3 Fatty-Acid Substrate Interactions with Cyclo-oxygenases

W. L. Smith, C. J. Rieke, E. D. Thuresson, A. M. Mulichak, and R. M. Garavito

3.1 Introduction

Prostaglandin endoperoxide H synthases 1 and -2 (PGHS-1 and -2) convert arachidonic acid and O2 (along with two reducing equivalents) to PGH2 – the committing step in the formation of prostanoids (Smith and DeWitt 1996; Smith et al. 1996). PGHS-1 is often referred to as the constitutive enzyme, whereas PGHS-2 is known as the inducible isozyme. They differ from one another mainly with respect to their temporal patterns of expression. The reason for the existence of the two PGHS isozymes is still unknown. One possibility is that PGHS-2 is induced and then functions at relatively low fatty-acid substrate and hydroperoxide-activator concentrations to generate prostanoid products during early stages of cell replication or differentiation, whereas PGHS-1 forms products that are involved in “housekeeping” functions when circulating...
hormones act on cells acutely to cause the release of higher concentrations of arachidonate (Capdevila et al. 1995; Kulmacz and Wang 1995; Kulmacz 1998; So et al. 1998).

Structurally (Picot et al. 1994; Kurumbail et al. 1996; Luong et al. 1996) and kinetically (Barnett et al. 1994; Laneuville et al. 1994), the two PGHS isoforms are remarkably similar. Both are homodimeric (~72 kDa/subunit), heme-containing, glycosylated proteins with independent but interactive peroxidase and cyclo-oxygenase (COX) active sites (Dietz et al. 1988; Smith et al. 1996). Moreover, the enzymes are novel integral membrane proteins, both of which are anchored to the luminal surfaces of the endoplasmic reticulum and both the inner and outer membranes of the nuclear envelope (Otto and Smith 1994; Spencer et al. 1998). This interaction involves the hydrophobic surfaces of amphipathic helices and only one leaflet of the lipid bilayer (Picot et al. 1994; Otto and Smith 1996). Both PGHS isozymes are important pharmacologically, because they are the major therapeutic targets of aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs). Common NSAIDs inhibit both isoforms (Meade et al. 1993; Mitchell et al. 1993; Barnett et al. 1994; Laneuville et al. 1994; O’Neill et al. 1994; Patrignani et al. 1994). However, PGHS-2 (COX-2)-selective inhibitors have recently been developed (Riendeau et al. 1997; Zhang et al. 1997; Smith et al. 1998). PGHS-1 is important in thrombosis, and its inhibition by aspirin acting on platelet cells is of cardiovascular benefit (Oates et al. 1988; Patrono et al. 1990; Funk et al. 1991; Willard et al. 1992; Patrignani et al. 1994; Patrono 1994). Inhibition of PGHS-2 is anti-inflammatory, analgesic and anti-pyretic (Riendeau et al. 1997; Zhang et al. 1997; Smith et al. 1998) and may prevent colon cancer (Thun et al. 1991; Levy 1997; Tsujii et al. 1998) and Alzheimer’s disease (Breitner 1996).

3.2 COX and Peroxidase Catalysis

PGHSs catalyze two reactions: a COX (bis-oxygenase) reaction in which arachidonate is converted to PGG$_2$ and a peroxidase reaction in which PGG$_2$ undergoes a two-electron reduction to PGH$_2$ (Fig. 1). PGHS-1 and PGHS-2 have similar COX turnover numbers (~3500 mol arachidonate/min/mol dimer; Barnett et al. 1994) and $K_m$ values for