

Research Article

MOLECULAR DYNAMICS SIMULATION OF KINETIC RESOLUTION OF RACEMIC ALCOHOL USING *BURKHOLDERIA CEPACIA* LIPASE IN ORGANIC SOLVENTS

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Abstract: Lipases, a subclass of hydrolases, have gained a lot of importance as they can catalyze esterification, transesterification and hydrolysis reaction in non-aqueous media. Lipases are also widely used for kinetic resolution of racemic alcohols into enantiopure compounds. The lipase activity is affected by organic solvents due to changes in the conformational rigidity of enzymes, the active site, or altering the solvation of the transition state. The activity of lipases strongly depends on the logP value of solvents. Molecular dynamics (MD) can help to understand the effect of solvents on lipase conformation as well as protein-ligand complex. In this work, MD simulations of *Burkholderia cepacia* lipase (BCL) and complex between R and S conformation of acetylated form of 1-phenylethanol with BCL using gromacs have been carried in various organic solvents. The RMSD values were within the range of 0.15 to 0.20 nm and radius of gyration was found to be with 1.65 to 1.9 nm. Major changes in the B factor compared to reference structure were observed between residues 60 to 80, 120 to 150 and 240 to 260. Higher unfolding was observed in toluene and diethyl ether compared to hexane and acetonitrile. R acetylated complex was found to favorably bind BCL compared to S form. The predicted enantioselectivity were in good agreement with the experimental data.

Keywords: *Burkholderia cepacia* lipase, (\pm)-1-phenylethanol, molecular dynamics, protein conformation, enantioselectivity

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Introduction

Enzymes are catalytic proteins in living organisms, which promote the chemical transformation of the substrates into value added products. Enzymes are highly selective and efficient biocatalysts. Lipases are particular class of enzymes which are tolerant toward organic solvents, accept a broad substrate range and are often selective, stable and readily available (Hietanen, 2012). Lipases catalyze esterification, transesterification and hydrolysis

reactions. The structural and thermodynamic origin of enantioselectivity of lipases has been studied in the literature (Cygler *et al.*, 1994).

Drug molecules have their effect related to particular diseases by interacting with a target molecule (such as receptor, enzyme, transporter protein or other proteins or occasionally nucleic acids) in a three-dimensional manner. The targets are typically single stereoisomer and if the drugs are also chiral then the interaction will be stereoselective. One stereoisomer of the drug will be bound to the target more effectively than the other. The other stereoisomer might bind to undesired location and can have severe side effects. Hence chiral resolution of drugs is very important in pharmaceutical field. The chiral resolution can

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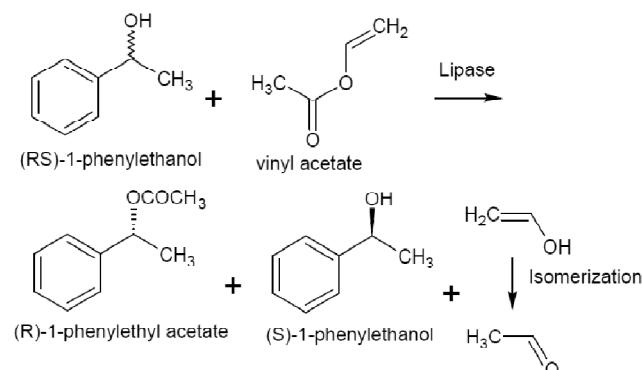
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be carried out by chromatographic techniques, selective crystallization or kinetic/dynamic resolution using chiral catalysts. Lipases are very efficient, cheap chiral catalysts which are widely being used for separation of seteroisomers of active pharmaceutical intermediates (Patel, 2002).

