Molecular modeling of LCAT and natural mutants

Peelman, F[¶], Vanloo, B.[¶], Verschelde,J.L.[§], Vandekerckhove, J.[§], Brasseur, R.[£], Tavernier, J.[§], and Rosseneu, M.[¶]

[¶]Laboratory for Lipoprotein Chemistry and the [§]Flanders Interuniversity Institute for Biotechnology, Department of Biochemistry, Universiteit Gent, B-9000 Gent, Belgium
[£]Centre de Biophysique Moléculaire Numérique, Faculté des Sciences Agronomiques de Gembloux, B-5030 Gembloux, Belgium.

The enzyme lecithin cholesterol acyltransferase(LCAT) shares the Ser/Asp/His triad with lipases, esterases and proteases, but the low level of sequence homology between LCAT and these enzymes did not allow an exact identification of the LCAT fold. We therefore relied upon structural homology calculations using the123D and THREADER fold recognition programs for prediction of the LCAT fold. These methods show that LCAT, like lipases, belongs to the α/β hydrolase fold family, and that the central domain of LCAT consists of seven conserved parallel beta-strands connected by four α-helices and separated by loops. Using site-directed mutagenesis, followed by activity measurements, we identified D345 and H377 as the catalytic residues of LCAT, together with F103 and L182 as the oxyanion hole residues. In analogy with lipases, we further proposed that a potential "lid" domain at residues 50-74 of LCAT might be involved in the enzyme-substrate interaction and showed that Trp61 plays a critical role in substrate recognition and specificity. Molecular modeling of human LCAT was carried out using human pancreatic and Candida antarctica lipases as templates. The 3-D model proposed here is compatible with the position of natural mutants for either LCAT deficiency or Fish-eye disease. We modeled 14 natural mutants where point mutations in the LCAT gene have lead to either FLD or FED phenotype. We show that most of the FLD mutants cluster around the active site of LCAT, while other mutations disrupt salt bridges and affect the enzyme structure. In contrast, FED mutants are localized on the outer hydrophilic face of the amphipathic helical segments, and are less detrimental for the protein structure. FED mutations seem more involved in the interaction of LCAT with the substrate and/or its co-factor.

Peelman, F., Vinaimont, N., Verhee, A., Vanloo, B., Verschelde, J.L., Labeur, C., Seguret, M.S., Duverger, N., Hutchinson, G., Vandekerckhove, J., Tavernier, J. and Rosseneu, M. Protein Sci., 7 (1998) 587-599.
Peelman, F., Vanloo,B., Perez-Mendez, O., Decout,A., Verschelde,J.L., Labeur,C., Vinaimont, N., Verhee, A., Duverger, N., Brasseur, R., Vandekerckhove, J., Tavernier, J. and Rosseneu, M. Protein Engineering, 12 (1999) 71-78