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Review

Structural and functional differences between cyclooxygenases: Fatty acid oxygenases with a critical role in cell signaling

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Abstract

Cyclooxygenase (COX) catalyzes the first two steps in the conversion of arachidonic acid (AA) to prostaglandins (PGs). The reaction mechanism is well-defined and supported by extensive structural data. There are two isoforms of COX, which are nearly indistinguishable in structure and mechanism, however, COX-2 oxygenates neutral derivatives of AA that are poor substrates for COX-1. The best neutral substrate is 2-arachidonylglycerol, oxygenation of which produces an array of prostaglandin glyceryl esters (PG-Gs) that is nearly as diverse as the PGs. The mobilization of Ca^{2+} by subnanomolar concentrations of PGE_2-G in RAW264.7 cells suggests the existence of a distinct receptor, and the formation of PG-Gs by zymosan-stimulated macrophages indicates that these species may be formed in vivo. These findings suggest that PG-Gs comprise a new class of lipid mediators, and that oxygenation of neutral derivatives of AA is a distinct function for the COX-2 isoform.

Keywords: Cyclooxygenase; Oxygenase; Mechanism; Arachidonic acid; Endocannabinoids; Glyceryl prostaglandins

Prostaglandins (PGs) were discovered by the American gynecologists, Ralph Kurzrok and Charles Lieb, in 1930 as muscle-contracting constituents of human semen [1]. The Swedish physiologist, Ulf von Euler, characterized the compounds as acidic lipids but their structures were not elucidated until 1960 when the Swedish biochemist, Sune Bergstrom, identified $PGF_{1\alpha}$ and PGE_1 as oxygenated fatty acids containing a cyclopentane ring and *trans*-dialkyl side chains (Fig. 1) [2–4]. Bergstrom and the Dutch biochemist, David Van Dorp, independently demonstrated that PGs are metabolites of polyunsaturated fatty acids [5,6]. Further studies by these same investigators indicated that all three of the oxygen atoms in PGE_1 derive from O_2 and that the two oxygen atoms of the cyclopentane ring originate from the same *molecule* of O_2 (Fig. 1) [7–12]. Bengt Samuelsson termed the oxygenase that catalyzes the biosynthesis of PGs "fatty acid cyclooxygenase (COX)," and performed extensive studies that defined the chemical mechanism of oxygenation of the fatty acid substrate [13,14]. The enzyme was ultimately purified by Osamu Hayaishi and Shozo Yamamoto and was demonstrated to be a membrane-bound hemeprotein [15]. A second isoform of the enzyme was discovered independently by Daniel Simmons and Harvey Herschman [16,17]. Sir John Vane had previously demonstrated that COX is the target for the pharmacological action of non-steroidal anti-inflammatory drugs (NSAIDs) and experiments by Philip Needleman indicated that the second form of COX (COX-2) is the principal target for the anti-inflammatory effects of NSAIDs [18–21].

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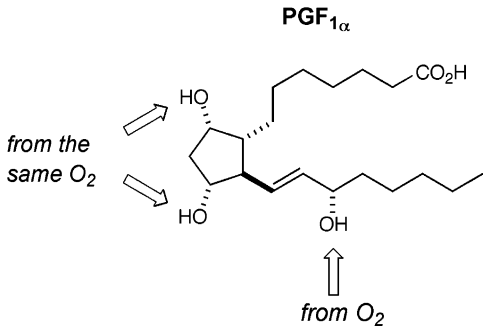

Fig. 1. Structure of $PGF_{1\alpha}$ and the sources of the three hydroxyl groups.

Structure of COX

COX enzymes contain two distinct active sites, which catalyze two sequential reactions—the double dioxygenation of arachidonic acid (AA) to a hydroperoxy endoperoxide, PGG_2 (cyclooxygenase reaction), and the reduction of PGG_2 to a hydroxy endoperoxide, PGH_2 (peroxidase reaction, Fig. 2) [22–25]. The cyclic peroxide of PGH_2 is converted to the ultimate PG derivative by one of a series of metabolizing enzymes (Fig. 2). The structure of a COX enzyme (COX-1) was first solved by Michael Garavito and the structure of COX-2 was solved independently by Michelle Browner and Ravi Kurumbail [26–28]. The proteins are 60% identical in amino acid sequence and nearly superimposable in overall folding. The enzymes are homodimers of 70 kDa subunits and each subunit is comprised of three domains—an epidermal growth factor domain, a membrane-binding domain, and a catalytic domain (Fig. 3). The membrane-binding domain contains four helices that lie along one leaflet of the lipid bilayer and project hydrophobic residues into the membrane. The helices