The whole catalytic process of lipase catalyzed reaction proceeds via formation of tetrahedral intermediates (Kraut, 1977). The lipase enantioselectivity is given by enantiomeric ratio (E) which is related to activation free energy difference ($\Delta\Delta G$) between the substrate enantiomers as $\Delta\Delta G = -RT \ln(E)$ (Overbeeke *et al.*, 1998). Solvents affect the enzymes or enzyme substrate complexes by producing changes in the conformational rigidity of enzymes, the active site, or altering the solvation of the transition state. Enantioselectivity depends on the substrate, enzyme, free/immobilized, acyl donor, temperature, solvent hydrophobicity ($\log P$), solvent size, water activity etc. solvent effects are well correlated with the polarity or hydrophobicity of the solvents (Secundo *et al.*, 1992; Li *et al.*, 2010; Trodler *et al.*, 2008; Chua *et al.*, 2006; Catoni *et al.*, 1996; Carrea *et al.*, 1995). However, the exact parametric dependency on the physicochemical properties of solvent such as size, $\log P$ etc. is yet to be established. Molecular dynamics simulation of lipase helps to understand the structural aspects of the protein responsible for interfacial activation, changes in active catalytic triads (Kaushik *et al.*, 2008; Rehm *et al.*, 2010). They have reported that the activity of *Candida rugosa* lipase increases with increase in solvent hydrophobicity due to increase in the movement of the flap. The lid is opening at a hydrophobic interface, making the active site accessible for substrates and enhancing the activity of the lipase (Verger, 1980; Trodler, 2009). In the literature, lipases such as *Candida rugosa* lipase (CRL), *Candida antarctica* lipase A (CAL-A) and B (CAL-B) and *Burkholderia cepacia* lipase (BCL) are used for kinetic resolution of racemic mixtures. CAL-B and BCL show higher enantioselectivity compared to other lipases for major classes of substrates. Trodler *et al.* (2009) carried out MD simulation of BCL in water and toluene. They observed that BCL is stable in toluene without significant loss of activity.

In this work, molecular dynamics (MD) simulations of *Burkholderia cepacia* lipase (BCL) using Gromacs have been carried out for 50 ns for solvents such as acetonitrile ($\log P -0.15$), diethyl ether ($\log P 0.8$), toluene ($\log P 2.7$) and hexane ($\log P$

3.5). The MD simulations were further extended to study the conformation of acetylated complex between BCL and chiral constituents of 1-phenylethanol. 1-phenylethanol is a very important pharmaceutical intermediate which is used in Ezetimibe, Prozac, Emend and Sotalol. The objective of the present work was to understand how the protein conformation changes in presence of solvents and possible effect on enantioselectivity. The reaction scheme of kinetic resolution of (\pm) -1-phenylethanol using lipase is given below. The enantioselectivity of kinetic resolution of (\pm) -1-phenylethanol using free *Burkholderia cepacia* lipase (BCL) is 43 and 88 in hexane and toluene respectively (More *et al.*, 2015; Mathpati and Bhanage, 2016).



Reaction Scheme 1: Lipase catalyzed kinetic resolution

Materials and Methods

Mezzetti *et al.* (2005) have studied in detail the conformation of BCL and its molecular basis for enantioselectivity. They have proved that the acyl enzyme complex is responsible for enantioselective transesterification. The PDB file of lipase was downloaded from RCSB protein database. For BCL, Protein Data Bank code 1YS1 was used (Mezzetti *et al.*, 2005). Firstly, the ligand and other non-protein molecules were removed from the protein structure. The omitted hydrogen atoms were then added and atom partial charges in protein were calculated using the Dock Prep utility in Chimera 1.10.1 software (Huang *et al.*, 1996).

In the first stage of dynamics simulations, solvents were simulated using OPLS force field parameters. The smiles structure of solvent was used in "Online SMILES Translator and Structure File Generator" (link- <https://cactus.nci.nih.gov/translate/>) to generate pdb structure. The pdb structures were renumbered at <http://>

erg.biophys.msu.ru/tpp/ using TPPnum tool and TPPmktop tool was used to obtain all-atom OPLS topology. The MD simulations were carried out using GROMACS which is a powerful tool to perform MD simulations and energy minimization (Berendsen *et al.*, 1995). The solvent box for all the simulation was 6 nm × 6 nm × 6 nm. The NVT simulations were carried out for 200 ps, followed by NPT simulation for 500 ps for equilibration. Energy minimisation was carried out using the steepest descent method. V-rescale temperature coupling and Berendsen pressure coupling methods were applied to keep the system in a stable environment (298K, 1 bar), and the coupling constants were set at 0.1 and 0.5 for temperature and pressure, respectively. Position Mesh Ewald method was employed for electrostatic and van der Waals interactions; cut-off distance for the short-range neighbour list (rlist) was set at 1.0nm, where Coulomb cutoff (rcoulomb) and VdW cut-off (rvdw) were fixed at 1.0nm. The LINCS algorithm was used to constrain the bonds (Berendsen *et al.*, 1995). The final molecular dynamics simulation was carried for 10000 ps. The solvent density was compared with experimental values to check the correctness of OPLS-AA topology. The resultant GRO and ITP files were used in protein-solvent interaction studies. The computational methodology was kept same for simulation of BCL in solvent. The MD step was carried out for 50000 ps. It was ensured that the box size is sufficient to account for at least 1000

