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Minireview

Cellular regulation of prostaglandin H synthase catalysis

Richard J. Kulmacz*

Department of Internal Medicine, University of Texas Health Science Center at Houston, 6431 Fannin St., Room 5.284 MSB, Houston, TX 77030, USA

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Abstract Prostanoids are a group of potent bioactive lipids produced by oxygenation of arachidonate or one of several related polyunsaturated fatty acids. Cellular prostaglandin biosynthesis is tightly regulated, with a large part of the control exerted at the level of cyclooxygenase catalysis by prostaglandin H synthase (PGHS). The two known isoforms of PGHS have been assigned distinct pathophysiological functions, and their cyclooxygenase activities are subject to differential cellular control. This review considers the contributions to cellular catalytic control of the two PGHS isoforms by intracellular compartmentation, accessory proteins, arachidonate levels, and availability of hydroperoxide activator.

Key words: Prostaglandin biosynthesis; Prostaglandin H synthase; Cyclooxygenase; Arachidonic acid; Hydroperoxide

Eicosanoids comprise a diverse group of oxygenated metabolites of polyunsaturated fatty acids (prototypically arachidonic acid), and include prostanoids (the various prostaglandins, prostacyclin, and thromboxane), the leukotrienes, and various epoxy, hydroperoxy, and hydroxy fatty acids [1]. Biosynthesis of each eicosanoid begins with oxygenation of the fatty acid. For the prostaglandins, thromboxane, and prostacyclin, this key committed step is catalyzed by the cyclooxygenase activity of prostaglandin H synthase (PGHS; also known as prostaglandin endoperoxide synthase), which converts arachidonic acid to prostaglandin (PG) G_2. PGHS also has an efficient heme-dependent peroxidase activity, which reduces the C15 hydroperoxide on PGG_2 to an alcohol, forming PGH_2.

Much early characterization of PGHS was done with an enzyme, now called PGHS-1, isolated from ovine and bovine seminal vesicles. A second isoform of PGHS, termed PGHS-2, was discovered in 1991 (reviewed in [2]). Both isoforms have cyclooxygenase and peroxidase activity and their basic reaction mechanisms are probably similar [3]. However, the isoforms have distinct genes and distinct regulation of gene expression. PGHS-1 message and protein are found in most cells at rather stable levels. As a result, PGHS-1 is considered a housekeeping enzyme, supplying prostanoids crucial for basal functions (e.g. hemostasis, gastric cytoprotection). On the other hand, PGHS-2 message and protein are at very low or undetectable levels in most quiescent cells, but are strongly and rapidly induced in some cells responding to cytokines and mitogens. PGHS-2 is thus referred to as the 'inducible' PGHS isoform, and has been assigned important roles in inflammation and proliferation [2].

PGHS-2 has emerged as an important pharmacological target because selective inhibitors of the PGHS-2 cyclooxygenase are expected to be effective anti-inflammatory agents while, by sparing PGHS-1 cyclooxygenase activity, avoiding the side effects common with the non-specific anti-cyclooxygenase agents currently in wide use, such as aspirin, naproxen, and indomethacin [2,3]. Interestingly, PGHS-2 appears elevated in many (but not all) human colon tumors, and use of anti-cyclooxygenase agents is associated with a dramatically reduced risk of colon cancer, although the identity of the pharmacological target remains controversial [4].

Prostanoids derived from PGH_2 are potent bioactive lipids, and so PGH_2 biosynthesis is tightly controlled. Studies in cells possessing both PGHS isoforms have demonstrated important regulation of prostaglandin synthesis occurs at the level of catalytic activity, with strikingly different control of PGHS-1 and PGHS-2 catalysis. The best studied examples are macrophages and fibroblasts, which have constitutive levels of PGHS-1 [2]. In these cells, mitogen stimulation leads to induction of PGHS-2, considerable arachidonate release, and conversion of a fraction of the arachidonate to prostaglandins. Antisense oligonucleotide against PGHS-2 sequences blocks PGHS-2 induction and the burst of prostaglandin synthesis, but it does not impede arachidonate release nor does it change the PGHS-1 protein level [5]. Selective PGHS-2 cyclooxygenase inhibitors have effects similar to PGHS-2 antisense oligonucleotide in mitogen-treated cells [2]. It is thus clear that the burst of prostaglandin synthesis triggered by cytokines or mitogens originates from PGHS-2 cyclooxygenase catalysis, with the large amount of PGHS-1 in the same cells remaining latent even though considerable unesterified arachidonate is released.

