The Structure of Arachidonic Acid Bound in the Cyclooxygenase Channel of Prostaglandin Endoperoxide H Synthase-1

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Introduction

Prostaglandin endoperoxide H synthase-1 and -2 (PGHS-1 and -2) are integral membrane, heme-containing proteins, that catalyze the committed step in prostaglandin and thromboxane biosynthesis.[1,2] PGHS-1 is constitutively expressed in most tissues and platelets and produces prostaglandins in response to stimulation by hormones. The resulting prostanoids mediate "housekeeping" functions required for maintaining organ and tissue homeostasis. Conversely, PGHS-2 is an inducible enzyme normally absent from cells but expressed in response to growth factors, tumor promoters, or cytokines. The prostanoids produced by PGHS-2 regulate cell replication, differentiation, and inflammation. PGHS-1 and PGHS-2 are the major targets for aspirin, ibuprofen, and other nonsteroidal anti-inflammatory drugs (NSAIDs).

The isoenzymes contain two spatially distinct catalytic sites — a cyclooxygenase (COX) site, and a peroxidase (POX) site. Both active sites depend upon a single heme group and thus are catalytically linked. The COX reaction converts arachidonic acid (AA) and two molecules of O_2 to the bicyclic hydroperoxide prostaglandin G_2 (PGG_2). This intermediate diffuses to the POX site where it undergoes a two electron reduction to the alcohol prostaglandin H_2 (PGH_2).

While the interactions of PGHS-1 and -2 with aspirin and other NSAIDs within the COX active site have been elucidated,[3-7] the binding modes of AA and other fatty acid substrates within this site have only been implied.[1,2] Contaminating exogenous peroxides and hydroperoxides make the formation of a stable PGHS:AA complex impossible. These contaminants generate tyrosyl and arachidonyl radicals that result in product formation and enzyme inactivation.[8] However, reconstitution of apo-ovine (o) PGHS-1 with Co^{3+}-protoporphyrin IX (Co^{3+}-oPGHS-1) overcomes the problem and leads to a catalytically inert enzyme.[9] Using this enzyme, we have determined the crystal structure of Co^{3+}-oPGHS-1 complexed with AA to 3.0Å resolution.[10]

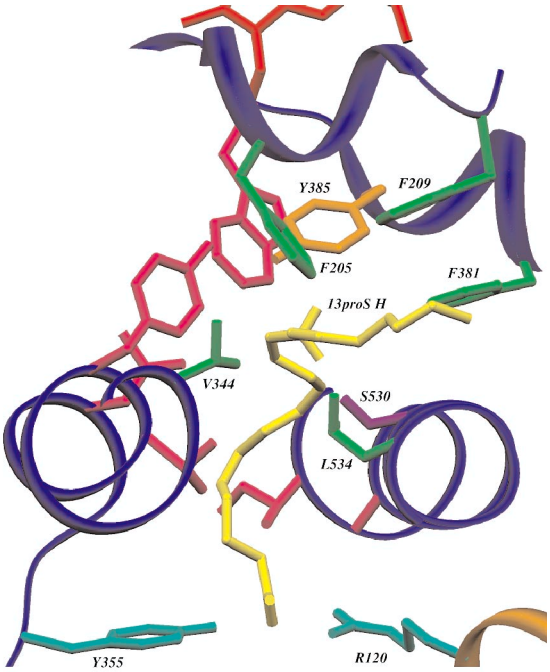

FIG. 1. AA bound in the COX channel of oPGHS-1. A view of the extended L-shaped conformation of AA (yellow) bound in the COX active site as generated by the program SETOR.[17] *Residues that contact C-1 through C-13 and C-14 through C-20 are colored pink and green, respectively. The carboxylate of AA is stablilized by interactions with Arg120 and Tyr355 (light blue). AA is positioned at the top of the COX channel such that Tyr385 (orange) can abstract the 13-proS hydrogen. Ser530, the residue acetylated by aspirin treatment, is shown in purple.*

