Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochemical and Biophysical Research Communications 338 (2005) 25–33
BBRC
www.elsevier.com/locate/ybbrc

Review

Regulation of cyclooxygenase catalysis by hydroperoxides ☆

Richard J. Kulmacz *

Department of Internal Medicine, University of Texas Health Science Center at Houston, Houston, TX 77030, USA

Received 24 July 2005
Available online 15 August 2005

Abstract

Activation of cyclooxygenase catalysis in prostaglandin H synthase-1 and -2 by peroxide-dependent formation of a tyrosyl radical is emerging as an important part of regulating cellular production of bioactive prostanoids. This review discusses the mechanism of tyrosyl radical formation and the influence of peroxide, fatty acid, peroxidase cosubstrate, and protein structure on the activation process and cyclooxygenase catalysis.

Keywords: Prostaglandin H synthase; Cyclooxygenase activation; Peroxide; Tyrosyl radical

Early investigations

Prostaglandin H synthase (PGHS) catalyzes the first irreversible step in the synthesis of prostanoids, a family of potent bioactive lipids that includes prostaglandins D_2, E_2, and F_{2a}, as well as prostacyclin and thromboxane [1]. Purification of one isoform, PGHS-1, revealed that the protein has two catalytic activities, a cyclooxygenase and a peroxidase, both dependent on heme [2–4]. The cyclooxygenase activity is a dioxygenase that converts arachidonate to PGG_2; the peroxidase activity reduces PGG_2 to the corresponding alcohol, PGH_2, at the expense of two electrons derived from reducing cosubstrate. The functional connection between hydroperoxides and cyclooxygenase catalysis was revealed by the observation that a hydroperoxide scavenger, glutathione peroxidase-1 (GPx-1), suppresses cyclooxygenase activity [5]. Several hydroperoxides, and not the corresponding alcohols, were found to be capable of activating the cyclooxygenase, indicating that the activation is tied to the chemistry of the hydroperoxide group [6,7]. Because the cyclooxygenase product, PGG_2, is itself a hydroperoxide and thus susceptible to decomposition by GPx-1, the major cytosolic peroxidase in many cells, this discovery led to the proposal that the cyclooxygenase activation by peroxide and peroxide reduction by cellular peroxidases are key elements in the cellular regulation of prostanoid biosynthesis [8]. This review will focus primarily on biochemical studies of cyclooxygenase activation in purified PGHS-1 and -2, as purified preparations avoid many complications associated with secondary reactions.

Elucidation of the cyclooxygenase activation mechanism

Conversion of arachidonate to PGG_2 during cyclooxygenase catalysis was proposed many years ago to begin with by abstraction of a hydrogen atom from C13 of the fatty acid [9], as shown in Fig. 1. This hydrogen abstraction clearly requires a strong enzyme oxidant. Peroxide-dependent generation of the enzyme oxidant could explain activation of the cyclooxygenase by peroxide, but it was not known just what the oxidant was or

☆ *Abbreviations:* PG, prostaglandin; PGHS, prostaglandin H synthase; GPx, glutathione peroxidase; TNM, tetranitromethane; EPR, electron paramagnetic resonance spectroscopy; 15-HPETE, 15-hydroperoxyeicosatetraenoic acid; LDS, linoleate diol synthase; PADOX, plant fatty acid alpha dioxygenase.

* Fax: +1 713 500 6810.
E-mail address: richard.j.kulmacz@uth.tmc.edu.