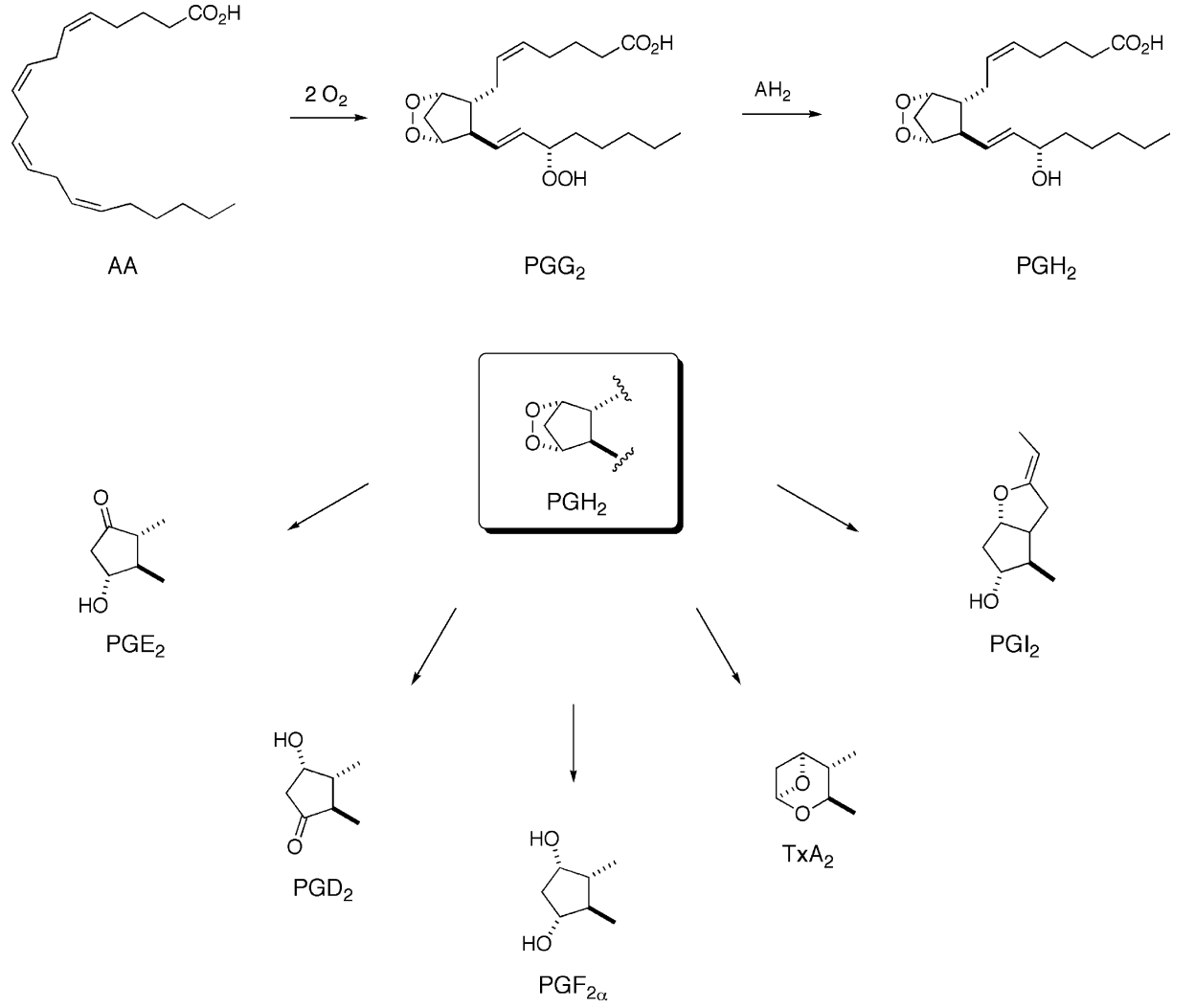

Fig. 2. Metabolic pathway for the conversion of AA to prostaglandins and thromboxane. COX enzymes catalyze the double dioxygenation of AA to PGG_2 (cyclooxygenase activity) and the reduction of PGG_2 to PGH_2 (peroxidase activity). PGH_2 diffuses from the COX protein and is converted in a tissue-specific fashion to the various prostaglandins or thromboxane. Each final metabolite activates one or more specific membrane-bound G-protein-coupled receptors to trigger a cellular response.

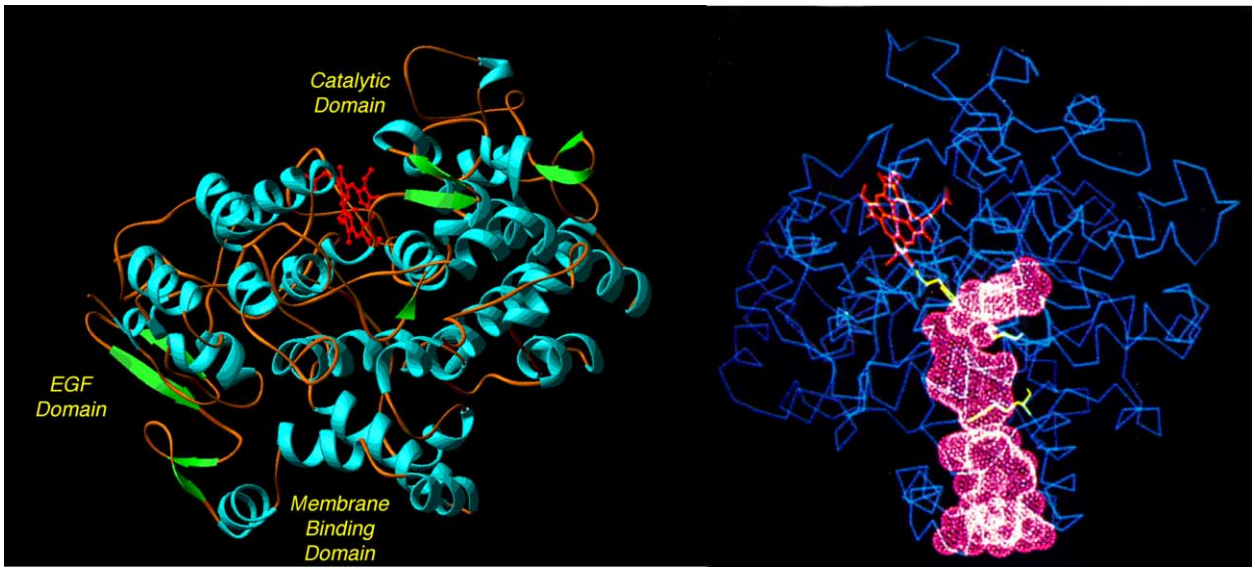

Fig. 3. Structure of a single subunit of COX. Both COX-1 and COX-2 are homodimers of ~70 kDa subunits. The left frame displays the overall domain structure of one subunit of COX-1 whereas the right frame displays the α-carbon backbone tracing of a subunit of COX-1. The substrate access channel and active sites are highlighted in pink. The constriction between the lower part of the channel and the active site is comprised of Arg-120 (shown in yellow), Tyr-355 (not shown), and Glu-524 (not shown). Other residues shown in yellow in the active site are Tyr-385 and Ser-530. Gly-533 is not shown but marks the upper end of the active site and is adjacent to the ω-end of AA [81,82]. The subunit displayed in the left and right frames is rotated 180° in the two frames. The figure in the right frame is reproduced from [26] with permission.

