Chapter 13

ACTIVATION OF PROSTAGLANDIN BIOSYNTHESIS: PEROXYNITRITE VS HYDROPEROXIDES

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13.1 Introduction

13.1.1 Overview of PGHS catalysis

Prostaglandins are an important family of lipid-derived molecules that play critical roles in physiological processes including platelet aggregation, vasoconstriction and inflammation. The committed step in prostaglandin biosynthesis is the oxygenation of the polyunsaturated fatty acid, arachidonic acid, by the enzyme prostaglandin endoperoxide synthase (PGHS), a membrane-bound hemoprotein. PGHS is a bifunctional enzyme with both peroxidase and cyclooxygenase activities. The reactions catalyzed occur at two structurally distinct sites on the PGHS protein called the cyclooxygenase and peroxidase active sites. The requisite heme prosthetic group is positioned between the two separate active sites. The cyclooxygenase activity of PGHS catalyzes the oxygenation of arachidonic acid to form the initial product, the hydroperoxy endoperoxide, \( \text{PGH}_2 \) (Scheme I). The peroxidase activity of PGHS reduces \( \text{PGG}_2 \) to the hydroxy endoperoxide, \( \text{PGH}_2 \).

\( \text{PGH}_2 \) serves as the precursor for all prostaglandins and thromboxanes. The specific prostaglandin product formed in a certain cell type depends on which \( \text{PGH}_2 \)-metabolizing enzyme is expressed therein. For example, platelets convert \( \text{PGH}_2 \) almost exclusively to thromboxane \( \text{A}_2 \), a potent activator of platelet

aggregation.\textsuperscript{50,51,52} Inhibition of the cyclooxygenase activity of PGHS is the basis for the pharmacological action of non-steroidal antiinflammatory drugs including aspirin, ibuprofen and indomethacin. Given its importance as a drug target, the cyclooxygenase activity of PGHS has received considerably more attention than the enzyme's peroxidase activity; thus, the PGHS is often referred to simply as "cyclooxygenase" or COX.\textsuperscript{45,46}

\subsection*{13.1.2 Peroxidase Activation of the Cyclooxygenase Reaction}

The peroxidase activity of PGHS is essential for activation of the cyclooxygenase reaction. The peroxidase activity of PGHS is typical of heme-containing peroxidases and spectroscopically distinct Compound I and Compound II intermediates have been detected (Scheme II).\textsuperscript{53} Hydroperoxides react with the resting Fe\textsuperscript{3+} form of the heme prosthetic group of PGHS to generate the corresponding alcohol and the higher oxidation state of the heme called Compound I that is consistent with the Fe\textsuperscript{4+}≡O porphyrin cation radical. One electron reduction of Compound I by an electron donor, represented by AH in Schemes I and II, leads to formation of Compound II, a Fe\textsuperscript{4+}≡O species with the porphyrin fully covalent. One electron reduction of Compound II regenerates the resting Fe\textsuperscript{3+} state of the enzyme. As depicted, reduction of the oxidized enzyme requires the input of two electrons in a sequential fashion (i.e. two one-electron transfers). For PGHS, two-electron reduction of a hydroperoxide to form Compound I, followed by intramolecular electron transfer, is required to generate a tyrosyl radical, Y•, that serves as the cyclooxygenase oxidant (Scheme II). The tyrosyl radical abstracts a hydrogen atom from arachidonic acid (AA) to initiate PGG\textsubscript{2} synthesis. Thus, an oxidized intermediate of peroxidase catalysis is required to activate cyclooxygenase catalysis.\textsuperscript{46}

Site-directed mutants of PGHS that have decreased peroxidase activity, and thus react much more slowly with hydroperoxides, typically have pronounced cyclooxygenase lag phases that can be overcome by adding high concentrations of

\begin{center}

\begin{tikzpicture}
  \node (arachidonic_acid) {\textbf{Arachidonic Acid}};
  \node (pghs_cyclooxygenase) at (1,0) {\textbf{PGHS cyclooxygenase}};
  \node (pghs_peroxidase) at (1,-2) {\textbf{PGHS peroxidase}};

  \draw [->] (arachidonic_acid) -- (pghs_cyclooxygenase) node [midway, above] {$\rightarrow 2 \text{O}_2$};
  \draw [->] (pghs_cyclooxygenase) -- (pghs_peroxidase) node [midway, above] {$\rightarrow 2 \text{AH}$};
  \draw [->] (pghs_peroxidase) -- (pghs_peroxidase) node [midway, above] {$\rightarrow 2 \text{A}^* + \text{H}_2\text{O}$};

  \draw [->] (pghs_peroxidase) -- (arachidonic_acid) node [midway, above] {$\rightarrow \text{PGH}_2$};

\end{tikzpicture}

\end{center}

\textbf{Scheme I.} Synthesis of \textbf{PGH}_2 from arachidonic acid by PGHS