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Binding orientation and interaction of bile salt in its ternary complex with pancreatic lipase-colipase system

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ABSTRACT

The interfacial activity of pancreatic lipases (PL) depends on the presence of colipase and bile salt. The activity of PL is inhibited by micellar concentrations of bile salt which can be restored by the addition of colipase. Though the formation of 1:1:1 tertiary complex by lipase-colipase-bile salt micelle is well accepted, the residue-level interactions between lipase-colipase and bile salt are yet to be clearly understood. Molecular dynamic simulations of lipase-colipase complex, lipase and colipase were performed in the presence of a model bile salt, sodium taurocholate (NaTC), at its near-CMC and supra-micellar concentrations. From the interactions obtained from the molecular dynamic simulations, the ternary complex was modelled and compared with earlier reports. The analysis suggested that a micelle of NaTC consisting of nine monomers was formed at the concave groove between lipase and colipase chain and it mainly interacted with the fourth finger of colipase. This complex was mainly stabilized by van der Waals interactions. Interestingly, the *C*-terminal domain of lipase which holds the colipase did not show any significant role in formation or stabilization of NaTC micelle.

1. Introduction

Lipases (E.C. 3.1.1.3) are ester hydrolases which are ubiquitous across bacteria, fungi and mammals. They all share certain common features such as interfacial activation and stability in organic solvents [1–3]. Pancreatic lipases (PL) aid in the hydrolysis of dietary triglycerides in animals [4]. Their activity is more at oil-water interfaces unlike the classical esterases. For their effective interfacial activity, they require a co-enzyme called colipase along with bile salts, a surfactant-like amphipathic molecule [3,5]. Colipase binds to the *C*-terminal domain of the lipase chain through non-covalent interactions [3,5]. Colipase is a stable protein with five disulphide bonds and shows substantial resistance against heat and surface denaturing agents. It has four finger-like loops projected outward with the tip of the fingers consisting of hydrophobic residues [6]. In the absence of colipase, an amphiphilic loop known as a lid covers the active site of the protein and limits its activity. Formation of PL-colipase complex moves the lid and makes the active site accessible to the substrate [3,5]. Lipases in lower organisms do not have *C*-terminal domain homologous to PLs and also do not require co-enzyme to stabilize the lid in its open conformation [1].

PL-colipase complex tends to dissociate in the absence of bile salt micelle [5]. At low concentrations, far below the CMC, bile salts could slightly activate PL in the absence of colipase [7,8]. However, at the concentrations near CMC, bile salts inhibit the catalytic reaction. The activity is completely inhibited at above the CMC of bile salts. This inhibitory effect can be reverted by the addition of colipase at all the concentrations of bile salts [5,7–9]. Also, the dialysis experiments show that a single micelle of bile salt binds to one molecule of colipase in the absence of PL [10]. Hence, the absence of inhibition at the higher concentration of bile salt could be attributed to the formation of 1:1:1 ternary complex of PL-colipase-bile salt micelle [5,9].

A structural model proposed for the ternary complex based on small-angle neutron scattering (SANS) experiments suggest that the bile salt forms a micelle at the concave junction created by the colipase and *C*-terminal domain of PL [9]. Pre-formed micelle interacts with PL-colipase at juxtaposition creating a non-centrosymmetric assembly. In addition, NMR structure shows 82° rotation of the loop in colipase covering the residues 70–85 compared to the crystallographically resolved colipase structure [11]. Nevertheless, the disorder of detergents in the crystal structures has limited the understanding of interactions between bile salt and lipase-colipase complex. In this report, all-atom molecular

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dynamic (MD) simulation has been used to reconstruct the ternary complex by collating the available primary information on each component of the enzyme complex from different experiments using porcine pancreatic lipase (PPL) as a model. As the structure and dynamic property of PPL in different conditions have been well studied, this aids us to construct a reliable model of the PPL-colipase-bile salt complex [12,13]. The developed complex structure is compared with earlier model [9] and the interaction energies are calculated from the MD simulation.

2. Experimental methods

PPL-colipase (PPL^{+colip}) complex was obtained from the crystal structure (PDB: 1ETH) after removing co-crystalized ligands. PPL was prepared by removing colipase from PPL^{+colip}. Colipase was extracted from PPL^{+colip} complex or from NMR solution structure (PDB: 1PCN [14]). Conjugated trihydroxy bile salt, sodium taurocholate (NaTC), was used in all the simulations with the physiological NaCl concentration (0.15 M). The parameters for NaTC were obtained from Automated Topology Builder ver 2.0 [15]. Two concentrations of NaTC, low concertation with 12 molecules and high concertation with 60 molecules, were used throughout the simulations for the box size of 1000 nm^3. All the MD simulations were performed on GROMACS 4.6.3. following the protocol as reported earlier [12]. The simulations carried out in this study are listed in Table S2. The analyses were performed with GROMACS, and in-house UNIX shell scripts and R-codes. Amino acid interaction analysis was performed on the last 10 ns and the interaction energies were the mean values of the energies obtained from last 100 steps each collected at the interval of 2 ps.