are nearly orthogonal to each other and define the outer edge of a channel that penetrates deep into the interior of the protein. The initial opening of the channel is wide but the channel narrows at a constriction comprised of Arg-120, Tyr-355, and Glu-524 (Fig. 3). This constriction separates the lower part of the channel from the cyclooxygenase active site. In the active site, the channel makes a sharp turn near Tyr-385 that terminates in an alcove near Gly-533. Each subunit contains a heme group (ferric protoporphyrin IX) that does not make direct contact with the cyclooxygenase active site. The heme of the resting enzyme appears to be six-coordinate with a proximal histidine residue (His-388) and a distal water ligand [29,30]. The distal face of the heme lies beneath a collection of residues that define the highly efficient peroxidase active site [31]. Unlike other heme peroxidases, the COX peroxidase prefers fatty acid hydroperoxides as substrates [32]. COX proteins are members of the peroxidase superfamily and are very similar to myeloperoxidase in overall folding pattern, with the exception of the absence of a membrane-binding domain in myeloperoxidase [33].

Mechanism of the cyclooxygenase reaction

Extensive studies, conducted in many laboratories, have defined the mechanism of action of COX enzymes (Fig. 4). These studies are summarized in recent, detailed reviews [34–37]. AA binds with its carboxylate ion-paired to Arg-120 and its ω-methyl group positioned near Gly-533. The 13-*pro*(*S*) hydrogen sits just below Tyr-385. A tyrosyl radical derivative of Tyr-385 abstracts this hydrogen generating a pentadienyl radical that is trapped by O_2 at carbon-11. The 11-peroxyl radical cyclizes at carbon-9 producing a cyclic peroxide and a carbon-8 radical that cyclizes at carbon 12 producing the bicyclic peroxide. Cyclization also generates an allylic radical that is trapped at carbon-15 to produce a peroxyl radical precursor to PGG_2. The PGG_2 peroxyl radical abstracts a hydrogen atom from Tyr-385 to produce PGG_2 and regenerate the tyrosyl radical for the next round of catalysis.

The tyrosyl radical is initially generated from Tyr-385 by intramolecular electron transfer to a ferryl-oxo derivative of the heme [38,39]. The latter is produced by reaction of the resting ferric heme with a hydroperoxide activator [40]. The initial hydroperoxide activator in lipopolysaccharide (LPS)-stimulated macrophages has been proposed to be peroxynitrous acid, the coupling product of nitric oxide and superoxide anion [41,42]. However once generated, PGG_2 can also serve this purpose, thereby recruiting additional COX enzymes [40,43]. Overall, the conversion of AA to PGG_2 is a controlled free radical chain reaction in which the stereochemistry of product formation is determined by the bound conformation of the oxidized arachidonate derivative in the cyclooxygenase active site [34]. In contrast to many oxygenases that work by O_2 activation, COX enzymes work by substrate activation.

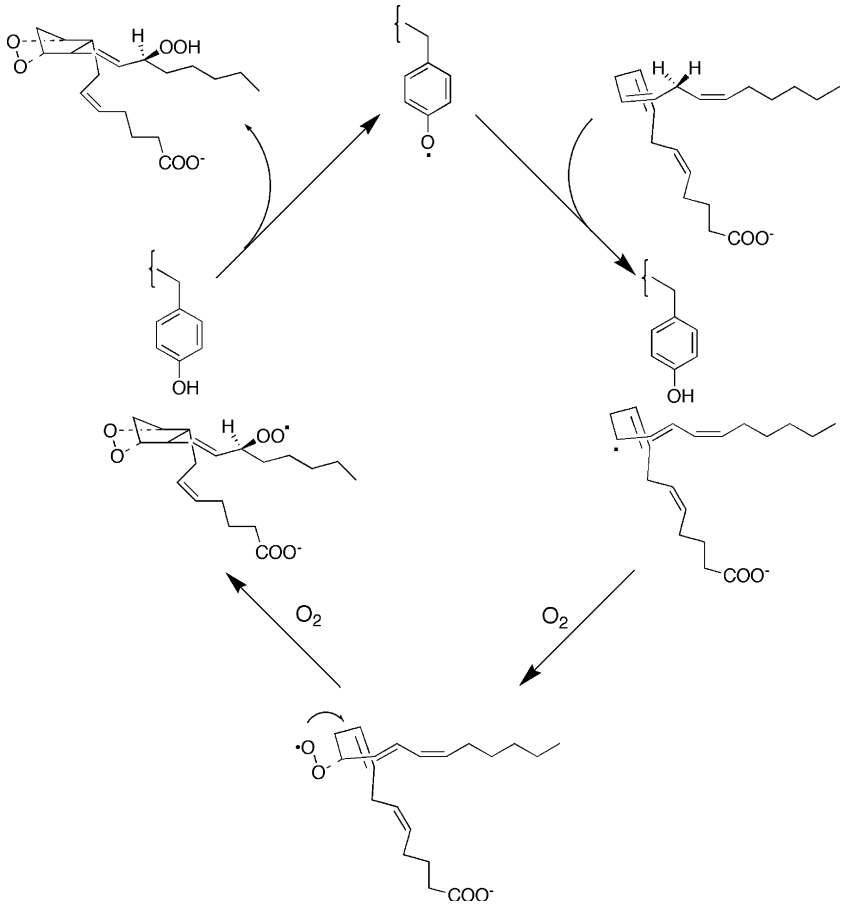

Fig. 4. Mechanism of oxygenation of AA to PGG_2 by COX. The tyrosyl radical derivative of Tyr-385 oxidizes the 13-*pro*(*S*)-hydrogen of AA to initiate a cascade of oxygenation and serial cyclization that results in the formation of a peroxyl radical precursor to PGG_2. This radical is reduced to PGG_2 by Tyr-385, which regenerates the tyrosyl radical for another round of catalysis.