solvent molecules in all the cases. Similar methodology was followed for MD simulation of acetylated complex in the solvent. The initial structure of acylated complex was generated using molecular docking simulations. Docking calculations were carried out using AutoDock 4.0. The torsional degrees of freedom of the ligand were explicitly considered, whereas the protein structure was kept frozen to X-ray atomic coordinates. The box dimension was set to 90 × 90 × 90 grids with 0.375 Å spacing. The lipase was placed at the centre of the grid box. Search for best poses was carried out using a Lamarckian genetic algorithm, using the following parameters; run: 100, population size: 200, maximum number of evaluations: 3500000. Best poses from each run were clustered using an RMSD tolerance of 2.0 Å. The structure having minimum free energy change was chosen for detailed MD simulation. 2-D representation of initial structure has been shown in Figure 1 using Ligplus software.

Results and Discussion

In the present work, solvents such as acetonitrile, hexane, diethyl ether and toluene were studied. The MD simulations were carried out with only pure solvent in box for 10000 ps. The MD predictions of density were compared with experimental values available in literature to validate the force field parameters used in the simulation (Table 1). It can

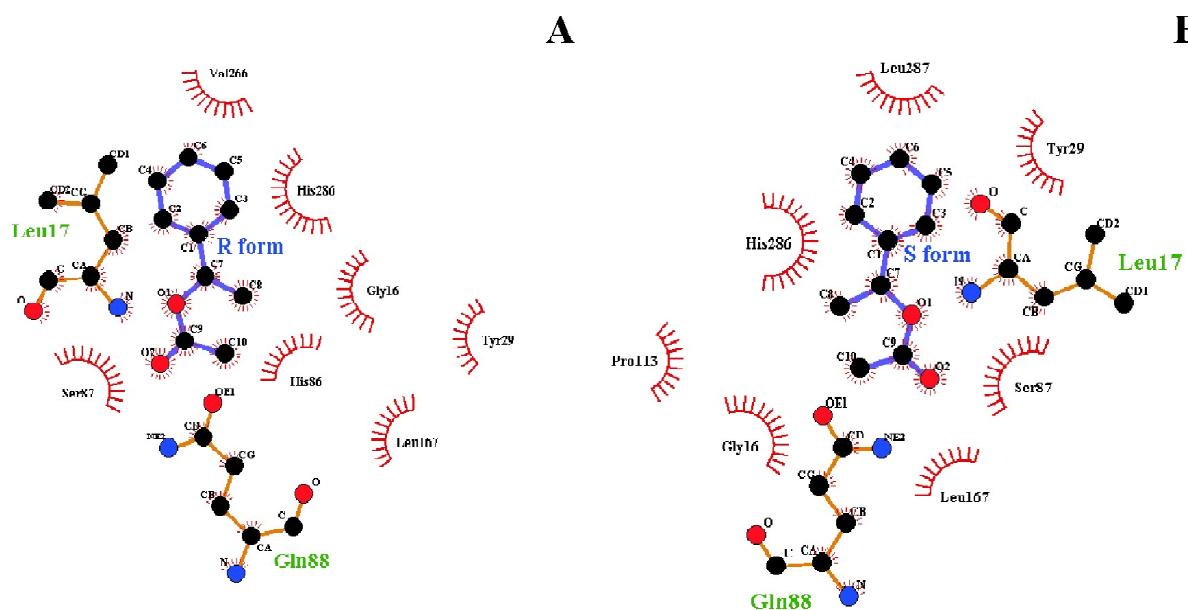


Figure 1: 2-D representation of docked conformation of (A) R form (B) S form of tetrahedral intermediate with BCL using ligplus

be seen that the % error are less than 5% and hence the force field parameters are suitable for further investigation.

