG ILs. The right panel represents HIV-1 protease dimer coordinates extracted from the beginning as well as end of the 500 ns MD simulations, describing the "open" and "closed" conformations. Similar binding pattern or conformational change for protease enzyme was traced in the system (CHL + EG or CHL + glycerol) with and without Darunavir. (E) Protein-protein interactions in the system that consist of choline chloride and glycerol. (F) H-bonds between monomers from the systems containing CHL and urea (with or without ligand). The right panel describes the conformational change of the protease enzyme over time. The donor-acceptor cutoff distance was set to 3.5 Å and angle was set to ≥160°–180°. The Darunavir compound is represented as cpk, excluding hydrogen atoms. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

demonstrated similar jumps in the RMSF plots for both CHL + EG and CHL + glycerol systems (Fig. 2A and B). Overlaying higher flexible regions (residue range: 10–21, 45–55, and 60–75) on the enzyme structure, suggest that these regions belong to the flaps, cheek turn, and cheek sheet domains. Distinctive behaviour of protease explains the effect of organic solvent over the structure folding (Fig. 2A and B). Whereas, both protease monomers with Darunavir have obtained a higher number of stable amino acids compared to the systems without ligands in the CHL + urea aqueous solution (Fig. 2C). Particularly, residues ranging from 45 to 55 amino acids from the 'flaps' were found to be more flexible in the system without ligands (Fig. 2C).

Overall, presence of Darunavir has induced the protein-protein intermolecular interactions between two protease monomers in different aqueous solutions (Fig. 2 and Table S2). In the model systems with CHL + EG and CHL + glycerol organic solvents, a declining trend of protein-protein interactions was observed over 500 ns of MD simulation time (Fig. 2D and E). Moreover, our previous study of protease enzyme in the water environment [34] have shown stable interactions throughout MD simulations. Visualizing the protease-Darunavir conformational changes in these systems highlighted that during the initial time of MDS the 'flap' domains retained a "closed" conformation, which "opened" up by the end of 500 ns. Due to such "open" flap domains from both protease monomers has resulted in expelling the ligand from the binding pocket (Fig. 2D and E). Whereas, in the systems containing CHL + urea organic solvent both monomers have retained a "closed" flap conformation in the presence of Darunavir (Fig. 2F). Tracing high occupancy protein-protein interacting amino acids suggest that most of them belong to the whiskers, eye, and nose domains of the HIV-1 protease (Table S2 and Fig. 1).

The presence of CHL + EG or CHL + glycerol in the simulation box hindered the binding of protease with Darunavir (Fig. 3A and B). This finding correlates with the protein-protein intermolecular interactions; due to 'open' conformation of flap domain from each monomer the ligand molecule exits the active site of the enzyme. On the contrary, both monomers were actively involved in the interaction with Darunavir for the CHL + urea system (Fig. 3C). In particular, the Monomer_1 (Chain A) formed more interactions with the ligand compared to that of the Monomer_2 (Chain B; Fig. 3C). In addition to these changes in the protein-ligand H-bonds over time, we identified high occupancy interacting protease residues with Darunavir (Table S3 and Fig. 3). For the system containing CHL + EG aqueous solution the following residues were involved in the binding with Darunavir; R8, D10, D30, G48, G49, and I50 (Chain A), and G49, C67, H69, and Q92 (Chain B; Fig. 3 and Table S3). Residues K14 / G48 / I50 and D30 from Chain A and Chain B, respectively were involved in binding with the ligand (Table S3 and Fig. 3) in the CHL + glycerol system. The following protease residues; G27 / I50 and R8 / D25 / D29 / D30 / I50 form Chain A and Chain B, respectively involved binding Darunavir (from CHL + urea system;