3. Results

3.1. Global changes

The preliminary analyses of MD simulations of lipase, colipase and their complex with bile salt were performed by calculating the root mean square deviations (RMSD) of the molecules from their respective initial structures (Fig. 1A–D) and the root mean square fluctuations (RMSF) of the residues (Fig. S1). The overall RMSD of lipase chains in different conditions suggested that the dynamics of PPL and PPL^{+colip} was more in the presence of NaTC. The analysis on the lid region alone showed that the lid had more deviation from the initial structure in the absence of NaTC when colipase was not present (PPL^{+TcL} and PPL^{+TcH}). In the lipase-colipase complex, the lid was more stable in the high concentration of NaTC ($PPL^{+colip+TcH}$) compared to PPL^{+colip} and $PPL^{+colip+TcL}$. Further, RMSF analysis also complemented the same. In the absence of NaTC, the lid fluctuations were higher for PPL than PPL^{+colip}. In the presence of low concentrations of NaTC, the lid fluctuations were comparable in the presence and the absence of colipase, and in higher concentrations, the movements around the lid were more in the absence of colipase. Overall, in the presence of NaTC increase in residue movements in *N*– and *C*-terminals were observed.

The conformational changes in colipase have significant effects on the interfacial activity of lipases [3,5]. In order to assess the role of orientation of finger 4 (residues 70 to 85), MD simulations of colipase were performed with two different initial conformations (Fig. S2): (i) colipase extracted from the lipase-colipase complex resolved by X-ray crystallography having PDB id: 1ETH ($colip_{xrd}$), and (ii) solution-NMR structure of colipase having PDB id: 1PCN ($colip_{nmr}$). The RMSF values indicated that the four hydrophobic finger tips had more fluctuations and the addition of high concentration of NaTC reduces their dynamics in both the cases (Fig. 1E and F). For further insight into the solvent exposure of PPL and the colipase in different states, hydrophilic and hydrophobic solvent accessible surface areas (SASA) were calculated. The hydrophobic SASA (Figs. S3A–C) decreased in the presence of NaTC for both PPL and PPL^{+colip} whereas it was not significantly changed for colipase chain. The hydrophilic SASA (Figs. S3D–F) increased for PPL and PPL^{+colip} in the presence of NaTC, but in the colipase, it slightly decreased in the presence of high concentration of NaTC.

3.2. Residue-specific interactions of bile salt

Interaction of NaTC with different types of residues, grouped into non-polar, polar and charged, in PPL and colilpase were analyzed (Fig. 2A). The results clearly suggested that NaTC at both the low and high concentrations largely interacted with the non-polar residues followed by polar residues. Only a small fraction of the charged residues was involved in the interaction with NaTC. Among the non-polar residues, Leu, Ile, Val and Pro in PPL and Leu, Ile, and Gly in colipase were found to be mainly interacting with NaTC (Fig. S4). Among the polar residues Asn, Gln, Ser and Thr were interacting more with NaTC. In PPL and PPL^{+colip} complex at the high concentration of NaTC, Lys was observed to participate considerably whereas at the low concentration of NaTC, PPL^{+colip} did not show any notable charged residue interaction. On the other hand, lipase at low concentration of NaTC showed a considerable fraction of interaction with Asp. Similarly, non-polar residues of colipase were majorly interacting with NaTC at higher concertation whereas at the lower surfactant concentration marginal increase in the fractions of interactions with lysine and cysteine were also noted.

3.3. Aggregation property of NaTC

NaTC is a conjugated, trihydroxy, amphiphilic bile salt molecule. During the MD simulation of NaTC in water, different levels of aggregations of NaTC were observed. For quantitative analysis, the aggregates were classified as small aggregates (1–4 molecules), medium aggregates (5–8 molecules) and micellar aggregates (9–12 molecules). All the atoms of NaTC within a cutoff of 4 Å were considered to be part of an aggregate and the fractions of different aggregate forms in each simulation were evaluated (Fig. 2B). When 12 molecules of NaTC were used to represent the low concertation (TcL), it formed only small and medium aggregates with a mean aggregation number of five which was similar to earlier experimental results [16]. When 60 NaTC molecules were simulated, along with small and medium aggregates, larger micelle-like aggregates were also observed. Therefore, it was assumed that the low concentration (TcL) of NaTC might represent sub-/near-micellar conditions whereas the high concentration (TcH) might represent supra-micellar conditions.

The fractions of distribution of NaTC (Fig. 2B) suggested that small aggregates were present in considerably larger number in all the forms of PPL and colipase. In the case of $colip^{+TcH}$ almost equal proportion of medium aggregates was also noticed; however, no medium aggregates were present in the cases of $PPL^{+colip+TcL}$ and $colip^{+TcL}$. Only at the high concentration of NaTC, micellar aggregates were observed in all the cases. The visual inspection of the trajectories provided further insight on NaTC localization on PPL and colipase surfaces (Fig. 3). At low concertation, NaTC preferably bound on the lid and *C*-terminal domain of the protein in the absence of colipase whereas in PPL^{+colip} complex, NaTC was found as small aggregates on the surface covering lid and colipase. Apart

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