COX-2-specific oxygenation of neutral substrates

COX-1 and COX-2 catalyze identical chemical reactions but there are subtle differences between the two proteins. For example, COX-2 is activated by hydroperoxide concentrations that are approximately tenfold lower than those that activate COX-1, which raises the possibility that under limiting concentrations of peroxide, COX-2 may be fully active whereas COX-1 is not [44,45]. More important for this review is the finding that COX-2 efficiently oxidizes ester and amide derivatives of AA whereas COX-1 does not. This finding is consistent with the observation that mutation of Arg-120 of COX-1 to Gln raises the K_m toward AA by approximately 1000-fold whereas the same mutation of COX-2 is without effect on the K_m for AA [46,47]. It appears that COX-1 relies mainly on ionic interactions whereas COX-2 uses H-bonding to bind substrate. Thus, neutral substrates can bind to and be efficiently oxidized by COX-2 but not COX-1.

Yu et al. [48] reported that purified human COX-2 oxidizes arachidonylethanolamide (AEA) whereas human COX-1 does not. The major product isolated after a 5 min incubation with 40 μM AEA was the ethanolamide of PGE_2 (PGE_2-EA), suggesting that COX-2 oxygenates AEA to PGH_2-EA. Intact HFF cells expressing COX-2 produced PGE_2-EA upon exposure to exogenous AEA, whereas cells expressing COX-1 did not. These results indicate that COX-2-dependent oxygenation of AEA can occur in an intracellular environment. The physiological significance of these findings is uncertain because the K_m of human COX-2 for AEA is 24 μM whereas the tissue concentration of AEA is reported to be in the range of 10–100 nM [48–52]. However, COX-2 also oxidizes 2-arachidonylglycerol (2-AG) and its K_m for this substrate (~5 μM) is within the range of concentrations reported in numerous tissues [53,54]. The k_{cat}/K_m for oxygenation of 2-AG by human or mouse COX-2 is comparable to that for AA, although the k_{cat} of mouse COX-2 is ~50% that for AA (Table 1) [53].

The range of arachidonate derivatives oxidized by COX-2 includes esters, amides, and amide conjugates to α-amino acids [55]. Within the ester and amide families, the presence of a hydroxyl group in the ester or amide side chain is critical for substrate activity (Fig. 5) [56,57]. 2-AG, which has two primary hydroxyl

Table 1
Steady-state kinetic parameters for COX-2-mediated metabolism of AA and alternative substrates

	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m (s^{-1} μM^{-1})
Human COX-2			
AA	15 ± 1	6.1 ± 0.6	2.4
2-AG	17 ± 1	4.4 ± 0.9	4.0
Murine COX-2			
AA	21 ± 2	8.2 ± 1.6	2.5
2-AG	11 ± 1	4.7 ± 0.8	2.3
AEA[a]	0.1	24	0.0042
NAGly	6.3 ± 1.1	12 ± 1	0.54

[a] Data from [48].

groups, and its amide analog, *N*-arachidonylserine, are the best substrates from both substrate classes [53,57].

Site-directed mutagenesis of residues that represent conserved differences between COX-2 and COX-1 indicates that the principal determinant of the ability of COX-2 to oxygenate ester and amide derivatives of arachidonate is an enlarged region of the cyclooxygenase active site that has been designated the side pocket (Table 2)[55–57]. This structure is conserved in all known COX-2s, but is missing in all COX-1s [28]. Among the three conserved "side-pocket" residues, Arg-513 appears to be the most important [56]. The

Table 2
Effects of side pocket mutations on the rates of oxygenation of various substrates by COX-2

Enzyme	AA	2-AG	AEA	NAGly
mCOX-2	100	100 (54)	100 (27)	100 (40)
V523I	96	86	91	49
R513H	92	41	43	27
V523I/R513H	88	29	42	20
V523I/R513H/V434I	81	20	36	45
oCOX-1	100	8	45	0

Data from [55–57].