0006-291X/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.bbrc.2005.08.030

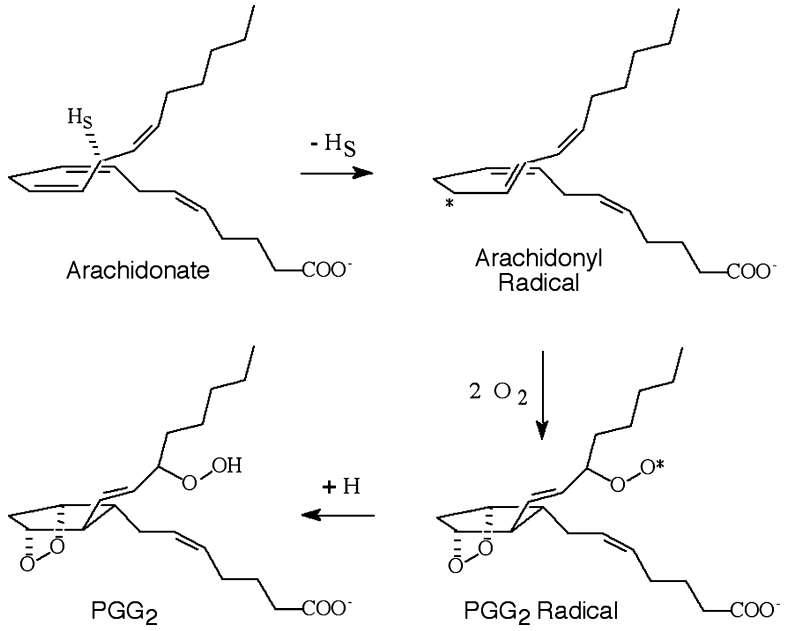

Fig. 1. Hypothetical steps in conversion of arachidonate to prostaglandin G_2 (PGG_2) during cyclooxygenase catalysis, beginning with stereospecific hydrogen abstraction from C-13; adapted from Hamberg and Samuelsson [9].

how peroxide might lead to its formation. Reaction of PGHS-1 with peroxides was found to produce highly oxidized intermediates similar to those observed with other heme-dependent peroxidases [10]. The involvement of peroxidase cycle intermediates in cyclooxygenase activation was suggested by similarities in the kinetic characteristics of the cyclooxygenase activation process and PGHS-1 peroxidase catalysis [11].

A major breakthrough in identifying the enzyme oxidant responsible for cyclooxygenase catalysis came with the realization that a protein-based radical, proposed to be a tyrosyl radical, is formed in PGHS-1 during reaction with hydroperoxide [12,13]. Later isotope replacement studies confirmed that the peroxide-induced radical in PGHS-1 is indeed a tyrosyl radical [14]. Ruf and co-workers [12] also proposed that this tyrosyl radical is generated by intramolecular electron transfer in PGHS peroxidase intermediate I (analogous to Compound I in other heme-dependent peroxidases), and that the tyrosyl radical is the enzyme oxidant that initiates cyclooxygenase catalysis (Fig. 2). This reaction mechanism functionally links cyclooxygenase and peroxidase catalysis and provides a satisfying explanation for the activation of the cyclooxygenase by peroxide. An alternative proposal, that tyrosyl radical generation is linked to the ferric/ferrous heme transition [15], appears unlikely because the ferric hemes in PGHS-1 and -2 are not strong enough oxidants to form a tyrosyl radical [16,17].

Involvement of a tyrosine radical in cyclooxygenase catalysis was supported by the results of experiments with tetranitromethane (TNM), a reagent that nitrates tyrosine sidechains. In PGHS-1, tyrosine residue nitration and cyclooxygenase inhibition by TNM occur only in the absence of cyclooxygenase inhibitors, and TNM treatment alters the peroxide-induced EPR signal [18]. TNM also inhibits the cyclooxygenase activity and alters the peroxide-induced free radical EPR signal in PGHS-1 substituted with mangano protoporphyrin IX and in PGHS-2 [19,20]. Direct sequencing showed that nitration of Tyr385 in PGHS-1 by TNM is blocked by cyclooxygenase inhibitors and mutagenesis of this residue results in selective inhibition of the cyclooxygenase activity, identifying Tyr385 as the likely site of the tyrosyl radical linked to cyclooxygenase activity [21]. Tyr385 of PGHS-1 can also be selectively nitrated by nitric oxide during reaction with arachidonate, further confirming this residue's participation in cyclooxygenase catalysis [22]. Both the accumulated biochemical evidence identifying Tyr385 as the site of the catalytically important tyrosyl radical and the Ruf mechanism are quite consistent with structural data from crystallographic studies in both isoforms [23–25], data that place Tyr385 between the heme and the fatty acid binding site, as shown in Fig. 3.