In the second step of simulations, the OPLS topology of solvents was for dynamic simulations of protein-solvent system. All the simulations were run for 50000 ps. To determine stability of conformations along MD simulation, we employed root mean square deviation (RMSD). The time evolution of the backbone atoms RMSD from the starting structure of production dynamics were calculated after least square fit. Figure 2 shows all values within the range from 0.05 to 0.2 nm, which indicate that all protein structures from protein-ligand complexes were stable during the simulation. The backbone RMSD gradually increased by 0.1 nm up to 2000 ps and reached final plateau by 10000 ps with increase up to 0.15 to 0.2 nm. This indicates that the BCL structure had undergone significant conformational change in the initial course of simulation. In case of toluene, large fluctuations were observed due to last opening of the lid. These results are in accordance with similar work available in literature (Trodler *et al.*, 2009). Table 2 shows the Lennard-Jones (LJ) and Coulomb interaction energy for BCL-solvent system. LJ interaction energy represents van der Waal (vdw) interactions. It can be seen that BCL has very strong vdw interactions in toluene compared to other solvents. The exact understanding can be obtained by quantification of binding free energies which can be estimated by PME with umbrella sampling simulations.

Radius of protein gyration (R_g) was used to observe the compactness of protein structure (Figure 3). The gyration values remain at 1.84-1.92 nm for all simulation times, which shows that all of the protein complexes are more compacted among MD simulation compared to original structure. In case of acetonitrile, radius of gyration was found to increase after 20000 ps indicating significant changes in BCL structure. The superimposed structures of solvated BCL with reference BCL are presented in Figure 4. It can be seen that maximum conformational changes take place in acetonitrile followed by hexane.

The B-factor (atomic displacement parameter) in protein crystal structures reflects the fluctuation of an atom about its average position. The distribution of B factors along a protein reflects its flexibility and dynamics. A large B-factor indicates

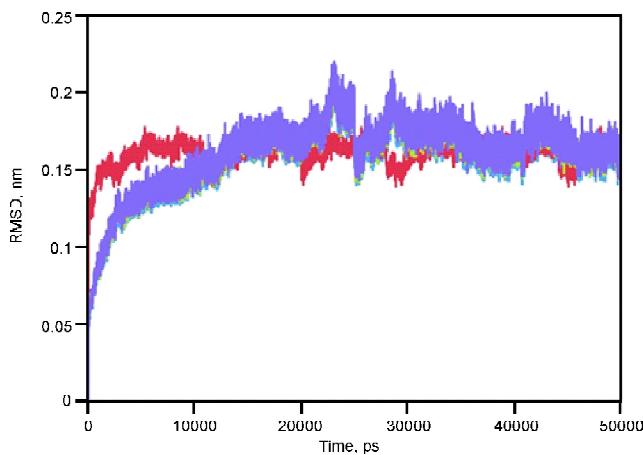


Figure 2: RMSD plot (— acetonitrile, — hexane, — diethyl ether, — toluene)

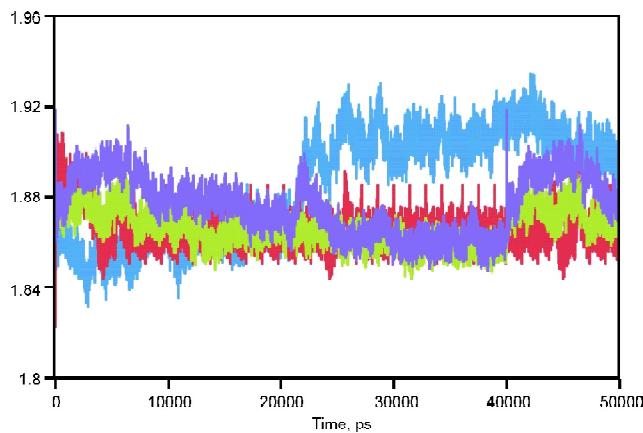


Figure 3: Radius of gyration plot (— acetonitrile, — hexane, — diethyl ether, — toluene)

