X-ray Crystallographic Studies of Prostacyclin Synthase

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Prostacyclin synthase (PGIS) is a cytochrome P450 enzyme which catalyzes production of prostacyclin from prostaglandin H2. PGIS is unusual in that it catalyzes an isomerization rather than a monooxygenation, which is typical of P450 enzymes. To understand the structural basis for prostacyclin biosynthesis in greater detail, we have determined the crystal structures of ligand-free, inhibitor (minoxidil) bound and substrate analog U51605-bound PGIS. These structures demonstrate a stereo-specific substrate binding and suggest features of the enzyme that facilitate isomerization (Fig. 1). Unlike most microsomal P450s where large substrate-induced conformational changes take place at the distal side of the heme, conformational changes in PGIS are observed at the proximal side and in the heme itself. The conserved heme propionate-protein interactions seen in all other P450s, which are absent in the ligand-free PGIS, are recovered upon U51605 binding accompanied by water exclusion from the active site (Fig. 2). In contrast, when minoxidil binds, the propionate-protein interactions are not recovered and water molecules are largely retained. These findings suggest that PGIS represents a divergent evolution of the P450 family in which a heme barrier has evolved to ensure strict binding specificity for prostaglandin H2, leading to a radical-mediated isomerization with high product fidelity. The U51605-bound structure also provides a view of the substrate entrance and product exit channels. (The X-ray diffraction data were also collected at NSRRC beamlines 13B1 and 13C1, and the SPring8 Taiwan Beamline SP12B2.)

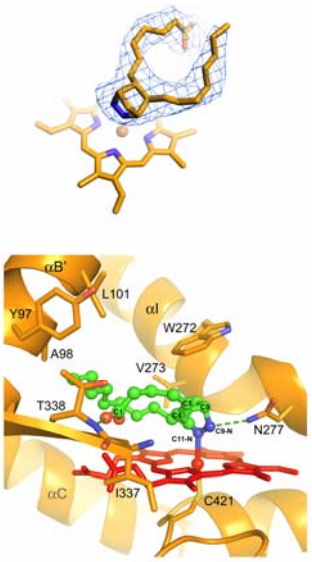

Figure 1. Experimental density and conformation of the active-site-bound substrate analog U51605.

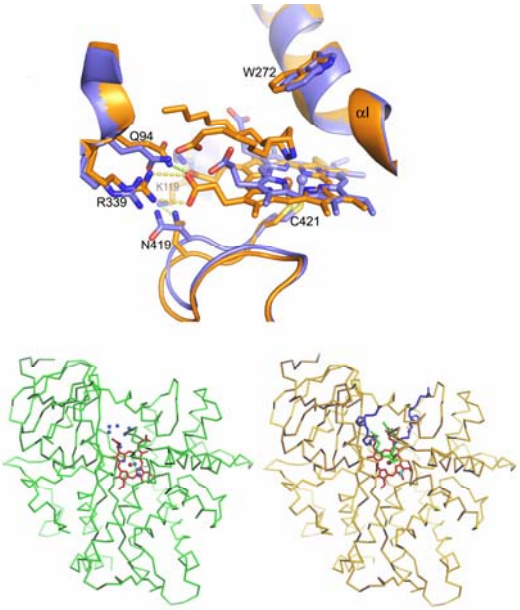

Figure 2. U51605-induced conformation change around the active site (top) and change in solvent structure upon U51605 binding.