Induction of PGHS-2 makes only a small impact on the overall prostaglandin biosynthetic capacity in macrophages and fibroblasts, and prostaglandin biosynthesis from endogenous substrate is only a small fraction of overall prostaglandin synthetic capacity regardless of whether one or both isoforms are present [5–7]. Induction of PGHS-2 thus is clearly not required to overcome insufficient catalytic capacity, but rather has the effect of supplementing a latent enzyme, PGHS-1, with a catalytically active enzyme, PGHS-2. This raises interesting questions of what keeps PGHS-1 latent while PGHS-2 is active, and what benefit induction of the second isoform might have for the cell. Several explanations for this differential catalytic regulation of the isoforms can be considered (see scheme in Fig. 1).

(a) Access of the isoforms to distinct arachidonate pools via differential compartmentation or coupling with distinct phos-

*Fax: (1) (713) 500-6810.
E-mail: kulmacz@heart.med.uth.tmc.edu

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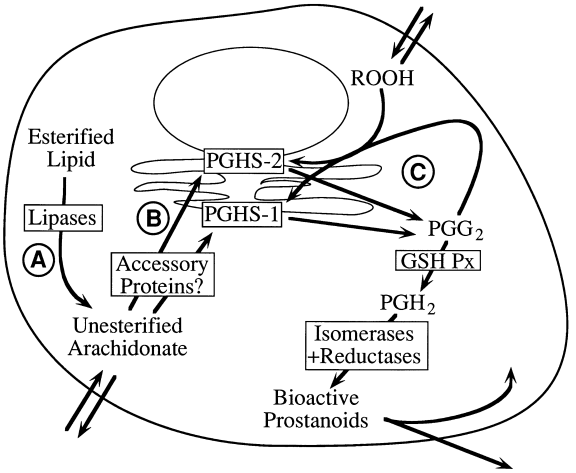

Fig. 1. Cartoon showing potential routes for differential cellular control of cyclooxygenase catalysis by PGHS-1 and -2. GSH Px, glutathione peroxidase; ROOH, hydroperoxide.

pholipases or accessory proteins (A and B in Fig. 1). This explanation was prompted by the observation that PGHS-2 formed prostaglandins from endogenous arachidonate, whereas PGHS-1 catalysis required exogenous arachidonate [5]. Immunofluorescence studies supported a differential compartmentation of the isoforms, with more PGHS-2 in the nuclear envelope than in the ER membrane, and PGHS-1 equally distributed between the two locations [8]. Very recent immunoelectron microscopy studies, however, have found roughly equal amounts of both PGHS-1 and -2 in the inner and outer nuclear membranes in several cell types [9]. Because only the outer nuclear membrane is contiguous with the ER membrane, this indicates that the two isoforms are similarly distributed (at least on a macro scale) in intracellular membranes. Several prostaglandin biosynthetic enzymes and substrate lipid precursors also are found in non-membranous, cytosolic lipid bodies which accumulate in cells associated with inflammation, although the amounts of each PGHS isoform present in these organelles have not been determined [10].

Data suggesting coupling of particular phospholipases with one or the other PGHS isoform have been reported, but a consistent pattern of coupling partners has yet to emerge [11–13]. Arachidonate binding accessory proteins analogous to the 5-lipoxygenase activating protein [14] could conceivably deliver fatty acid specifically to one of the isoforms, but candidate proteins have not been identified, and neither cyclooxygenase activity is impaired by purification to remove other cellular components. Channelling of arachidonate to a particular PGHS isoform seems difficult to reconcile with the observation that arachidonate release is in large excess over prostaglandin synthesis, with much of the excess escaping from the cell [5]. In addition, extracellular fatty acid scavengers, such as serum albumin and cyclodextrin, inhibit prostaglandin synthesis from endogenous substrate in some cells [15], indicating that the intracellular arachidonate available for prostaglandin synthesis equilibrates with the extracellular fatty acid pool.