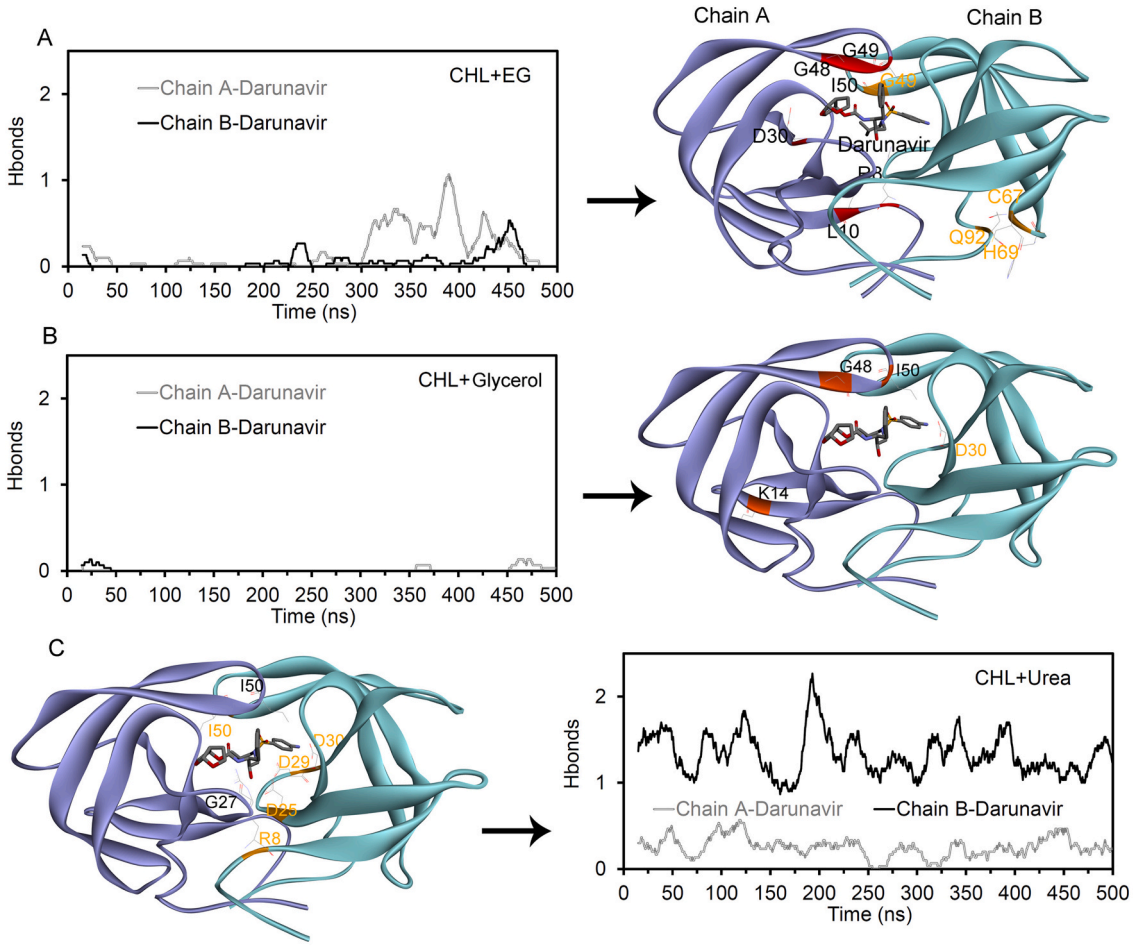

Fig. 3. The protease-Darunavir intermolecular hydrogen bond interactions measured considering hetero atoms. (A) Interactions between individual protease monomers with Darunavir in the system that consist of CHL and EG organic solvents. (B) Protein-ligand interactions in the system that consist of CHL and glycerol. (C) Change in the protein-ligand interactions over time for the systems containing CHL and urea. The donor-acceptor cut-off distance was set to 3.5 Å and angle was set to ≥160°–180°. The Darunavir compound is represented as a stick, excluding hydrogen atoms. The right panel represents HIV-1 protease stable interactions with the ligand; red colour residues from Chain A and orange colour residues belong to Chain B. Darunavir is represented as cpk, choline chloride is presented in line and urea as a stick. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Table S3 and Fig. 3). Residues D25 and G27 are conserved among the aspartic protease enzymes [13] that were found binding with the Darunavir molecule. Furthermore, several residues were found common binding Darunavir in the presence of distinct sets of organic solvent (R8, D30, G48, and I50; Table S2).

