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Lid closure dynamics of porcine pancreatic lipase in aqueous solution

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ABSTRACT

Background: Pancreatic lipases hydrolyze fatty acids in dietary pathway. The activity of porcine pancreatic lipase (PPL) is controlled by lid domain along with a coenzyme, colipase. The active open-state conformation of the protein could be induced by detergents or bile salts which would be further stabilized by binding of colipase. In the absence of these interactions, the lid preferably attains a closed conformation in water.
Methods: Molecular dynamic simulation was used to monitor the lid movement of PPL in open and closed conformations in water. Free energy surface was constructed from the simulation. Energy barriers and major structural changes during lid opening were evaluated.
Results: The lid closure of PPL in water from its open conformation might be initiated by columbic interactions which initially move the lid away from domain 1. This is followed by major dihedral changes on the lid residues which alter the trajectory of motion. The lid then swirls back towards domain 1 to attain closed conformation. This is accompanied with conformational changes around β5- and β9-loops as well. However, PPL in closed conformation shows only the domain movements and the lid remains in its closed conformation.
Conclusions: PPL in closed conformation is stable in water and the open conformation is driven towards closed state. The lid follows a swirling trajectory during the closure.
General significance: Conformational state of the lid regulates the activity and substrate specificity of PPL. Hence, it is essential to understand the lid dynamics and the role of specific amino acid residues involved.

1. Introduction

Lipases [EC 3.1.1.3] are a subclass of esterases, isolated from various species and share a well-conserved α/β-hydrolase fold [1–3]. Lipases hydrolyze naturally occurring oils of plant seeds and fats of animals which on further oxidation release huge amount of their stored energy [4]. Lipases are activated at oil–water interface to access water insoluble substrates for hydrolysis [5–8]. Like other esterases, lipases also follow the well-characterized acyl enzyme pathway for hydrolysis reaction [7]. The activity of lipases is reported in a range of solvents, from polar to nonpolar, such as ethanol, heptane, and octanol [8–13]. Lipases break (hydrolysis) [5–8], and make (esterification) [9–13] ester bonds in polar and non-polar solvents, respectively. Due to their ability to catalyze the organic reactions such as hydrolysis, acidolysis, aminolysis, esterification, and transesterification, lipases are widely used in food, pharmaceuticals, leather, and cosmetics industries [14,15].

Pancreatic lipases are crucial enzymes for the digestion of dietary long chain triglycerides. Upon digestion in the duodenum these bulky nonpolar molecules are broken down into small polar β-monoglycerides and fatty acids. These polar fractions are then easily transited to the enterocytes [16]. Optimal activity of the pancreatic lipases could be obtained in the presence of a coenzyme known as colipases [17]. Surfactants such as sodium deoxycholate and sodium taurodeoxycholate, at above their critical micelle concentration (CMC), inhibit the activity of pancreatic lipases which can be restored in the presence of colipases [18–20]. Colipases are highly homologous across mammals and a colipase from one mammal can activate the lipase of another mammal [21].

Porcine pancreatic lipase (PPL) is a 50 kDa protein, with 448 amino acid residues. The crystal structure of PPL-colipase complex in the open state was resolved in 1996 by Hermoso et al. (PDB id: 1ETH) [22]. PPL consists of two domains: α/β-fold *N*-terminal domain (residues: 1–336) and β-sandwich *C*-terminal domain (residues: 337–448) named domain 1 and domain 2, respectively. The domains are linked through a hinge residue Ala333. The active site, constituted by Ser153, Asp177, and His264, is covered by a flap or lid region stretched between Cys238 and Cys262. The conformational changes around the lid control the enzyme activity of the lipase. The open conformation of lid allows substrates to reach the active site residues which are otherwise not accessible in the closed conformation [23–27]. Open conformation of PPL could be stabilized by either ionic/non-ionic detergents or bile salts in aqueous solution. Addition of colipase could further enhance the stabilization of open conformation [22,28]. Colipase is held with the lipase

Abbreviations: PPL-Cl, procine pancreatic lipase in closed conformation; PPL-O, lipase in closed conformation; PPL^{+colip}, lipase–colipase complex; PPL^{-colip}, only lipase chain of lipase–colipase complex; RMSD, root mean square deviation; RMSF, root mean square fluctuation; R_g, radius of gyration; SASA, solvent accessible surface area; PCA, principal component analysis.

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through non-covalent interactions across the lid and C-terminal domain [22].

The structural stability and plasticity of the various lipases have been investigated by both experimental and computational approaches [11–13,29–32]. Studies on the atomistic-level changes in lipases with respect to their structure, solvent environment, and substrate specificity are getting momentum in the recent years [29–34]. However, to the best of our knowledge, the lid dynamics of mammalian lipase has not been investigated at atomistic-detail. This report attempts to identify the kinetic pathway and the possible barriers during the lid closure of PPL from its open conformation (PPL-O) in aqueous environment and to compare its dynamics with colipase bound PPL (PPL^{+colip}) and PPL in closed conformation (PPL-Cl). The results suggest that the open conformation is less stable in water, in the absence of colipase, and moves towards domain 1 to attain the closed conformation. The lid first moves away from domain 1 and later alters its trajectory and moves towards domain 1. Also, the conformational fluctuations in PPL-Cl are mainly due to domain–domain movement, and the lid dynamics of PPL-Cl is highly constrained in water.