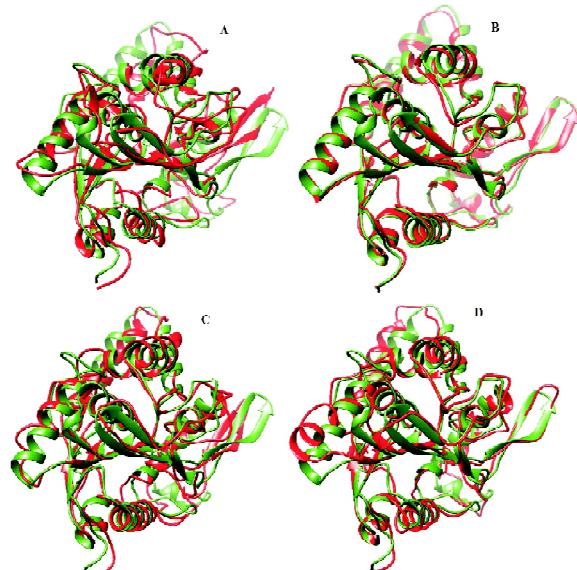


Figure 4: BCL conformation in presence of solvent (A: acetonitrile, B: hexane, C: diethyl ether, D: toluene) Green: reference BCL structure (1YS1), Red: BCL structure in presence of solvent

Table 1
Experimental and Predicted Density of Solvents

Sr. No.	Solvent	$\log P$	Solvent size (nm ³)	Density (kg/m ³) at 298 K and 1 atm		
				Experimental	MD prediction	% Error
1	acetonitrile	-0.15	0.048	786	759	3.43
2	hexane	3.50	0.112	655	652	0.45
3	diethyl ether	0.80	0.086	713	726	1.82
4	toluene	2.70	0.098	867	863	0.46

high mobility of individual atoms and side chains and generally belongs to part of the structure that is very flexible. Atoms with low B-factors belong to a part of the structure that is well ordered. Figure 4 shows the B factor of BCL in various solvents. Major changes in the B factor compared to reference structure were observed between residues 60 to 80, 120 to 150 and 240 to 260. Higher unfolding was observed in toluene and diethyl ether compared to hexane and acetonitrile. Minor variations were observed in B factor at catalytic triad (Ser87, Asp264 and His286) for solvents with positive logP (i.e. diethyl ether, hexane and toluene). In case of acetonitrile, significant changes were observed at Ser87 and His286. These changes may have impact on the enantioselectivity of the lipase. Based on experimental studies in literature, solvents having logP higher than 3 are suitable for kinetic resolution. Table 3 shows solvent accessible surface area for BCL in various solvent. Maximum change in solvent accessible surface area was observed in ACN (11%) followed by DEE (6%), which indicates that BCL undergoes large conformational changes in presence

of solvents having lower logP. The estimation of solvent accessible surface area was done using a web based tool <http://cib.cf.ocha.ac.jp/bitool/ASA/>.

The lipase conformation can further get affected by the presence of ligand. The lipase catalyzed transesterification proceeds via formation of an acylated complex. The solvent can have significant impact on the productive docking of acylated complex. In order to understand the 3-D conformation of BCL with R and S acylated complex, additional MD simulations were carried out for 12000 ps. The conformation of R and S form in various solvents is shown in Figure 6. The most productive binding of the R form can be seen in toluene as the positively charged C atom of carbonyl group in ligand is close to negatively charged Serine 87 O_y atom. In lipase catalyzed transesterification, various bonds are formed in the residues involved in catalytic triad and the acylated complex. These important bonds are a) between Gln88-N and acylated complex O atom b) His286-Nδ2 and ASP264-Oδ2 c) His286-Nε2 and Ser87-O d) Leu17N