The general features of the tyrosyl radical mechanism in Fig. 2, sometimes termed the "branched chain" radical mechanism because it allows PGG_2 to accumulate and increase the number of active cyclooxygenase reaction centers with time, have come to be generally accepted. An alternative, "tightly coupled" mechanism requiring PGG_2-consuming reformation of the tyrosyl radical after each cycle of cyclooxygenase catalysis [26] is less consistent than the branched chain mechanism with key experimental observations [17,27,28]. Nevertheless, there have been refinements and elaborations

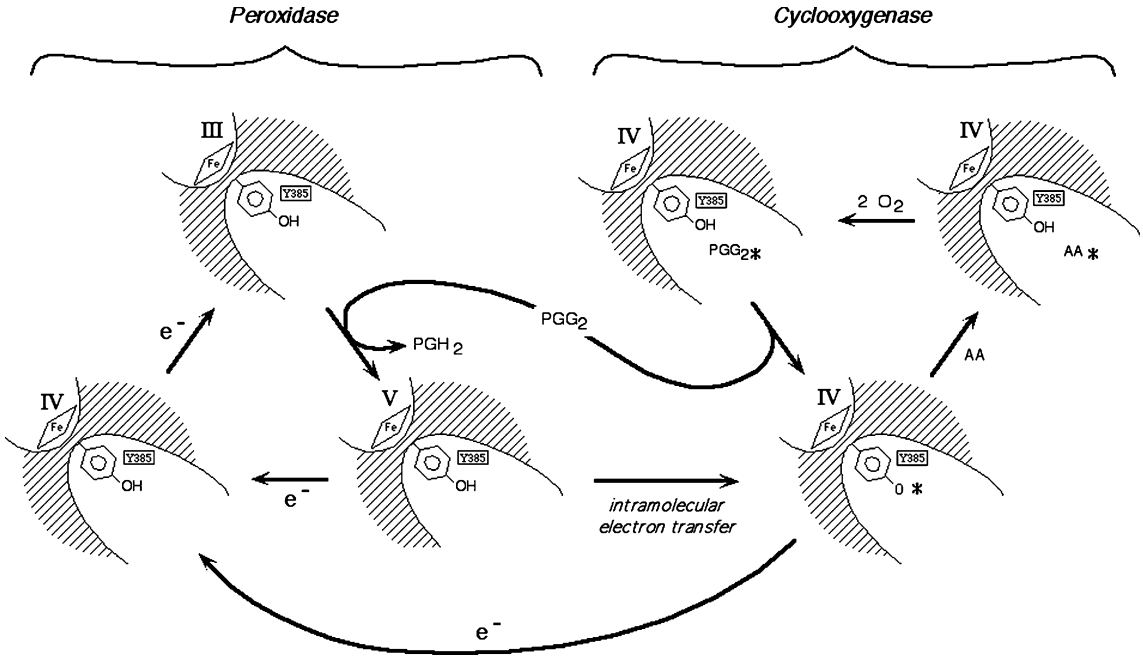

Fig. 2. Integrated mechanism for PGHS catalysis, adapted from Dietz et al. [13], to depict events in the peroxidase site (identified by the heme icon) and in the cyclooxygenase site (identified by the Tyr385 sidechain). III, IV, and V represent the formal oxidation state of the heme; AA and AA*, arachidonic acid and the corresponding radical; PGG_2 and PGG_2*, prostaglandin G_2 and the corresponding radical; e^-, reducing equivalent from a peroxidase cosubstrate; and the Tyr385 radical is indicated by an "–O*" on the tyrosine sidechain.

