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Homology modeling of human serum paraoxonase1 and its molecular interaction studies with aspirin and cefazolin

Mohammed Salman[1, 2], Chandramouli Malleda[3], Narayanavari A Suneel[4], Insaf A Qureshi[5], Elizabeth A Frank[1], Cletus JM D'Souza[1]*

[1]Department of studies Biochemistry, University of Mysore, Mysore 570006, India; [2]Anthropological survey of India, Southern regional center, Bogadi, Mysore 570006; [3]Department of Biochemistry, University of Hyderabad, Hyderabad 500046, India; [4]Department of Animal Sciences, University of Hyderabad, Hyderabad 500046, India; [5]Department of Biotechnology, University of Hyderabad, Hyderabad 500046, India; Cletus J.M. D'Souza* - Email: cletus@biochemistry.uni-mysore.ac.in; Phone: +91 821 2419621; *Corresponding author

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Abstract:
Human serum paraoxonase1 (HuPON1) belongs to the family of A-esterases (EC.3.1.8.1). It is associated with HDL particle and prevents atherosclerosis by cleaving lipid hydroperoxides and other proatherogenic molecules of oxidized low density lipoproteins (LDL). Since the precise structure of HuPON1 is not yet available, the structure-function relationship between HuPON1 and activators/inhibitors is still unknown. Therefore, a theoretical model of HuPON1 was generated using homology modelling and precise molecular interactions of an activator aspirin and an inhibitor cefazolin with PON1 were studied using Autodock software. The ligand binding residues were found to be similar to the predicted active site residues. Both cefazolin and aspirin were found to dock in the vicinity of the predicted active sites of PON1; cefazolin bound at residues N166, S193 and Y71, while aspirin at residues N309, I310 and L311. Binding region in the PON1 by prediction (3D2GO server) and docking studies provide useful insight into mechanism of substrate and inhibitor binding to the enzyme active site.

Keywords: Human Paraoxonase1; prediction; molecular docking; homology modeling; secondary structure

Background:
Paraoxonase (EC 3.1.8.1) belongs to the family of A-esterases consisting of three members namely PON1, PON2 and PON3. PON1 and PON3 are produced in liver and secreted into blood. PON2 is not present in blood, but is expressed in tissues like liver, kidney, intestine, brain and heart. The genes coding for these enzymes are located adjacent to each other on the long arm of chromosome 7 (7q21.3-q22.1) **[1]**. PON1 is the most abundant form and hence extensively investigated. HuPON1 consists of 355 amino acids exclusively associated with high-density lipoprotein (HDL) in association with human phosphate binding protein (HPBP). ApoA1 is major protein in HDL which stabilizes PON1 and binds it with very high affinity **[2]**. HuPON1 plays a major role in the prevention of atherosclerosis by protecting HDL and LDL against oxidative stress mediated through the uptake of oxidized-LDL by macrophages, inhibition of macrophage cholesterol biosynthesis and stimulation of HDL mediated cholesterol efflux from macrophages **[3]**. HuPON1 activity is reduced in type 1 and type 2 diabetes independent of individual's genotype; hence diabetic patients are at high risk to develop atherosclerosis **[4, 5]**. The low serum paraoxonase activity in type 2 diabetes was recently shown to be correlated with the levels of oxidized LDL and vascular complications **[6]**. A study demonstrated that HDL from type 1 diabetic patients showed higher levels of lipid hydroperoxides and lower activity of HDL-PON1 than healthy

subjects. Moreover, HDL from these patients was unable to protect erythrocyte membranes against oxidative damage. In diabetic patients, significant decrease in HuPON1 activity in the lipoprotein deficient serum (LPDS) fraction in comparison with healthy subjects was reported, where most of HuPON1 activity was in HDL **[7]**. The interest in HuPON1 is mainly because of its implications in cardiovascular diseases. HuPON1 being an antioxidant enzyme, also has a thiolactonase activity and prevents homocysteinylation of proteins by homocystein thiolactone **[8]**. It can also activate statin prodrugs, many of which are lactones. Hence, the cardioprotective functions of HDL are attributed to the HDL associated PON1. Because of its great biological diversity, the active site of paraoxonase has received much attention from researchers for understanding the active site conformation capable of handling multiple substrates. Site directed mutagenesis and kinetics have identified various amino acids necessary for the different substrate binding and catalysis **[9]**. Since crystal structure of HuPON1 was not available, it has been modeled using crystal structure of rePON1 (rabbit PON1, PDB ID: 1V04).