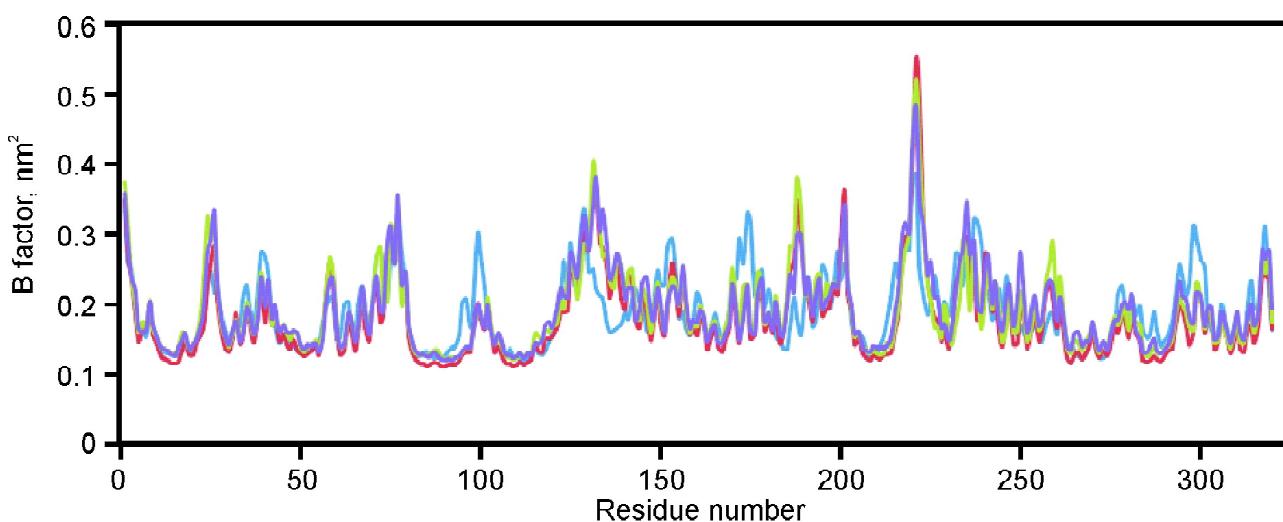


Figure 5: B factor plot (— acetonitrile, — hexane, — diethyl ether, — toluene)