Self-aggregation by the amphiphilic nature of the cations among the solvents has been reported earlier [45–49]. In our study, a similar clustering of organic solvents was observed for the systems containing choline chloride + ethylene glycol or choline chloride + glycerol (Figs. 3 and 4). Specifically, ethylene glycol and glycerol were found forming clusters in the presence of choline chloride (1:2 ratio making 20% of solvent in the simulation box) and water molecules (Fig. 4A and B). On the contrary, the system containing choline chloride and urea lacked such formation of self-aggregation (Fig. 4C). To understand such effect of organic solvents in detail, we simulated the protease enzyme (presence / absence of darunavir) ethylene glycol + choline chloride in low concentration (1:2 ratio making 4% of solvent in the simulation box), as well as without water in the solvent (Figs. S1 and S2). In the presence of water molecules (without protease or darunavir) the ethylene glycol + choline chloride organic solvents have formed self-aggregation (Fig. S1). The systems with ethylene glycol + choline chloride (without water molecules) lacked sef-aggregation formation of solvent observed in both ligand bound and unbound systems (Fig. S2). However, the 'flap' domains were found in the close state in the presence of Darunavir and in the "open" state in the apo-form. Whereas, in the system with 4% deep eutectic solvents (~0.05 M DES concentration), self-aggregation was observed in the system without the drug molecule.

