Structural Characterization and Interaction Studies of Human Lipocalin-type Prostaglandin D Synthase (L-PGDS)

M. Perduca, M. Bovi, S. Capaldi and H.L. Monaco

Biocrystallography Laboratory, University of Verona, Department of Biotechnology, 37134 – Verona.

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 in the presence of sulphydryl compounds. PGH2 is a common precursor of all prostanoids, which include thromboxanes, prostacyclins and prostaglandins. PGD2 is synthesized in both the central and peripheral nervous system and it is 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. L-PGDS is also detected in brain, testis and prostate, endothelial cells, placenta and heart tissue and even in macrophages infiltrated in atherosclerotic plaques. In these tissues it participates in many physiological activities as well as in the response to diseases. Currently the main structural and biochemical studies, present in the literature, concern recombinant rat and mouse L-PGDS. In this work we use recombinant human L-PGDS in order to solve its three-dimensional structure by X-ray diffraction and test its affinity for several ligands using Surface Plasmon Resonance (SPR). Wild type human L-PGDS and three mutants (C65A; C65A-K59A; C89/186A) were expressed using *E. coli* cell strains and subsequently purified by a chitin affinity column, size exclusion and hydrophobic interaction chromatography. Large and highly ordered crystals were used to collect X-ray diffraction data using either a rotating-anode generator or a synchrotron source. The multiple isomorphous replacement method was used to solve the phase problem. In the electron density maps an unidentified density was observed apparently interacting with lysine 59 inside the L-PGDS-C65A cavity; the foreign molecule is probably PEG, an additive present in the crystallization liquors. This hypothesis is supported by the fact that the L-PGDS-C65A/K59A crystals, which grow without PEG, show a completely free protein cavity. A seeding experiment of L-PGDS-C65A/K59A crystal, grown in L-PGDS-C65A crystallization conditions, partially confirmed this hypothesis since the foreign molecule was present in the L-PGDS-C65A/K59A cavity. Another crystal form was obtained by mixing L-PGDS-C65A/K59A with the amyloid β peptide (1-40). Although the amyloid β peptide is not visible in the maps, the packing of the protein molecules has changed in the presence of the peptide suggesting interaction of the two molecules. Wild type L-PGDS small crystals were recently obtained and will be tested as soon beam time at a synchrotron source becomes available.

SPR experiments are also in progress and will be used to verify interaction of L-PGDS with PEG, the amyloid β peptide and other ligands and to determine their binding constants.