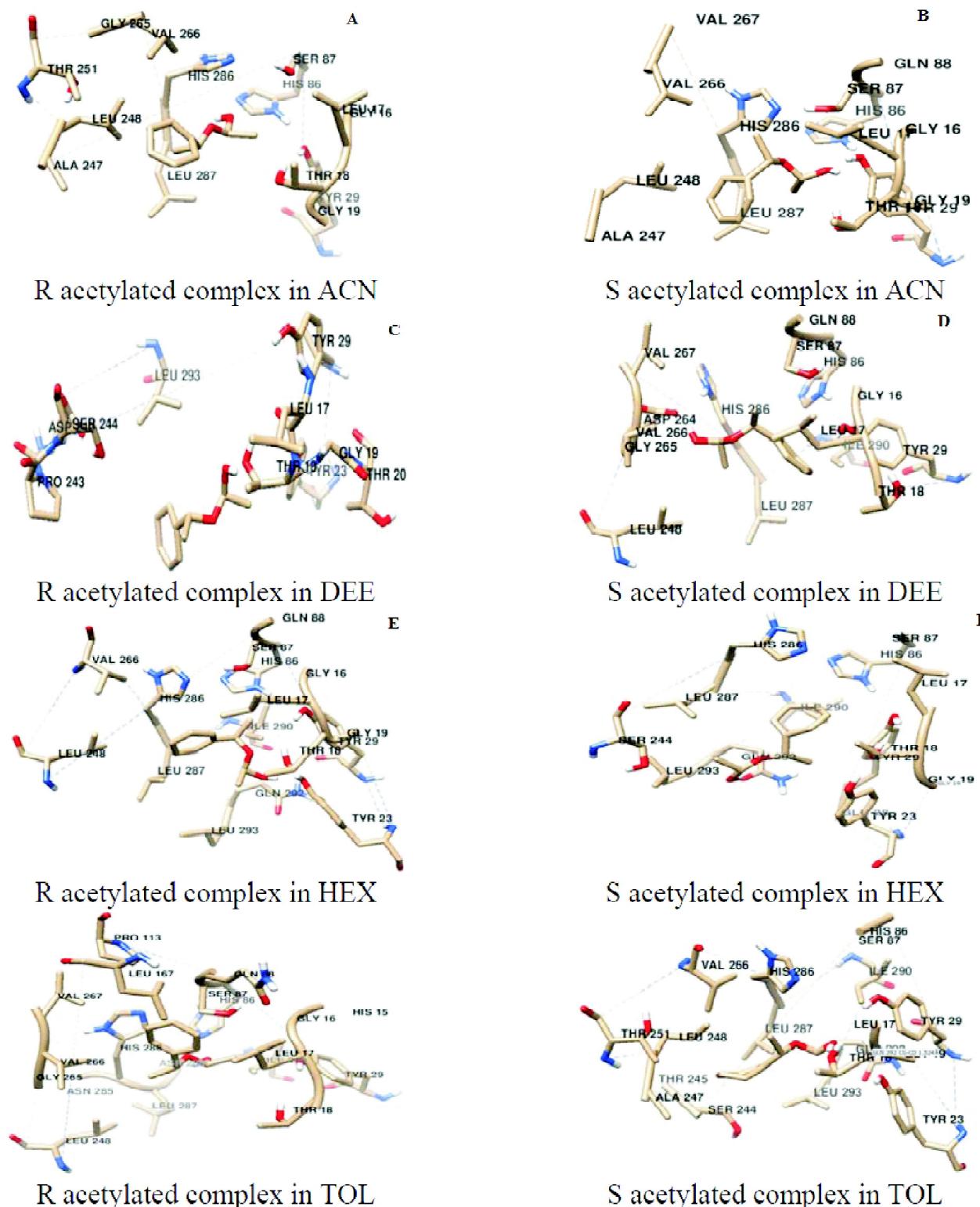


Figure 6: 3-D conformation of R and S for acetylated complex in various solvents

and THI-O e) Ser87-O and acylated complex C atom. In the acylation step, covalent bond forms between Ser87-Oy and C atom (connected to oxygen atoms) in the ligand. The time evolution of distance

between Ser87-O and carbonyl carbon of acylated complex is shown in Figure 7. The acylation step proceeds via formation of a covalent bond between Ser87-O and carbonyl carbon of ligand. Hence

Table 2
Interaction Energy between protein and solvent

Solvent	Lennard-Jones (LJ) kJ/mol	Coulomb kJ/mol
ACN	-24.5	-121.8
DEE	-62.1	-88.0
Hexane	-30.9	-95.8
Toluene	-51.4	-71.3

Table 3
Solvent accessible surface area

Solvent	solvent $\log P$	protein total surface (nm ²)	% Change
Crystal structure	—	128.0	0
ACN	-0.15	142.9	11.64
DEE	0.80	119.8	6.44
Hexane	3.50	125.7	1.83
Toluene	2.70	124.4	2.84

Table 4
Critical bond distances in catalytic triad for R acetylated complex

Bond distance (nm)	acetonitrile	diethyl ether	hexane	toluene
His286(N82)-Asp264(O82)	0.55	0.52	0.37	0.28
His286(Nε2)-Ser87(Oγ)	0.68	0.88	0.38	0.28

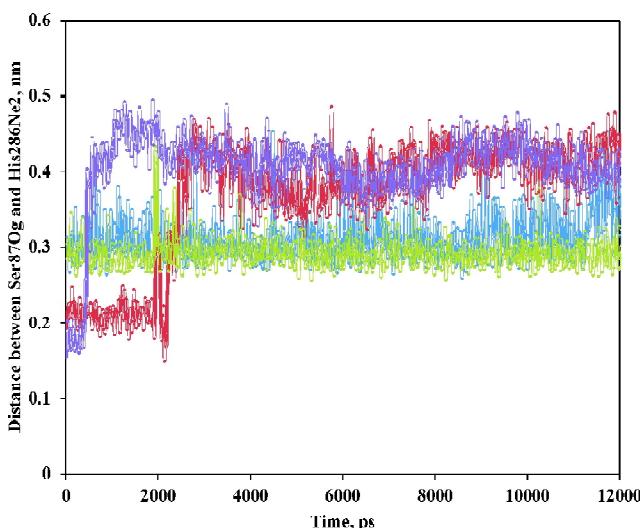


Figure 8: Time evolution of distance between Ser87O and His286Ne2 from MD simulation of R acetylated BCL complex