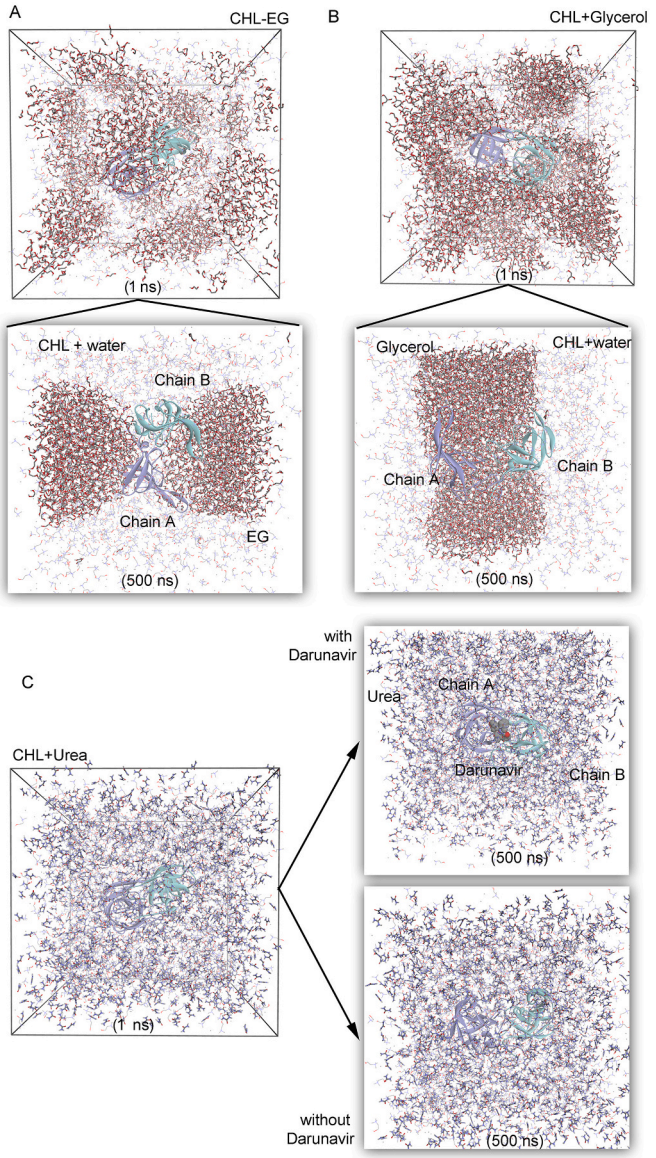

Fig. 4. Distinctive behaviour of ionic liquids when complexed with the HIV-1 protease dimer. (A) Conformational dynamics of protease in the system that consist of CHL and EG organic solvents; protein coordinates retrieved from the beginning and end of the MD simulation. Choline chloride is presented in line and ethylene glycol as a stick. Similar behaviour of the solvents and protease was analysed in the systems with Darunavir. (B) The protease enzyme in the system that consist of CHL and glycerol; protein coordinates retrieved from the beginning and end of the MD simulation. Choline chloride is presented in line and glycerol as a stick. Similar behaviour of the organic solvent and protease was analysed in the systems with Darunavir. (C) "Open" conformation for the HIV-1 protease enzyme in the absence of compound, and a "closed" conformation in the presence of Darunavir for the systems containing CHL and urea. Darunavir is represented as cpk, choline chloride is presented in line and urea as a stick. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Interestingly, this self-clustering among the organic solvents has shown a significant effect over the structural folding of the HIV-1 protease, as well as its binding with the Darunavir molecule. This self-clustering (Fig. 4A and B) induced an 'open' conformation for the flap domains of the protease, in the presence or absence of the ligand. Lacking aggregation in the CHL + urea aqueous solution, the protease enzyme showed conventional structural folding, i.e., presence of ligand 'closed' conformation and in absence of ligand 'open' conformation for the flap domains (Fig. 4C). However, previous studies [50–53] have reported that higher concentration of urea can influence the proper folding of the secondary structures. Particularly, urea was found binding with the protease enzyme in our analysis, and this finding correlates with the work by Brien et al. [50], describing that urea can directly interact with polar residues stabilizing nonnative conformations. Several studies have been reported describing that increased concentration can unfold protein structure, and low urea concentration can trigger protein aggregation [54–56]. Moreover, it has been reported that urea may implement its effect directly by binding protein, or indirectly by altering the solvent environment [50,56,57]. In particular at 3–8 M urea concentrations, a urea molecule in solution was proposed to suppress peptide aggregation and a weak enhancement in secondary structure at 5 M urea [54]. Nemergut et al. reported that increased urea concentration can delay / prevent aggregation [56]. However, in our study with the protease enzyme we have considered 20% deep eutectic solvents (Fig. 1D) of the total system, which is ~0.20 M DES concentration. At this concentration in the CHL + urea aqueous solution the protease enzyme behaves differently in presence / absence of drug molecules, which could be further investigated in different urea concentrations.

In this direction, to investigate the conventional behaviour (open and close flap domain conformation) of the protease enzyme in the CHL + urea aqueous solution, we performed MD simulations of protease in the urea solvent only (with water). Exceptionally, both monomers bound with Darunavir were found more stable in the urea solvent, compared to that when with CHL + Urea solvent (Fig. 1G and 5A). Like the protease enzyme, the Darunavir molecule was found comparatively more stable (Fig. 1H and 5B). Considering the inter monomer interactions, a similar trend in the binding pattern was observed in both urea or CHL + urea systems (Fig. 2F and 5C). Darunavir in the CHL + urea system was found making dominant interactions with only one monomer form the dimer (Fig. 3C), whereas in the system with urea solvent by the end of MD simulations it formed interactions with both monomers (Fig. 5D). The system containing urea solvent lacked any self-aggregation formation similar to that with the CHL + urea system (Fig. 5D and E). In addition, the flap domains formed an "open state" in the absence of the drug molecule (Fig. 5E).

