Northeast Structural Genomics Consortium

Structural and Functional Studies of Tryptophan 2,3-Dioxygenase: NESG Target XcR13

Tryptophan 2,3-dioxygenase (TDO) and indoleamine 2,3-dioxygenase (IDO) catalyze the oxidative cleavage of the L-tryptophan (L-Trp) pyrrole ring, the first and rate-limiting step in L-Trp catabolism through the kynurenine pathway (Fig. 1A). Both enzymes constitute an important, yet poorly understood, family of heme-containing enzymes. TDO is a homotetrameric enzyme and is highly specific for L-Trp and related derivatives such as 6-fluoro-Trp as the substrate. In comparison, IDO is monomeric, and shows activity toward a larger collection of substrates, including L-Trp, D-Trp, serotonin, and tryptamine. In a collaboration with Chapman group at the University of Edinburgh, United Kingdom, we succeeded in conducting extensive structural and biochemical studies of the *Xanthomonas campestris* TDO, including the structure at 1.6-A resolution of the catalytically active, ferrous form of TDO in a binary complex with the substrate L-Trp (Fig. 1B&C). The carboxylate (Fig.1D) and ammonium moieties (Fig.1E) of tryptophan are recognized by electrostatic and hydrogen-bonding interactions with the enzyme and a propionate group of the heme, thus defining the L-stereospecificity (Fig. 1B). A second, possibly allosteric, L-Trp-binding site is present at the tetramer interface (Fig. 1C). The sixth coordination site of the heme-iron is vacant, providing a dioxygen-binding site that would also involve interactions with the ammonium moiety of L-Trp and the amide nitrogen of a glycine residue. The indole ring is positioned correctly for oxygenation at the C2 and C3 atoms. The active site is fully formed only in the binary complex, and biochemical experiments confirm this induced-fit behavior of the enzyme. The active site is completely devoid of water during catalysis, which is supported by our electrochemical studies showing significant stabilization of the enzyme upon substrate binding.

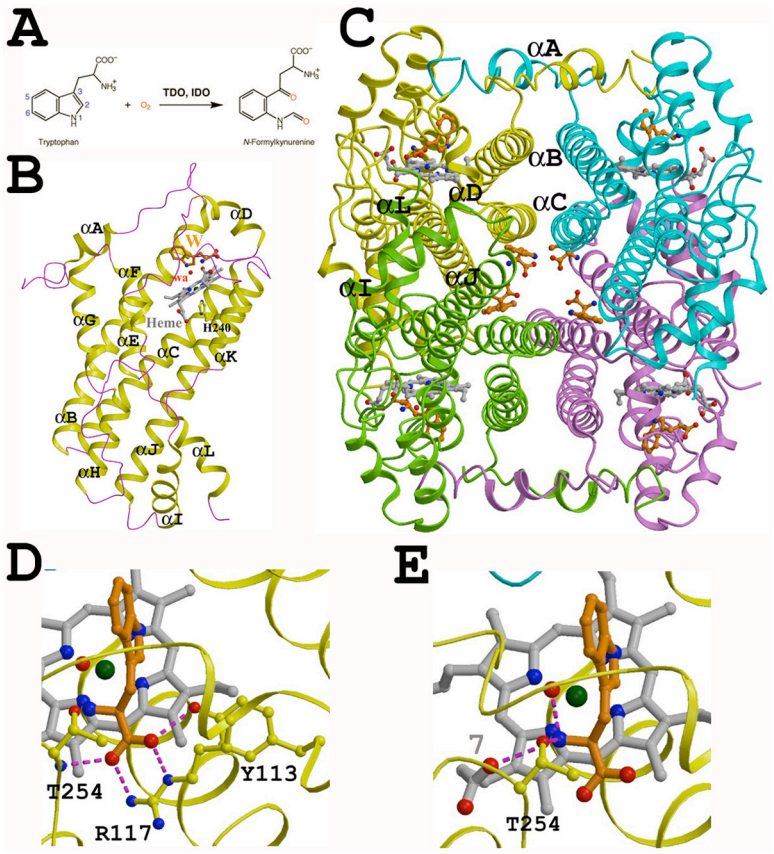

Fig. 1. **(A).** Reaction catalyzed by TDO & IDO. **(B).** Structure of TDO in complex with heme (in gray for carbon atoms) and its natural substrate L-Trp (in orange). **(C).** The tetramer is the biological unit of TDO. The four protomers are shown in yellow, cyan, green and magenta. Four tryptophan molecules (in orange) at the interface of the TDO tetramer possibly have an allosteric role in TDO activity. **(D).** Recognition of carboxyl group of L-Trp by the backbone amide of T254, and invariant residues Y113 and R117. The ferrous ion of the heme and a water molecule in the active site are shown in dark green and red solid spheres. **(E).** Recognition of ammonium ion of L-Trp by invariant residue T254 and propionate group of heme.

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