Role of Tyr348 in Tyr385 Radical Dynamics and Cyclooxygenase Inhibitor Interactions of Prostaglandin H Synthase-2

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Prostaglandin H synthases (PGHS) are hemoproteins that utilize a tyrosyl radical at residue 385 to abstract a hydrogen atom from arachidonic acid, initializing prostaglandin synthesis. A Tyr348-Tyr385 hydrogen bond is conserved in both isoforms of PGHS and may modulate the reactivity and conformation of the Tyr385 radical. The EPR signal of the Tyr385 radical undergoes a time dependant transition from a wide doublet to wide singlet species. In PGHS-2 this transition is the result of tyrosyl radical migration from Tyr385 to Tyr504; in the Y504F PGHS-2 mutant only a wide doublet signal from Tyr385 is observed. This simplification of EPR signals in the Y504F mutant was exploited to examine the effects of disrupting the hydrogen bond by introduction of a Y348F mutation. Removal of the Tyr348-Tyr385 hydrogen bond maintains cyclooxygenase and peroxidase activities, but allows the Tyr385 radical greater rotational freedom: a wide doublet EPR species forms first but converts to a wide singlet species. Pre-incubation with cyclooxygenase inhibitors results in a third type of EPR signal, a narrow singlet, which is not observed in the Y504F PGHS-2 mutant. Analysis of the spectra suggests two distinct conformations of Tyr385 are responsible for the wide doublet and narrow singlet species and that the wide singlet signal is a mixture of these two conformations. The effect of disruption of the Tyr348-Tyr385 hydrogen bond on the cyclooxygenase active site in PGHS-2 also was probed by examination of cyclooxygenase inhibitor kinetics. Aspirin treatment eliminated all oxygenase activity in the double mutant similar to PGHS-1, whereas in wild-type PGHS-2 acetylation results in lipoxygenase activity. In addition, the sensitivity to time dependant inhibition by nimesulide was increased by the Y348F mutation. These results suggest that removal of the Tyr348-Tyr385 hydrogen bond allows greater conformational flexibility of the cyclooxygenase active site in PGHS-2, resulting in tyrosyl radical behavior and inhibitor interactions resembling those in PGHS-1.

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