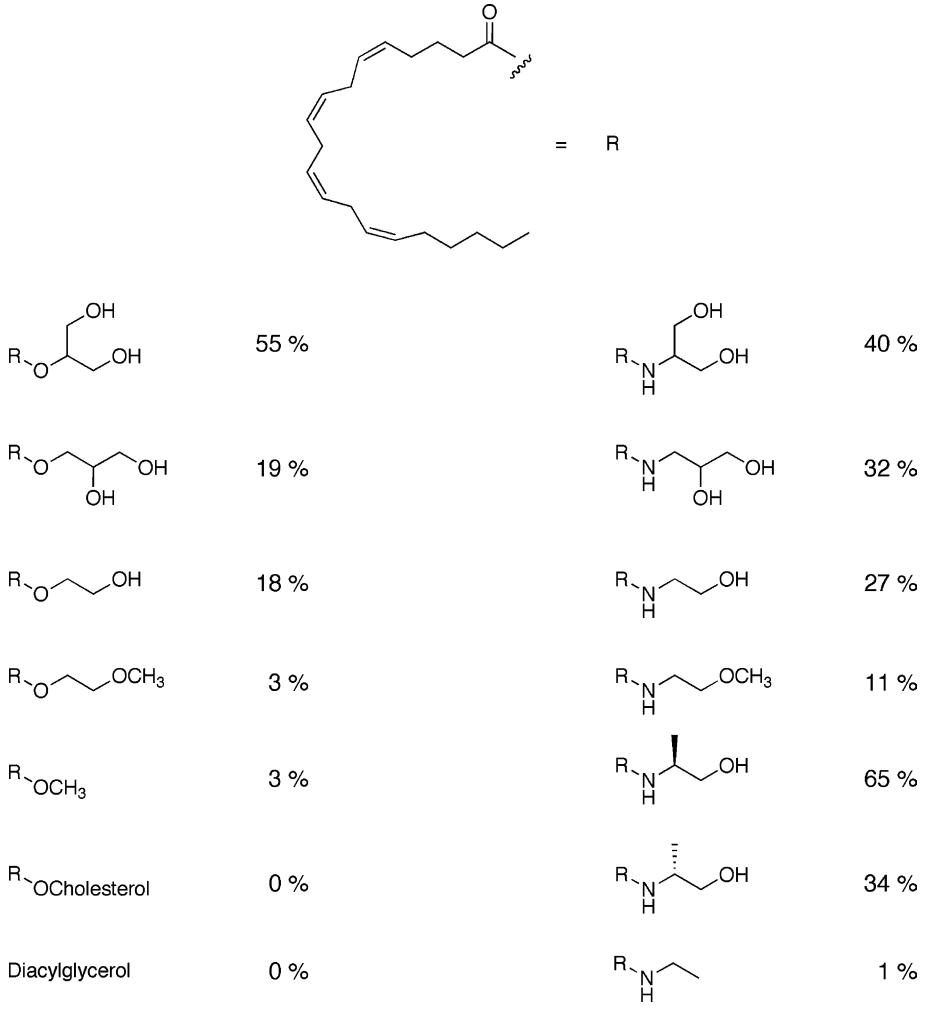

Fig. 5. Efficiency of oxygenation of a series of 2-AG and AEA analogs by COX-2. The number next to each structure represents the relative k_{cat} for oxygenation of the analog by mouse-COX-2 compared to AA. The absence of a hydroxyl group in the ester or amide side chain drastically reduces substrate oxygenation. Data from [56,57].

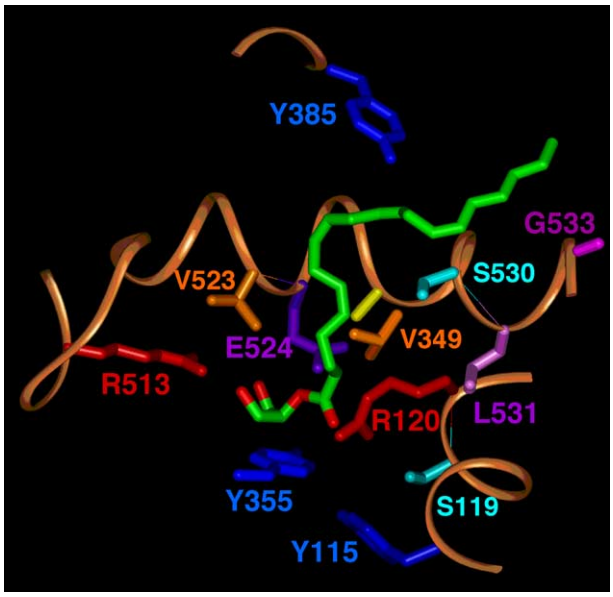

Fig. 6. Model for binding of 2-AG to COX-2. This model is based on the crystal structure of AA bound to sheep COX-1 modified to reflect the results of functional analysis of substrate oxygenation as determined by site-directed mutagenesis [56,81].

combination of substrate structure-activity and protein structure-activity enables a model to be constructed for the binding of 2-AG and AEA to COX-2 (Fig. 6) [56]. The carbonyl oxygen of the ester or amide is H-bonded to Arg-120 while the hydroxyl group in the side chain is H-bonded to Arg-513. Another feature of this model is that 2-AG and AEA appear to be displaced slightly toward the side pocket relative to arachidonate. This accommodates the finding that mutations in Leu-531, which sits opposite the side pocket, negatively affect arachidonate oxygenation but have minimal impact on 2-AG or AEA oxygenation [56].

In vitro, purified COX-2 oxygenates 2-AG to the glyceryl ester of PGG_2 (PGG_2-G) and reduces it to PGH_2-G in amounts similar to those observed in the oxygenation of AA to PGG_2 and PGH_2 [53]. In addition, COX-2 produces small amounts of 11-HETE-G and 15-HETE-G similar to those observed with AA. Thus, the overall profile of 2-AG (and presumably AEA) oxygenation by COX-2 is very similar to that observed with AA [53]. The downstream metabolites of PGH_2-G and PGH_2-EA are very much similar to those observed with PGH_2 [58]. Both endoperoxides are nearly equivalent to PGH_2 as substrates for PGE synthase, PGD synthase, PGF synthase, and PGI synthase. The sole exception is TxA synthase. Both PGH_2-G and PGH_2-EA are converted by TxA synthase to TxA_2 analogs at rates corresponding to approximately 3% that of PGH_2. This suggests that if 2-AG or AEA oxygenation occurs in intact cells, the ultimate pattern of eicosanoids synthesized will be nearly as diverse as that observed with AA, with the exception that TxA_2-G/TxB_2-G or the corresponding ethanolamide derivatives are not expected to be formed or will be formed in very low yield (see below).