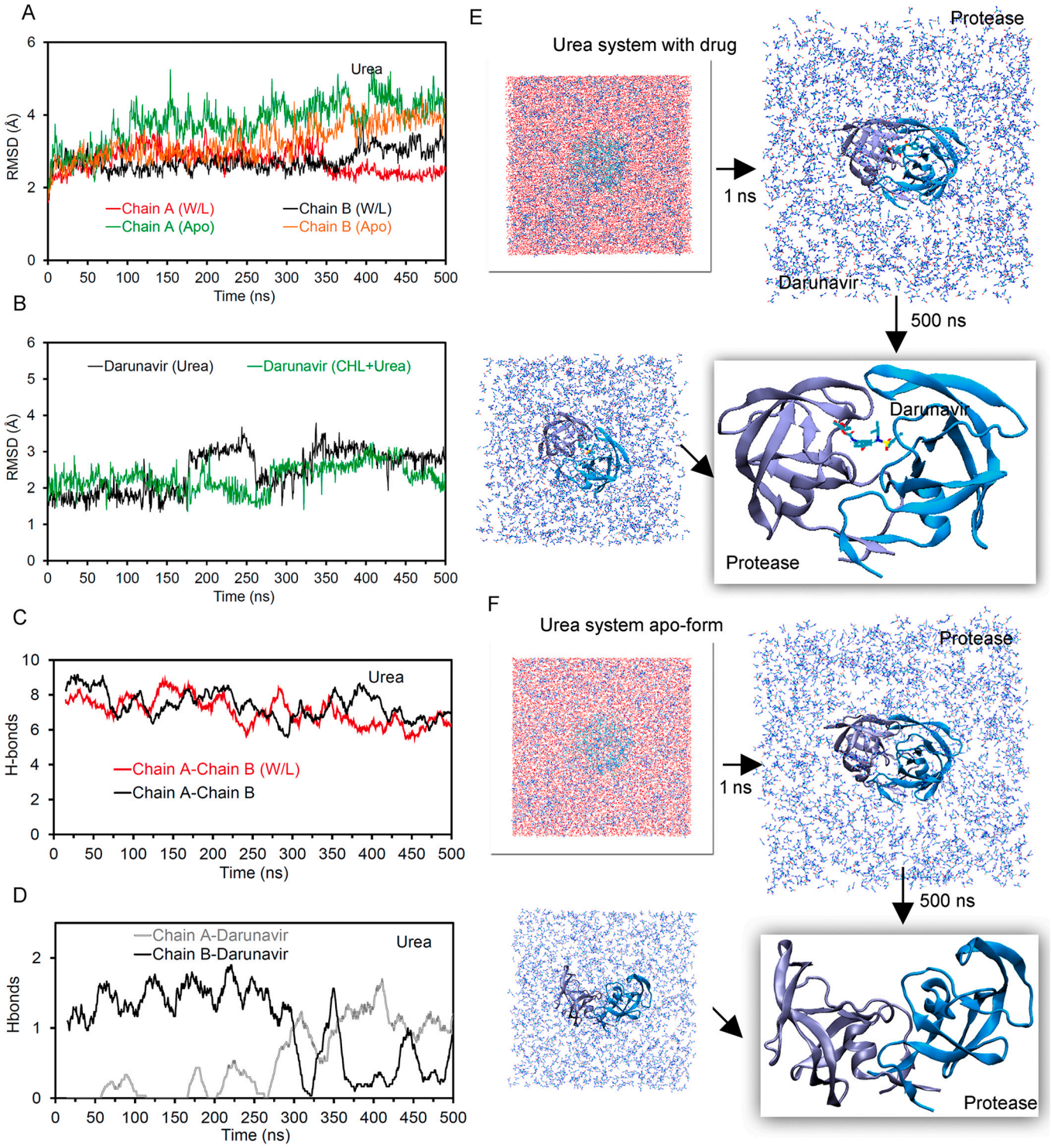

Fig. 5. Effects of Urea over the conformational dynamic of the protease enzyme. (A) The RMSDs for protease enzyme in presence / absence of Darunavir. (B) RMSDs for the Darunavir molecule complexed with the protease enzyme. (C) H-bonds between monomers from the systems containing urea (with or without ligand). (D) Change in the protein-ligand interactions over time for the systems containing urea. (E) and (F) Conformational dynamics of protease in the system that consist of urea solvents; protein coordinates retrieved from the beginning and end of the MD simulation. The Darunavir compound is represented as a stick. The donor-acceptor cutoff distance was set to 3.5 Å and angle was set to ≥160°–180°. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

4. Conclusions

Alteration in the enzymatic activity, bio-catalysis selectivity or enzymatic stability can be obtained by changing the micro-solvation environment of the enzymes or by other solvents than native ones. To understand such an effect over the protease enzyme we implemented the MD simulations approach, and traced significant changes in the enzyme when placed in highly concentrated solutions of OS and ILs. Proteases in the CHL + urea aqueous solution, showed a greater stability compared to when with other solvents (CHL + EGl or CHL + glycerol). Higher

flexible regions / amino acids belong to the flaps, cheek turn, and cheek sheet domains of the protease enzyme. Darunavir ligand induced protein-protein interactions between monomers of protease in different aqueous solutions. Majority of residues involved in such interactions come from the whiskers, eye, and nose domains of the protease enzyme. Exclusive for the systems containing urea ILs, both monomers were actively involved in the interaction with the Darunavir molecule. In systems with the CHL and urea, the D25 and G27 amino acids conserved among the aspartic protease enzymes were found binding with Darunavir. In addition, the residues R8, D30, G48, and I50 were found to be common binding the Darunavir ligand in the presence of different organic solvent. Our findings suggest that self-aggregation within a particular type of organic solvents has a significant effect over the folding of the HIV-1 protease and its binding with Darunavir. Hence, to maintain such nonpeptidic inhibitor activity towards the protease enzyme organic solvents can have a significant influence, and our current findings can guide further in vitro or in vivo experiments in this direction. However, we believe that our novel findings shall help to better understand the HIV-1 life cycle maintained by protease enzymes, and its interactions with inhibitors, which could be controlled by deep eutectic solvents.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bpc.2022.106909.

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