Aspirin is analgesic, anti-inflammatory and anti-pyretic drug, well known for treatment of cardiovascular diseases. A recent study reported *in vitro* and *in vivo* induction of PON1 and ApoA1 gene by aspirin and salicylate **[10]**. Studies have also shown aspirin competes with ρ-nitrophenylacetate and paraoxon for hydrolysis by PON1 in human plasma and isolated HDL preparations **[11]**. It also inhibits cyclooxygenase (COX) enzyme by acetylation of the active site and prevents the formation of cyclooxygenase products like prostaglandins, thromboxanes and prostacyclin **[12]**. Cefazolin sodium is a known cephalosporin antibiotic to inhibit *Enterobacter aerogenes*, *Escherichia coli*, *Haemophilus influenza*, *Klebsiella species*, *Proteus mirabilis* and *Streptococcus pneumonia*. Cefazolin sodium significantly decreases liver glucose 6- phosphate dehydrogenase and human carbonic anhydrase I and II. A study has shown that cefazolin sodium causes a dose and time dependent decrease on PON1 activity in HepG2 cells and effectively inhibit purified human serum PON1 **[13]**. Though studies involving the effect of aspirin as an activator and cefazolin as an inhibitor of rePON1 have been reported **[10, 13]**, no reports are available on the 3D model of HuPON1 with aspirin and cefazolin. In view of the above, the present study establishes the active site amino acid residues by *in silico* approaches and docking studies and also deduces the structural interaction between PON1 and one inhibitor cefazolin and an activator aspirin.

Methodology:

Sequence alignment and Structure prediction:

The FASTA sequence of query protein (HuPON1) was retrieved from NCBI Entrez sequence search (http://www.ncbi.nlm.nih.gov). Following BLASTp run (http://blast.ncbi.nlm.nih.gov/Blast.cgi), a Serum paraoxonase by directed evolution (PDB ID: 1V04) was selected as template sequence (http://www.rcsb.org/pdb). The sequences were subjected to pairwise alignment using ClustalW followed by secondary structure prediction using ESPript2.2 (http://espript.ibcp.fr/ESPript). The 3D-structure of query protein was predicted by automated homology modeling program, Modeller9v8 **[14]**. For Modeller, the template and query sequences were carefully aligned to remove potential alignment errors. The default modeling process did end up with a loop. The model obtained was further submitted to modbase server (http://modbase.compbio.ucsf.edu/modloop/server) to rebuild the loop (Met1-Phe17) into its secondary structure. Validation of the model was done by Ramachandran plot analysis **[15]**. Structural models were visualized by PyMol™ Molecular Graphics System version 0.97 (http://www.pymol.org).

Docking studies and Binding site prediction:

Two molecules, aspirin (activator) and cefazolin (inhibitor) were chosen for docking studies. Docking study was carried out using Autodock software, which uses Genetic algorithm (GA). HuPON1 was loaded into AutoDock Tools (ADT) (http://autodock.scripps.edu/ resources) as a receptor and made ready for docking by the addition of hydrogens which any PDB file of the molecule usually does not contain, using the edit option in ADT. The ligands aspirin and cefazolin were separately and individually docked with HuPON1, grid for dock search was built for the whole molecule to find the most probable binding site in HuPON1 and to measure its interaction parameters with aspirin and cefazolin. The docking process was carried out in the default parameters of ADT. Binding sites has been predicted by submitting the model to 3D2GO binding site prediction server (http://www.sbg.bio.ic.ac.uk).