Biological significance of 2-AG oxygenation

As noted above, COX-1 and COX-2 are very similar enzymes, both structurally and in terms of their reaction with AA. The most notable difference between the two isoforms is their genetic regulation. COX-1 is found in a wide range of tissues where it is usually constitutively expressed, whereas COX-2 is found in a limited number of tissues (nervous, immune, and renal) where its expression is induced in response to inflammatory and proliferative stimuli [16,17,37,59–62]. This difference provides the basis for the hypothesis that COX-1 serves predominantly homeostatic functions whereas COX-2 primarily plays a role in pathophysiological responses such as pyrexia, algesia, inflammation, and tumorigenesis [62–64]. However, the development of isoform selective inhibitors and the availability of mice bearing targeted deletions of the genes for each isoform have allowed more detailed studies that indicate this scenario is an oversimplification [65–69]. Therefore, the question of why there are two isoforms of COX remains, and the search for distinct roles for each isoform continues [70].

The oxygenation of neutral derivatives of AA by COX-2 may be a distinct physiological function of this isoform. This possibility is particularly intriguing in light of the fact that AEA and 2-AG are bioactive lipids in their own right since they are the two known endogenous ligands for the cannabinoid receptors (CB1 and CB2) [71–73]. These receptors mediate the psychotropic and immunomodulatory effects of the active component of marijuana—Δ^9-tetrahydrocannabinol [74,75]. One can readily propose three potential roles for 2-AG oxygenation. These include: (1) inactivation of 2-AG's endocannabinoid activity leading to regulation of cannabinoid tone; (2) formation of PG-Gs which may serve as precursors of PGs through hydrolysis; and (3) formation of a new class of lipid mediators if PG-Gs are found to possess biological activities distinct from those of PGs. Data are available that support all three potential roles.

PG-Gs are inactive at the cannabinoid receptor, and Alger and coworkers have shown that COX-2 inhibitors potentiate endocannabinoid-mediated depolarization-induced suppression of inhibition in the hippocampus [76]. These results suggest the possibility that COX-2 acts in this system to inactivate and thereby regulate endocannabinoid function.

PG-Gs are hydrolyzed in rat ($t_{1/2} = 14$ s) and human ($t_{1/2} > 10$ min) plasma and in human whole blood ($t_{1/2} = 7$ min), confirming the possibility that they may serve as precursors to PGs [56]. This possibility is

strengthened by the fact that metabolism of PG-Gs by 15-hydroxyprostaglandin dehydrogenase (the major PG catabolic enzyme) is slower than that of PGs [77]. Therefore, prior to hydrolysis, PG-Gs could be expected to avoid inactivation during transport to another location in the body where they could then be hydrolyzed to PGs.

PGE_2-G induces mobilization of Ca^{2+} in the mouse macrophage-like cell line, RAW264.7; the concentrations that induce Ca^{2+} release are in the pM-nM range [78]. In contrast, PGE_2 does not induce Ca^{2+} release in the same cell line at concentrations approaching 1 μM. Ca^{2+} mobilization in response to PGE_2-G is associated with a transient increase in IP_3 and is inhibited by treatment with TMB-8, an IP_3 receptor antagonist. Treatment of the cells with PGE_2-G in the absence of extracellular Ca^{2+} reduces but does not eliminate Ca^{2+} release. These observations suggest that PGE_2-G induces hydrolysis of PIP_2 to IP_3, which stimulates release of Ca^{2+} from the endoplasmic reticulum followed by storage-dependent uptake of Ca^{2+} through the plasma membrane. Consistent with this putative mechanism, PGE_2-G induces protein kinase C translocation to the membrane fraction of RAW264.7 cells and phosphorylation of model substrates. This implies release of diacylglycerol concomitant with hydrolysis of PIP_2 and generation of IP_3. PGE_2-G induces increased ERK phosphorylation (ERK-1 and -2) in RAW264.7 cells, which leads to transcriptional activation via the serum response element. It appears that PGE_2-G binds to a unique receptor on RAW cells that is linked to Ca^{2+} mobilization and downstream signaling [78]. Together the data on the effects of COX-2 inhibitors on endocannabinoid action, the rapid hydrolysis of PG-Gs to PGs, and the biological effects of PGE_2-G suggest multiple ways in which COX-2-dependent oxygenation of 2-AG may be important physiologically.

2-AG metabolism in macrophages

In order to adequately evaluate the potential biologic importance of COX-2-dependent oxygenation of 2-AG, it is necessary to determine whether PG-Gs are produced in normal cells and/or tissues in response to physiologically relevant stimuli. We have used murine resident peritoneal macrophages treated with LPS to induce COX-2 expression as a model system to study PG-G production. Although LPS treatment induces increases in intracellular AA levels and the formation of large amounts of PGs (6900 ± 1400 pmol/10^7 cells), only trace amounts of PG-Gs were detected [79,80]. This observation is consistent with the fact that no change in intracellular 2-AG levels occurs during the incubation with LPS. In contrast, exposure of LPS-pretreated macrophages to unopsonized zymosan results in marked increases in intracellular AG and concomitant PG-G formation. The time course of PG-G synthesis is similar to that of PG synthesis (Fig. 7), and the major products formed, PGI_2-G and PGE_2-G, correspond to the major PGs produced by the cells [80].