(b) Differences in the dependence of the isoforms' cyclooxygenase activities on arachidonate concentration. Purified PGHS-1 was found to have a sigmoidal dependence on arachidonate concentration, whereas PGHS-2 displayed simple saturable behavior [16]. This was interpreted as evidence for allosteric activation of PGHS-1 cyclooxygenase by the fatty acid. Sigmoidal kinetics also have been observed for PGHS-1 activity in intact cells [17]. In principle, the difference between the isoforms in response to substrate level could give PGHS-2 a 2–4-fold catalytic advantage over PGHS-1 at the low arachidonate levels expected within cells [16]. The structural basis for cooperative behavior in PGHS-1 is not well defined, although a sigmoidal response to arachidonate was induced in PGHS-2 by mutation of Tyr^{341} to phenylalanine [18].

(c) Differences in hydroperoxide activator requirement (C in Fig. 1). The cyclooxygenase activity in both PGHS-1 and -2 is inherently latent. Cyclooxygenase activation requires reaction of the PGHS peroxidase with hydroperoxide, which in one proposed mechanism [19,20] entails generation of peroxidase compound I and its subsequent conversion to a tyrosyl radical species (steps 1 and 4 in Fig. 2). The tyrosyl radical is probably on Tyr^{385} in PGHS-1 and on the corresponding residue, Tyr^{371}, in PGHS-2 [21–25]. $Tyr^{385/371}$ lies at the end of the cyclooxygenase channel, oriented toward the fatty acid binding site [26–28], a spatial arrangement consistent with the mechanism in Fig. 2.

With adequate arachidonic acid and oxygen present, activation of a single PGHS molecule produces about 10^3 molecules of PGG_2, itself a hydroperoxide [29]. Diffusion of this PGG_2 to latent PGHS nearby can progressively activate the bulk PGHS in a chain reaction with powerful feedback characteristics (Fig. 2). The requirement for activation provides a means for cellular control of cyclooxygenase catalysis because the bulk enzyme can be kept latent if sufficient PGG_2 is intercepted by peroxide scavengers such as glutathione peroxidase, a common cytosolic peroxidase [29]. Cyclooxygenase activation in PGHS-2 has a peroxide requirement of about 2 nM, an order of magnitude lower than that in PGHS-1, and the two isoforms respond independently to the hydroperoxide level even when both are in the same compartment

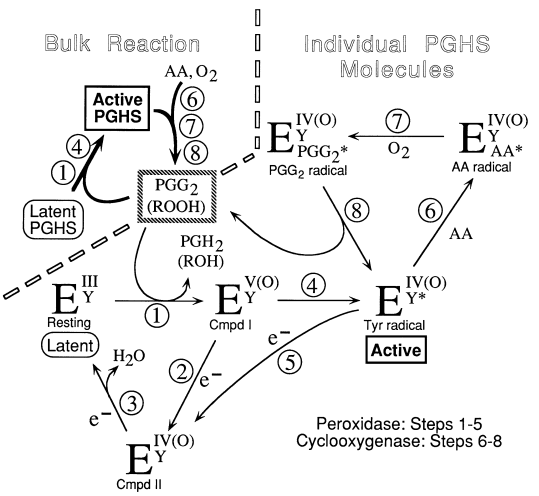

Fig. 2. Hypothetical tyrosyl radical reaction mechanism for individual PGHS molecules (based on [19]), and feedback activation scheme for bulk PGHS cyclooxygenase by the cyclooxygenase product, PGG_2. Enzyme symbols indicate the overall oxidation state of the peroxidase heme (III, V(O), or IV(O)), and whether Tyr^{385} (Tyr^{371} in PGHS-2) is in the ground state (Y) or carries a tyrosyl radical (Y*). ROOH and ROH, hydroperoxide and the corresponding alcohol; Cmpd I and Cmpd II, peroxidase compounds I and II; e^-, reducing equivalent; AA, arachidonic acid.

[30,31]. As a result, a considerable fraction of PGHS-2 cyclooxygenase can be in the catalytically active state at hydroperoxide levels where the large majority of PGHS-1 activity is latent. Release of arachidonate by cellular phospholipases under such circumstances would permit prostaglandin synthesis via PGHS-2 but not by PGHS-1.