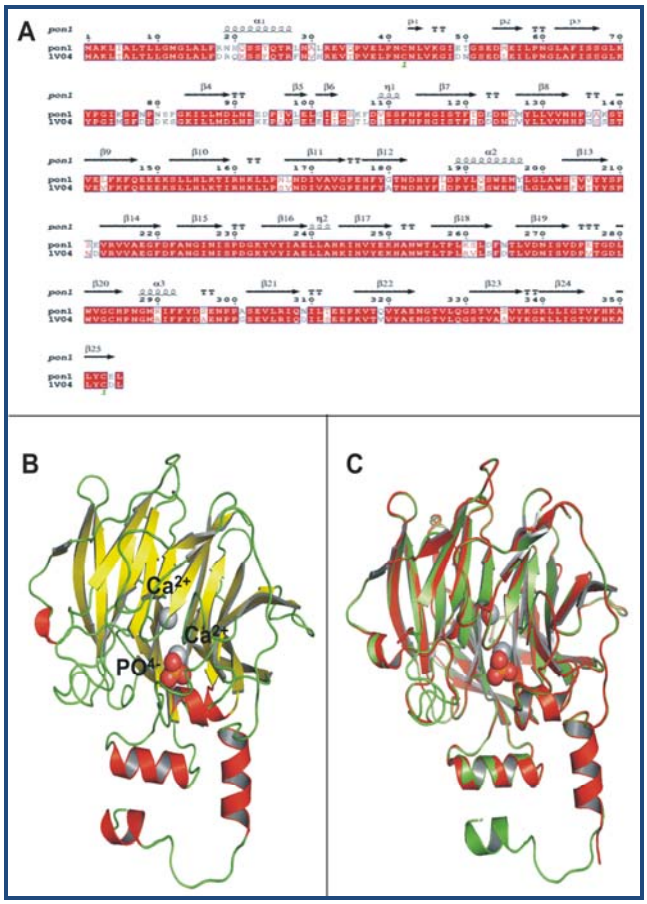

Figure 1: (a) Sequence alignment of HuPON1 with rePON1 (PDB: IV04). Strictly conserved residues are highlighted in red and partially conserved residues in white boxes. **(b)** Theoretical model of HuPON1 predicted using homology modelling. **(c)** Superimposition of HuPON1 (colored in green) with rePON1 (colored in red).

Results and Discussion:

Structural evaluation:

Present study reports the structural interaction between HuPON1 with one inhibitor and one activator separately. rePON1 (PDB ID: 1V04) having high degree of homology with HuPON1 was used as a template with identity of 83% with atomic resolution of its x-ray crystal structure at 2.2 Å and R value being 0.189. The secondary structure alignment obtained between the query and template sequence is shown in **Figure 1a**. The query (HuPON1) subjected for homology modeling by Modeller ended up with a loop by default modeling process. Then, it was submitted to http://modbase.compbio.ucsf.edu/modloop/ server to rebuild into secondary structure, where loop attained alpha helix conformation **(Figure 1b)**. Root mean square deviation (RMSD) value between the template and predicted loop model was found to be 0.202, indicative of a good model. The G-factors indicating the quality of covalent and bond angle distance were -0.09 for dihedrals, -0.32 for covalent, and overall -0.14. The predicted model was subjected to PROCHECK analysis to determine psi and phi torsion angles, the comparable Ramachandran plot characteristics and G-factors confirm the quality of predicted model **(Table 1, see Supplementary material)**.

The overall topology of modeled structure is a β propeller consisting of five alpha helices with multiple beta folds. Top view of PON1 appears to be a 6 bladed β propeller; each blade is composed of four β strands. The residues comprising the β-sheets of the propeller were highly conserved among all PON sequences, while the majority of the variation among the paralogs was located primarily in the 3 helices forming a unique lid over the active site of PON1. The crystal structure of the rePON1 also revealed a six bladed β-propeller protein centrally arranged around two calcium ions (PDB: 1V04), which is similar to the DFPase-based homology model **[16, 17]**. The N-terminal sequence (helix1) is the main determinant for PON's cell distribution, translocation and secretion; both Helix H1 and H2 provide hydrophobic anchors for binding to the HDL particle and retain its hydrophobic N terminus which resembles a signal peptide. It is believed that PON1 anchors to HDL through this peptide and the other helices are also likely to have a profound effect on their protein-lipid and protein-protein interactions **[18]**. HuPON1 is always found in association with ApoA1 of HDL suggesting strong protein-protein interaction. Insights into the 3D structure and mechanistic studies also enabled a detailed description of PON1 active site. PON1 has two calcium atoms, one of which probably has catalytic and the other has structural role. These are 7.4 Å apart and are located in the central tunnel of the β propeller. The catalytic calcium is within 2.2-2.5 Å from oxygens of N224, N270, N68, D269 and E53 **[16]**. HuPON1 is considered to be a complex in substrate recognition and catalytic reactions mediated through multiple mechanisms and residues. Substantial efforts have focused on identifying amino acid residues important for HuPON1 enzymatic activity and have revealed that the residues affecting the diverse activities of HuPON1 (lactonase/esterase and phosphotriesterase) are located in different regions of the active site **[19]**. The enzymatic catalysis of various substrates by PON1 is known to be mediated by a unique H115–H134 dyad, which deprotonates a water molecule to generate the attacking hydroxide ion and accounts for the hydrolytic activity of PON1 **[9, 20]**. Kinetic and site-directed mutagenesis studies have been extensively investigated to delineate substrate specificity and identified number of residues important for esterase and PON1 activities, These residues include L69, H115, H134, D169, F222, D269, H285, F292, T332, V346 and W281, and substitution of these residues results in diminution or loss of function **[21]**. A disulphide bridge formed between C42 and C353 stabilizes the 3D structure. Moreover, eight amino acids have been identified as essential for PON1 activities, which are W280, H114, H133, H154, H242, H284, E52 and D53 **[16]**. Sequence variation between HuPON1 and rePON1 found in regions that do not affect their active sites. Since HuPon1 has been modeled after the template (PDB: 1V04), it is likely that these interactions which are predicted may also hold good for the HuPON1-HDL interaction as predicted by the superposition analysis **(Figure 1c)**.