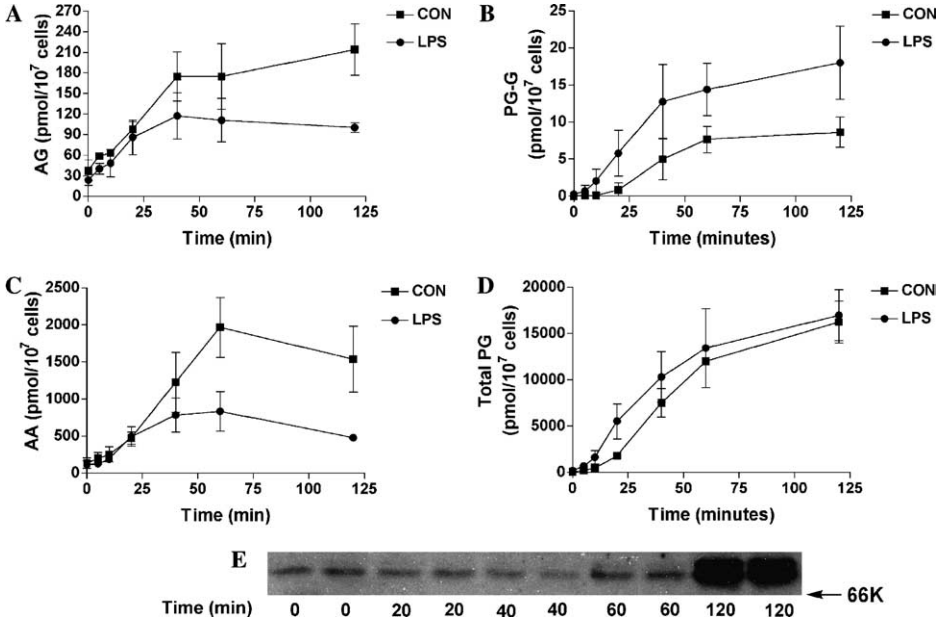

Fig. 7. Time course of the synthesis of PG-Gs and PGs in resident peritoneal macrophages in response to zymosan. Macrophages were preincubated for 6 h in the absence (control, CON) or presence (LPS) of LPS to induce COX-2. The cultures were then washed and placed in fresh serum-free medium containing zymosan. At the indicated times, the cells were harvested for the analysis of total AG (1(3)-AG and 2-AG) (A) and AA (C), and the medium was harvested for the analysis of PG-Gs (B) and PGs (D). (E) Control cell lysates were analyzed by immunoblot for the induction of COX-2 by zymosan, which occurred after the maximal rate of PG and PG-G formation. Reproduced with permission from [80].

An unexpected finding was that the quantity of PG-Gs produced by macrophages pretreated with LPS (16 ± 6 pmol/10^7 cells) was only 1.3-fold higher than the quantity produced by cells that had not been pretreated with LPS (12 ± 5 pmol/10^7 cells). Equally unexpected was the finding that LPS pretreatment elicited no increase in zymosan-dependent PG synthesis in macrophages ($21{,}000 \pm 7000$ pmol/10^7 cells versus $21{,}000 \pm 6000$ pmol/10^7 cells in LPS-pretreated versus non-pretreated macrophages, respectively), despite the marked induction of COX-2 protein resulting from the LPS exposure [80]. These results suggest the possibility that COX-1, which is strongly and constitutively expressed in the cells, could be a major contributor to both PG and PG-G formation in response to zymosan. This hypothesis is supported by studies using the COX-2 selective inhibitor, SC236, which show that 51 and 74% of the zymosan-dependent PG-G synthesis are COX-1 dependent in LPS-pretreated and non-pretreated cells, respectively. COX-1 plays a greater role in PG synthesis, producing an estimated 83% and 96% of the PGs formed by LPS-pretreated and non-pretreated macrophages [80].

Based on the in vitro data, COX-2 utilizes 2-AG with much greater efficiency than COX-1 [53]. Therefore, it is difficult to explain why as much as half of the PG-G synthesis in zymosan-challenged LPS-pretreated macrophages is COX-1-dependent. Studies in which exogenous AA is provided as substrate demonstrate that LPS pretreatment increases the PG synthetic capacity of macrophages by 1.8-fold. When exogenous 2-AG is provided, both PGs and PG-Gs are formed, and LPS pretreatment increases the synthetic capacity by 3.2- and 4.8-fold, respectively [80]. These results confirm that the LPS-induced COX-2 protein is active and are consistent with the expectation that COX-2 can utilize 2-AG for PG-G formation more efficiently than COX-1. Of note is the finding that PG synthesis from 2-AG results from hydrolysis of 2-AG followed by oxygenation of the product, AA, rather than oxygenation of the 2-AG followed by hydrolysis of the product, PG-G. Since the ratio of PGs to PG-Gs formed from 2-AG is approximately 9:1, these results suggest that in the intact cell, AA is preferred over 2-AG as substrate, even when COX-2 is present at high levels [80].

Careful analysis of the kinetics of PG and PG-G synthesis in zymosan-stimulated cells indicates that LPS-pretreated macrophages exhibit higher rates of PG and PG-G synthesis (2.9- and 5.1-fold, respectively) than non-pretreated cells. However, these elevated rates are only sustained for the first 20 min of the response, after which PG and PG-G synthesis in LPS-pretreated cells decreases to rates below those of non-pretreated cells [80]. These results suggest that LPS pretreatment increases PG and PG-G synthetic capacity as a result of COX-2 induction, but that the COX-2 is rapidly inactivated during the zymosan response. Further work is required to test this hypothesis and to determine the mechanism of putative inactivation.

The studies described above confirm that primary peritoneal macrophages produce PG-Gs in response to an inflammatory stimulus, a finding that supports a physiologically relevant role for PG-G formation. However, the relatively low levels of PG-Gs that are formed by these cells call into question their importance as compared to those of PGs. The fact that PG-G formation in macrophages is significantly lower than PG formation is likely due to multiple factors, including higher levels of AA than 2-AG, the preference of AA over 2-AG as substrate, and the relatively low contribution of COX-2 to both PG and PG-G formation. Thus, it is possible that PG-G formation may be higher in another cell type in which these conditions are different. It should be noted, however, that PGE_2-G-dependent Ca^{2+} mobilization in RAW264.7 cells occurs at picomolar concentrations of the ligand [78]. Therefore, it is possible that PG-Gs are biologically active at very low concentrations and that a low level of biosynthesis will support this activity.

