High resolution structures of mutants of residues that affect access to the ligand-binding cavity of human lipocalin-type prostaglandin D Synthase

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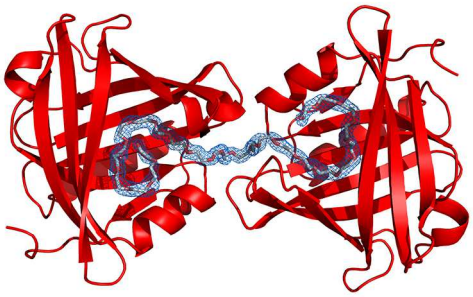
Lipocalin-type prostaglandin D synthase (L-PGDS) catalyzes the isomerisation of the 9,11-endoperoxide group of PGH2 (Prostaglandin H2) to produce PGD2 (Prostaglandin D2) with 9-hydroxy and 11-keto groups. The product of the reaction, PGD2, is the precursor of several metabolites involved in many regulatory events. L-PGDS, the first member of the important lipocalin family to be recognized as an enzyme, is also able to bind and transport small hydrophobic molecules and was formerly known as β-trace protein, the second most abundant protein in human cerebro-spinal fluid. Previous structural work on the mouse and human proteins has focused on the identification of the amino acids responsible and the proposal of a mechanism for catalysis. In this paper we present the X-ray structures of the apo and holo forms (bound to PEG) of the C65A mutant of human L-PGDS to 1.40 Å resolution and of the double mutant C65A K59A to 1.60 Å resolution. We have also studied the apo forms of the double mutants C65A W54F and C65A W112F and the triple mutant C65A W54F W112F. Mutation of the lysine residue does not seem to affect the binding of PEG to the ligand-binding cavity and mutation of a single or both tryptophanes appears to have the same effect on the position of these two aromatic residues at the entrance of the cavity. We have also identified a solvent molecule in an invariant position in the cavity of virtually all the molecules present in the 9 asymmetric units of the crystals that we have examined. Taken together our observations indicate that the residues we have mutated appear to indeed play a role in the entrance-exit process of the substrate and/or other ligands to the binding cavity of the lipocalin.



Binding of polyethylene glycol to the C65A mutant of human L-PGDS.