Differential control of catalysis in PGHS-1 and PGHS-2 via cellular peroxide level has several appealing features which accord with experimental observations. Regulation by peroxide does not require physical sequestration of the proteins, peroxide activator, or arachidonate. This fits with the ease with which fatty acids cross lipid membranes [32] and the apparent equilibration of intracellular and extracellular arachidonate pools [15]. The ratio of peroxide scavenging capacity (measured as glutathione peroxidase activity) to cyclooxygenase capacity in tissues ranges from 10 in cells with active prostaglandin synthesis to near 2000 in tissue with little prostaglandin synthesis [29]. This range of glutathione peroxidase/cyclooxygenase capacity ratio in tissues spans the ratios of 70 observed for complete suppression of purified PGHS- 1 and 700 for complete suppression of purified PGHS-2 [31], suggesting that the amounts of cellular peroxidases are sufficient to poise intracellular peroxides at levels consistent with catalytic control. Control of cyclooxygenase catalysis via peroxide level also provides an attractive rationale for the observed induction of PGHS-2 in cells already containing PGHS-1. Induction of the more readily activated PGHS-2 would allow a cell to dramatically increase the rate of prostaglandin synthesis without increasing the cellular peroxide tone, thereby avoiding the increased risk of damage to DNA, protein, and lipid which accompanies elevated peroxide levels.

A hydroperoxide-based cyclooxygenase regulatory regime could conceivably be linked to the nitric oxide (NO) pathway because peroxynitrite, a NO metabolite, is a cyclooxygenase activator [33]. This indirect cyclooxygenase activation via peroxynitrite requires appreciable levels of superoxide [33], which is not produced during catalysis by purified PGHS unless particular cosubstrates, such as NADH or NADPH, are added [34]. Direct activation of PGHS-1 and -2 cyclooxygenase activity by nitric oxide or NO donors has been reported (e.g. [35,36]). However, such activation has proven difficult to reproduce, even with enzyme from the same sources (e.g. [37,38]). In addition, a biochemical basis for direct cyclooxygenase activation by NO has yet to be demonstrated convincingly. NO has only very weak affinity for the ferric heme in PGHS-1, with a K_d value of 0.9 mM [37]. NO does bind tightly to ferrous PGHS-1 heme [37], but the ferrous state is rapidly oxidized in air and is not observed during catalysis [19]. These properties make it very unlikely that NO is a significant PGHS heme ligand at the low micromolar levels expected in vivo. Furthermore, PGHS heme ligands generally inhibit, rather than activate, cyclooxygenase activity [39]. Nitrosothiol formation on PGHS cysteine residues has been proposed as a mechanism for cyclooxygenase activation by NO [36], but the published data yield an implausibly high stoichiometry of PGHS nitrosothiol formed/NO added (>4), indicating that the issue needs to be re-examined.

The actions of each of the three individual factors described above in differential catalytic control of the PGHS isoforms in vivo are being actively examined. Some of the recent evidence suggests a new hypothesis needs to be considered: that fatty acid and hydroperoxide levels interact to play the dominant role in regulation of cyclooxygenase catalysis. In the prototypical fibroblast model, PGHS-1 cyclooxygenase activity is released from the latent form by raising the hydroperoxide level [[40]; R. Kulmacz and W. Chen, unpublished observations], and PGHS-2 cyclooxygenase activity is suppressed by decreasing cellular hydroperoxides [17]. In the same intact cell model, PGHS-2 catalysis occurs readily with low arachidonate levels, whereas PGHS-1 catalysis requires higher fatty acid substrate levels [17]. However, the hydroperoxide and fatty acid levels do not exert their influences independently, as can be appreciated from the feedback activation scheme shown in Fig. 2. Activation of latent cyclooxygenase requires hydroperoxide (PGG_2) accumulation, and formation of that PGG_2 requires arachidonate. Thus, catalysis by the cellular cyclooxygenases could be expected to be modulated in an interdependent fashion by both the hydroperoxide level and the supply of arachidonate. Further investigation of the dynamics of this interplay between the substrates and products of the two PGHS isoforms should help to elucidate how cellular prostanoid synthesis is controlled.

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