2. Experimental methods

2.1. Structure of PPL in open and closed conformations

The crystal structure of colipase bound PPL in open conformation was obtained from protein databank (PDB id: 1ETH). The structural coordinates of lipase–colipase complex (PPL^{+colip}) were extracted by removing other co-crystallized molecules. Further, colipase free PPL with open conformation was obtained from PPL^{+colip} by removing the structural coordinates of colipase (PPL-O) which is shown in Fig. 1. Since, the crystal structure of closed conformation of PPL without colipase (PPL-Cl) is not available, it was modeled using Modeller 9.11[35] against rat pancreatic lipase-related protein 2 (RPLRP2) in closed conformation (PDB id: 1BU8) [36] as a template. RPLRP2 shows 84% similarity and 99% sequence coverage with PPL and the structure was resolved with 1.8 Å resolution in the absence of colipase. The quality of the modeled structure was further validated by verify 3D [37] and procheck [38].

2.2. Molecular dynamics (MD) simulation

MD simulations of PPL-Cl, PPl-O, and PPL^{+colip} were carried out using GROMACS 4.5.4 [39–42] with OPLS-AA force field. The protein was initially solvated in a cubic box with SPC/E water. The structures were energy minimized and equilibrated under NVT followed by NPT conditions. After the equilibration, the production simulations were carried out at 300 K for 50 ns. 1 nm cutoff was used for short range columbic and Lennard-Jones interactions. Long range electrostatic interactions were treated by Particle mesh Ewald (PME) scheme [43]. Stochastic velocity rescaling scheme with time constant of 0.1 ps was used for temperature control. Pressure was maintained at 1 bar by Parrinello–Rahman [44] type pressure coupling with a time constant of 2.0 ps. For all bond constraints, LINCS algorithm [45] was used. Coordinates, velocities, trajectories, and energies were collected at every 2 ps for further analysis.

GROMACS utility tools were used for analyzing the trajectories. For structure visualization and analyses PyMol [46] and UCSF chimera [47] were used. PDBsum web server [48] was used for analyzing 2D representation of the protein structures. PDB2PQR [49,50] and APBS [51] were used to calculate solvation energies and electrostatic forces,

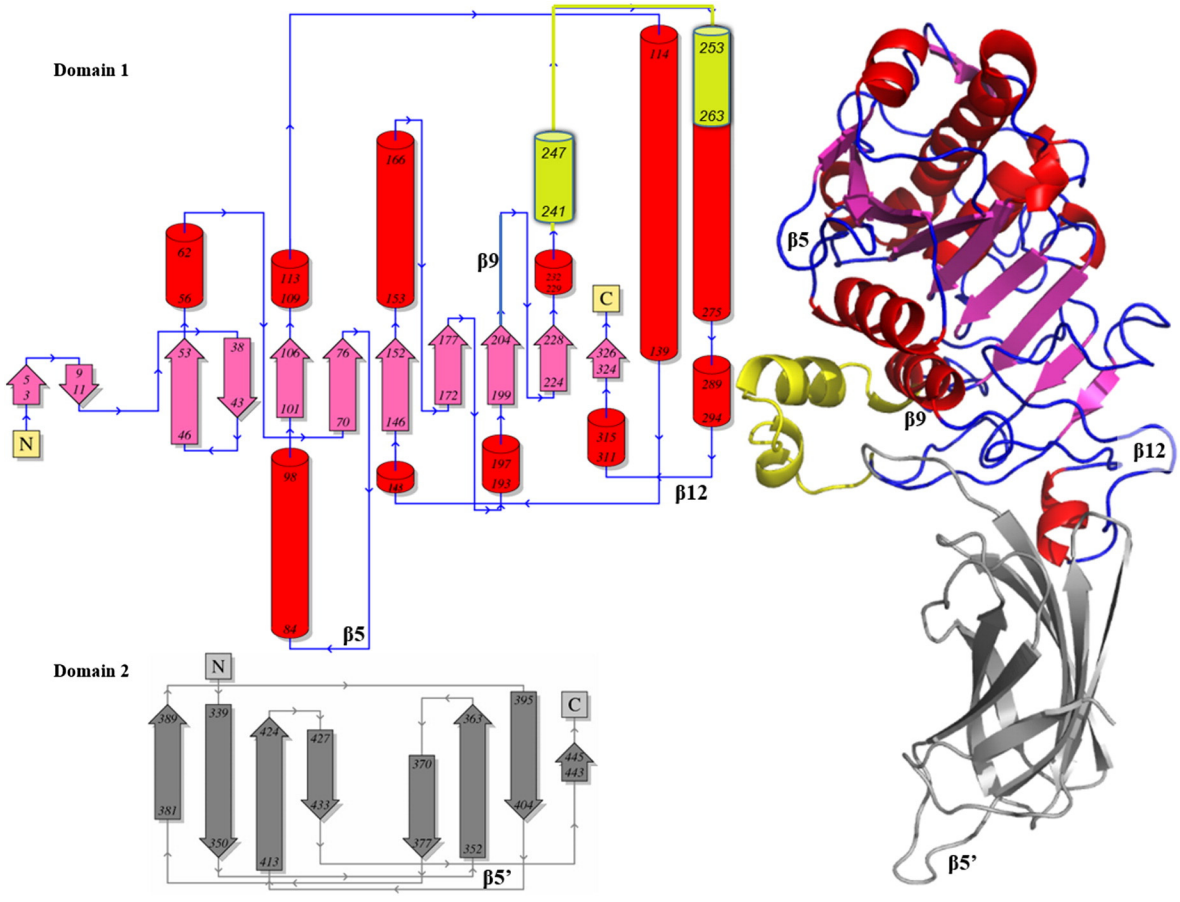

Fig. 1. Topology diagram of PPL in open conformation without colipase (left panel). Rods represent α-helices and arrows represent β-strands. Lid region is marked in green and domain 2 in gray. Right-side panel presents cartoon diagram of PPL-O with the same color codes as in topology diagram. Major β-loops are labeled.

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