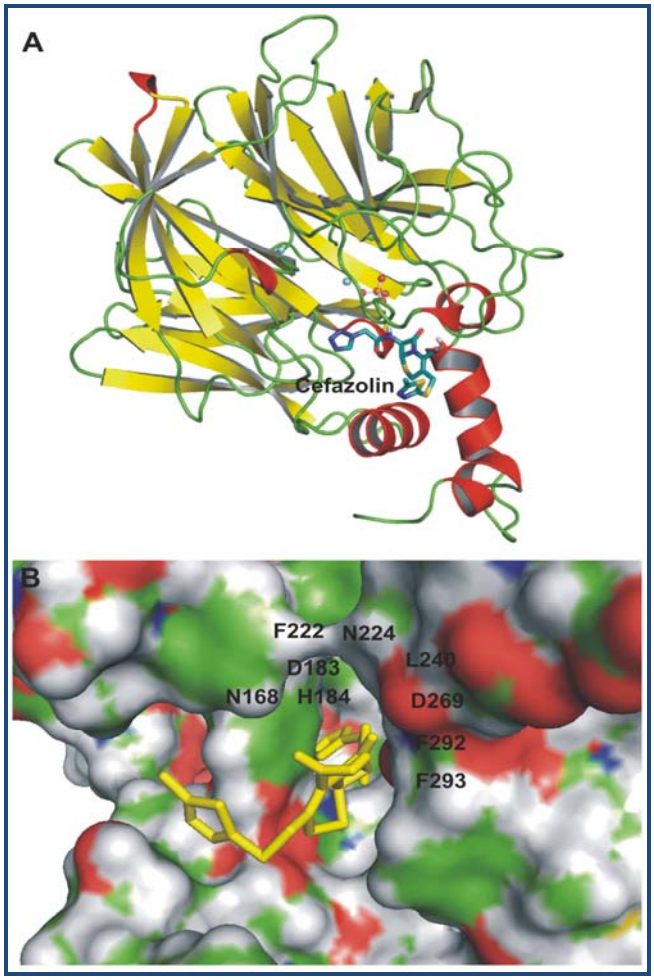

Figure 2: Receptor-ligand interaction between cefazolin and modeled HuPON1 enzyme obtained using Autodock 4.0; **(a)** Active site of HuPON1 with cefazolin; **(b)** Electrostatic surface of HuPON1 binding pocket and interacting residues for cefazolin drug.

Docking study and binding site prediction:

Docking was performed using AUTODOCK 4.0 for the receptor of PON1 with ligands aspirin and cefazolin, loaded individually into ADT to analyze ten best conformations. The study showed that the conformation of cefazolin with PON1 has binding energy of -5.5k.cal M-1 and binding constant/inhibitory constant of 80.71μM. While, the conformation of aspirin had lower binding energy than that of

cefazolin (-4.85k.cal M-1) and higher binding constant/inhibitory constant (276.3μM). The ligand binding residues of cefazolin and aspirin showed similarity with binding site predicted in our model and those predicted by the 3D2GO **(Table 2, see Supplementary material)**. Cefazolin was found to dock in the vicinity of predicted binding site 1 of HuPON1 (**Figure 2a**) and restored all its H-bond contacts with predicted site residue by residue with slight variations from 3D2GO. However, aspirin was found to dock in vicinity of the predicted binding site 2 of HuPON1 (**Figure 3a**) and restored the geometrical premises of the predicted binding site but not the residues **(Table 3, see Supplementary material)**. Cefazolin shares three hydrogen bonds and aspirin shares four hydrogen bonds with HuPON1. Furthermore, cefazolin docks at residues N166, S193 and Y71 **(Figure 2b)** and aspirin at residues N309, I310 and L311 **(Figure 3b)**.