Summary

COX is one of the most thoroughly studied and best understood of all of the oxygenases due to a unique confluence of circumstances. The discovery that COX is the target of the NSAIDs made it an immediate focus of intensive research that was aided by relatively easily accessible sources of purified protein, first from ram seminal vesicles (COX-1) and later from recombinant expression. The spectral properties of the enzyme facilitated complex kinetics studies that were crucial for determining the reaction mechanism, and the fact that COX is one of the few membrane-bound proteins that has successfully been crystallized allowed X-ray diffraction studies that provided detailed structural confirmation for the mechanism. Equally important since the discovery of COX-2 have been the extensive genetic studies that have defined the differences in the regulation of expression of the two isoforms. Despite all these advances, however, it is still not entirely clear why there are two isoforms of COX. Therefore, the discovery that COX-2 is capable of oxygenating neutral derivatives of AA that are only poorly oxygenated by COX-1 is of particular interest as a possible answer to this conundrum. Results to date indicate that the oxygenation of 2-AG may represent a distinct pathway for the generation of COX-2 metabolites (Fig. 8). 2-AG is believed to be produced from the hydrolysis of an AA-containing diacylglycerol, which in turn is formed from the action of either phospholipase C or the combination of phospholipase D and phosphatidic acid phosphatase on membrane phospholipid. This is in contrast to the

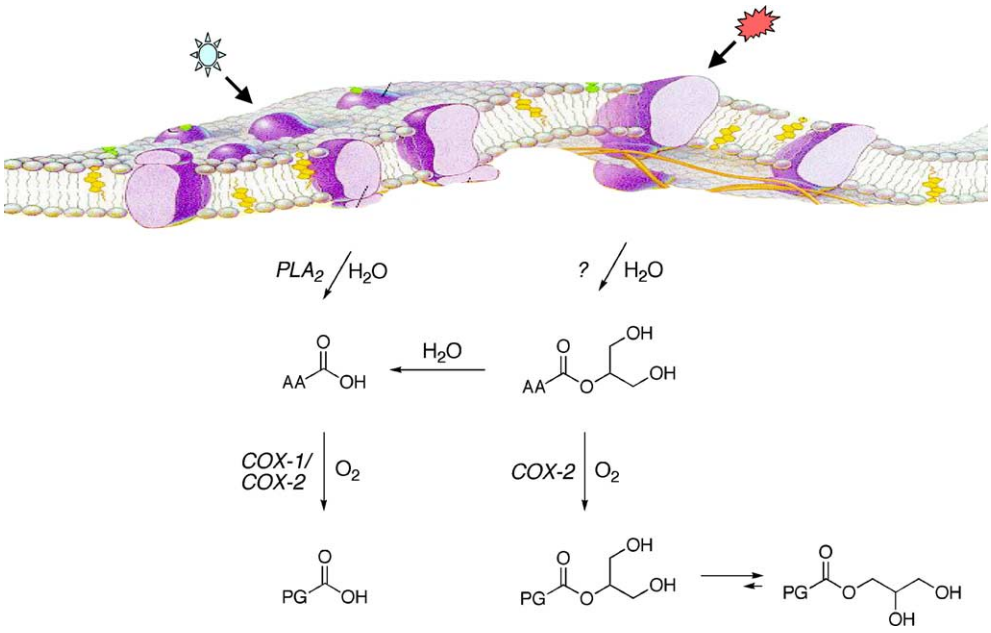

Fig. 8. Overview of AA and 2-AG metabolism by COX enzymes. Agonists that activate signaling via phospholipase A_2 (PLA_2) stimulate release of AA, which is oxygenated by COX-1 or COX-2 to PGs. Agonists that activate signaling via a yet-to-be-determined phospholipase simulate release of 2-AG, which is oxygenated mainly by COX-2 to PG-Gs or hydrolyzed to AA. PG-Gs exist as an equilibrium mixture of the initially formed 2-AG and the thermodynamically more stable 1-AG isomer. The equilibrium population is ~10% 2-AG and 90% 1-AG.

production of AA, which primarily comes from the direct action of cytosolic phospholipase A_2. AA is equally subjected to oxygenation by both COX-1 and COX-2, whereas 2-AG is primarily a COX-2 substrate. The PGH_2 produced from the direct oxygenation of AA is further converted to PG metabolites that carry out their physiologic functions through interaction with specific G-protein-coupled receptor(s). Available data suggest that PGH_2-G is subjected to the same pattern of metabolism as PGH_2, with the exception that it is not converted readily to TXA_2-G. The resulting PG glyceryl esters (PG-Gs) may exert distinct physiologic functions through their own specific receptors. Note that there are two mechanisms by which 2-AG can give rise to free acid PGs. One is through the hydrolysis of 2-AG to AA followed by oxygenation, a process that has been demonstrated to occur in peritoneal macrophages. The second is through the oxygenation to PG-Gs followed by hydrolysis, a process that has been shown to occur in both rodent and human blood. Thus, although PG and PG-G formation occurs via distinct pathways, those pathways may be interconnected in vivo.

The selective oxygenation of neutral derivatives of AA by COX-2 is the only known functional difference between the two COX isoforms. It remains to be determined whether this unique feature is the foundation for a distinct physiologic role for COX-2. However, the findings that subnanomolar concentrations of PGE_2-G invoke Ca^{2+} mobilization in RAW264.7 cells and that PG-Gs are formed by primary cells in response to physiologic stimuli strongly suggest that PG-G formation may be of biologic importance. Clearly there remain many avenues of interesting research in this exciting new field of lipid mediator biochemistry.

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