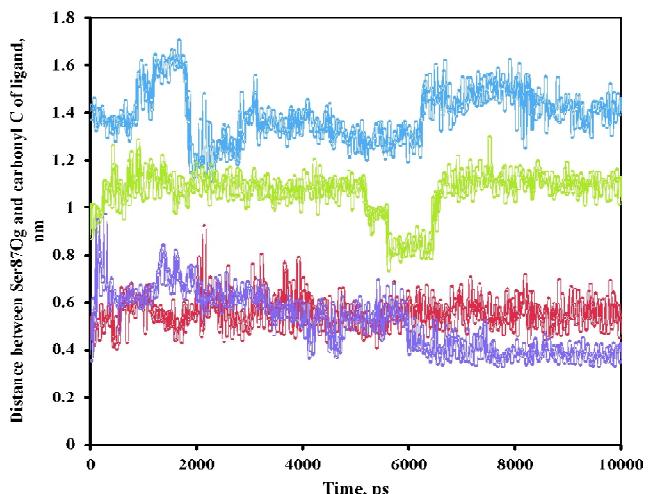


Figure 7: Time evolution of distance between Ser87Oγ and C atom of R form of acetylated complex

shorter the distance between two, better the chances of transformation. It can be seen that diethyl ether and toluene provide suitable environment in terms of formation of a covalent bond. Similarly, distance between Ser87O and His286Ne2 (Figure 8) and distance between Asp264 and His286 (Figure 9) also play a vital role. Productive docking takes place when these distances are smaller (preferably below 0.5 nm). Table 3 shows these critical bond distances for BCL in different solvents. It can be seen that toluene and diethyl ether provide favorable environment for productive docking compared to hexane and acetonitrile. Hence it is expected that toluene and DEE will provide higher enantioselectivity compared to hexane and acetonitrile. This is in accordance with the available

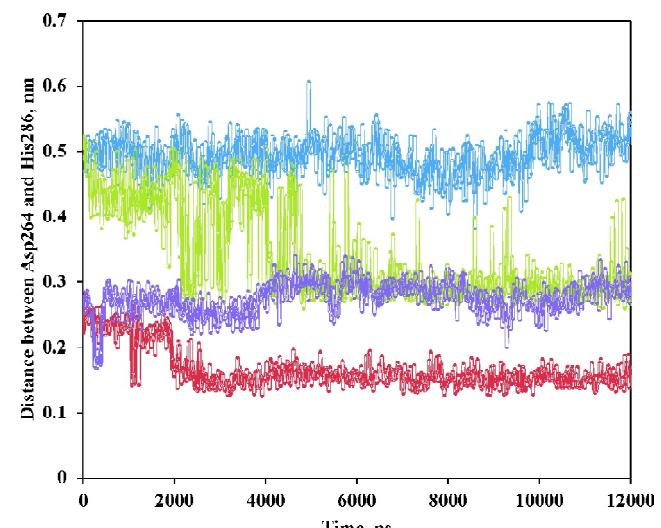


Figure 9: Time evolution of distance between Asp264 and His286 from MD simulation of R acetylated BCL complex

experimental data of E value for hexane ($E = 43$) and toluene ($E = 88$).

Conclusion

Molecular dynamics protocol is a useful tool for screening solvents for biochemical reactions. This can help in terms of cost saving in experimentation as well as time required for process optimization. The conformational changes in protein structure are captured accurately by molecular dynamics. The major changes in protein conformation were observed with acetonitrile compared to other solvents. B factor analysis shows that protein undergoes favorable conformational changes in presence of toluene and diethyl ether. The critical bond distances between Ser87, Asp264 and His286 were favorable for productive binding for cases of toluene followed by diethyl ether. The MD simulations of R and S form of acylated complex of 1-phenylethanol show that R form binds favorably in active site compared to S form. The active site undergoes favorable conformational changes in toluene to improve the enantioselectivity.

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Conflict of Interest

The authors have no conflict of interest.

Abbreviations

BCL, *burkholderia cepacia* lipase; CAL, *candida antarctica* lipase; CRL, *candida rugosa* lipase; MD, molecular dynamics; OPLS-AA optimized potentials for liquid simulations (all atoms); RMSD, root mean square deviation

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