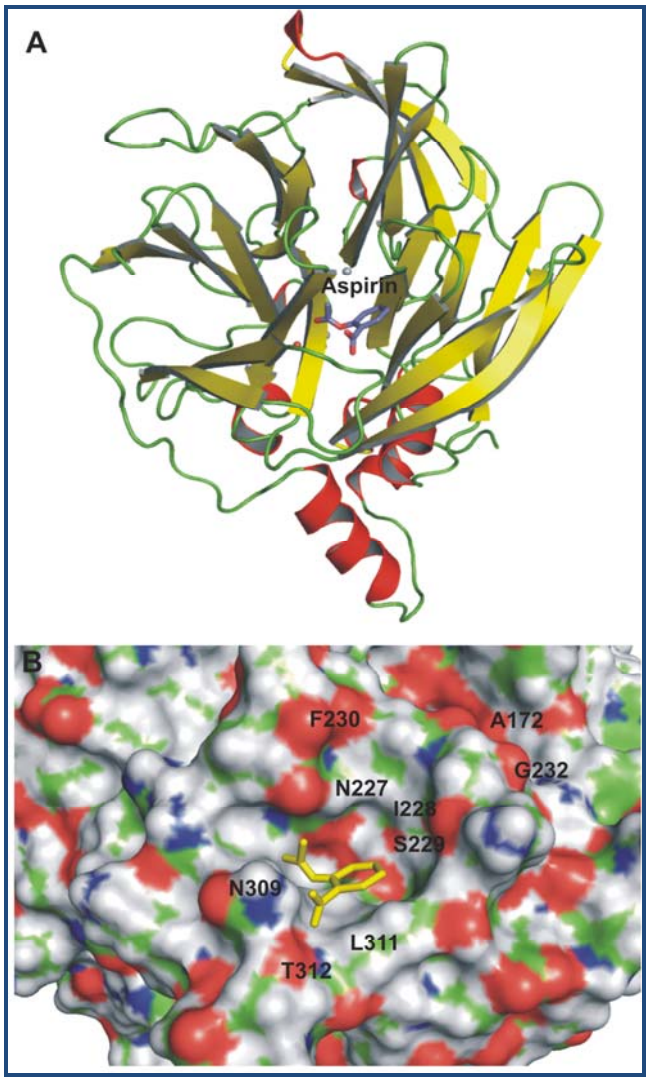

Figure 3: Receptor-ligand interaction between aspirin and modeled HuPON1 enzyme obtained using Autodock 4.0; **(a)** Active site of HuPON1 with aspirin; **(b)** Electrostatic surface of HuPON1 binding pocket and interacting residues for aspirin drug.

Conclusion:

In conclusion, model building and docking study were used to attain details of interaction between protein and ligands-aspirin and cefazolin. Docking studies of aspirin and cefazolin into the active site of HuPON1 revealed two binding sites; the first region is occupied by cefazolin and the second with aspirin. The multiple binding sites may possibly explain the multifunctional nature of HuPON1 towards structurally unrelated substrates. Furthermore, site directed mutagenesis has identified distinct amino acids for PON1 and phenyl acetate binding which are distinct from the residues important for binding aspirin and cefazolin. Therefore, screening many more substrates and inhibitors might provide the answers to the multi-factorial nature of HuPON1 and may help understand its cardio-protective function.

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Supplementary material:

Table 1: Validation statistics for theoretical model of PON1 by procheck analysis

Plot statistics		
Residues in most favored regions	275	89.0%
Residues in additional allowed regions	30	9.7%
Residues in most generously allowed regions	3	1.0%
Residues in disallowed regions	1	0.3%
Number of non-glycine and non-proline residues	309	100.0%
Number of end –residues (excl. Gly and Pro)	2	
Number of glycine residues	24	
Number of proline residues	20	
Total number of residues	355	

Table 2: Predicted binding sites of PON1 by 3DGO server (http://www.sbg.bio.ic.ac.uk/phyre/pfd/)

Binding site	Residues
Site 1	N224, D269, N168, F222, L240, D183
Site 2	I228, S229, N227, P230, A172, G232

Table 3: Residues interacting in binding sites of PON1 with cefazolin and aspirin

Molecule	Amino acid residues involved in binding pocket of model	Atoms involved in bond formation	Distance (Å)
Cefazolin	ASN166	LIG: N-HD22	2.1Å
	SER193	LIG: S-HG	2.2 Å
	TYR71	LIG: N-HH	2.2 Å
Aspirin	ASN309	LIG: C:O-HN	2.1 Å
	ASN309	LIG: C:0-HD22	1.8 Å
	ILE310	LIG: C:0-NH	2.0 Å
	LEU311	LIG: C:0- NH	2.2 Å