Methods and Materials

Crystals of Co^{3+}-oPGHS-1 complexed with AA were prepared and flash-frozen for data collection as described in (9). Data were collected at 100 K on beamline 19-ID of the Structural Biology Center at the Advanced Photon Source and processed using HKL2000.[11] The structure of the Co^{3+}-oPGHS-1:AA complex was solved by the molecular replacement method using CNS_SOLVE version 0.9a[12] and the protein portion of the refined oPGHS-1 structure complexed with flurbiprofen (PDB entry 1CQE) as the search model. The space group was determined to be hexagonal ($P6_522$) with cell dimensions of a=b=182.10 Å, c=103.64 Å, and a monomer in the asymmetric unit. Subsequent refinement in CNS, employing the maximum likelihood target using amplitudes, a bulk solvent correction, and an overall B-factor correction, resulted in R and free-R values of 21.6% and 29.0%, respectively.

Results

AA is oriented within the COX channel in an extended L-shaped conformation (Fig. 1). The apical portion of the substrate binds in the channel such that the carboxylate is positioned to interact with the guanidinium group of Arg120 and the phenolic oxygen of Tyr355. Of the 50 contacts made between enzyme and substrate, only these interactions are hydrophilic. This is to be expected due to the hydrophobic nature of the residues that line the COX channel. The interaction of AA with Arg120 has been shown to be a major determinant in substrate binding in PGHS-1 but not in PGHS-2.[13,14] C-7 through C-14 of AA form an S-shaped kink that weaves the substrate around the side chain of Ser530 (Fig. 1), the residue acetylated by aspirin treatment.[4,15] In this conformation, AA is positioned such that C-13 is oriented near the phenolic oxygen of Tyr385, where the proS hydrogen can be abstracted to begin the COX reaction. In addition, the kink positions carbon 11 above a small pocket into which O_2 could migrate from the lipid bilayer. Thus, C-11 would be accessible to O_2 on the side opposite (antarafacial) to hydrogen

abstraction, a known aspect of the COX reaction mechanism.[2,16] Although the side chain atoms of Ser530 make close and intimate contacts with AA, a S530A mutant retains up to 80% of native COX activity with no change in Km for AA.[15] Thus, the hydroxyl group is not essential for COX catalysis. The ω-end of the substrate (C-14 through C-20) binds in a small hydrophobic groove above Ser530, where it is stabilized by Phe205, Phe209, Phe381, and Leu534 (Fig. 1).

Discussion

In addition to complimenting ongoing functional studies that have defined the key players involved substrate binding and catalysis,[1,2,10] elucidation of the structure of Co^{3+}-oPGHS-1:AA has allowed for the proposal of a valid sequence of catalytic events for the COX reaction.[10] As mentioned above, AA is positioned in the COX channel such that a Tyr385 phenolic radical can abstract the 13-proS hydrogen and subsequent formation of the 11R-peroxyl radical achieved via O_2 addition from the lipid bilayer on the side antarafacial to that of hydrogen abstraction. In the next step, the 11R-peroxyl radical attacks C-9 to form the endoperoxide, with isomerization of the radical to C-8. However, completion of the next step, ring closure and bond formation between C-8 and C-12, is not possible due to the extended structure of AA. Therefore, a conformational transition involving significant movement of the ω-end towards the apical end of the substrate must occur. This conformational movement would bring C-8 and C-12 in close proximity for facile ring closure. Additional mechanistic consequences would include: a) the repositioning of C-13 through C-20 such that C-15 becomes optimally positioned for addition of the second molecule of O_2; and b) the placement of the 15S-peroxyl radical for donation of the radical back to Tyr385 to complete COX catalysis.

Acknowledgments

This work was supported by National Institutes of Health grants PO1 GM57323 (W.L.S. and R.M.G.), R01 HL56773 (R.M.G.), and F32 HL10170 (M.G.M.). Use of the Advanced Photon Source was supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences, under Contract No. W-31-109-Eng-38.

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