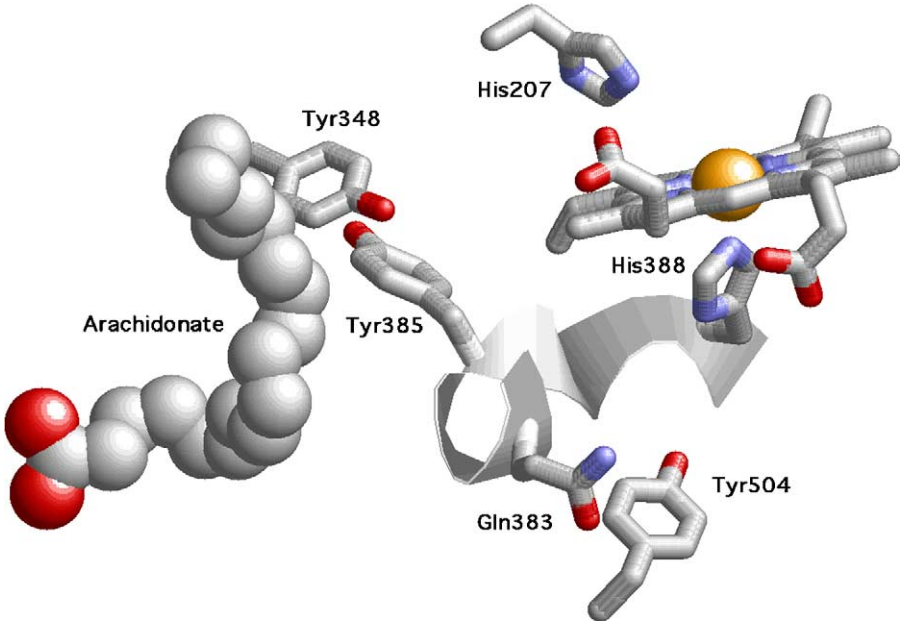

Fig. 3. PGHS-1 structure showing the positions of arachidonate and key amino acid sidechains; coordinates from PDB 1DIY [96].

to the branched chain mechanism since its introduction in 1988. As is evident from Fig. 2, the original mechanism predicts that PGHS occupied with cyclooxygenase catalysis has heme fixed in the ferryl state and is thus not capable of peroxidase catalysis. However, peroxidase catalytic rates are not changed by ongoing cyclooxygenase catalysis [29], indicating that enzyme intermediates in the cyclooxygenase catalytic cycle are capable of simultaneously going through the peroxidase catalytic cycle. This behavior can be accomodated by postulating additional enzyme intermediates [30]. Branches to the mechanism in Fig. 2 have been added to account for cyclooxygenase and peroxidase self-inactivation during catalysis [31–33]. Overall, the Ruf mechanism continues

to be very useful as the starting point for detailed mechanistic studies of PGHS-1 and -2.

Peroxide structure and cyclooxygenase activation

The branched chain mechanism (Fig. 2) predicts that any peroxide that is a substrate for the PGHS peroxidase will be an activator of the cyclooxygenase, and experimental observations bear this out. The relative potencies of peroxides as PGHS-1 peroxidase substrates parallel their potencies as cyclooxygenase activators, with a preference for aliphatic hydroperoxides, such as PGG_2 and 15-hydroperoxyeicosatetraenoic acid (15-HPETE), and a bias against sterically hindered peroxides, such as *t*-butyl hydroperoxide, and against smaller, water-soluble peroxides, such as ethyl hydroperoxide and hydrogen peroxide [18,34,35]. Activation potencies of hydroperoxides (including those that are not cyclooxygenase products) can be evaluated from their effects on the acceleration of the cyclooxygenase reaction in a system where normal activation is impaired [35]. Among positional isomers of HPETE, PGHS-1 cyclooxygenase activation efficiency is greatest for the 15-hydroperoxy isomer and decreases progressively as the hydroperoxy group moves towards the carboxyl group [36]; this follows the efficiency of formation of the tyrosyl radical EPR signal by the HPETE isomers [18]. Hydroperoxides of fatty acids esterified in phosphatidylcholine, trilinolein, and cholesterol linoleate are ineffective as PGHS-1 cyclooxygenase activators unless the fatty acyl groups are hydrolyzed [35].

Several other oxidants have been examined as cyclooxygenase activators. Superoxide can be ruled out as a direct cyclooxygenase activator because superoxide dismutase does not inhibit PGHS-1 cyclooxygenase activity [37]. Results from many laboratories indicate that nitric oxide is not capable of activating PGHS-1 or -2 cyclooxygenase via action at the peroxidase heme [38]. However, peroxynitrite, an inorganic peroxide that results from reaction of superoxide and nitric oxide, is a peroxidase substrate and a cyclooxygenase activator for both PGHS-1 and -2 [39]. Peroxynitrite ranks intermediate between hydrogen peroxide and 15-HPETE as a PGHS peroxidase substrate [39], suggesting it has moderate potency as a cyclooxygenase activator, but its rapid decomposition made characterization difficult. Iodosobenzene forms peroxidase spectral intermediates in PGHS-1 similar to those formed by PGG_2 [40] and so presumably activates the cyclooxygenase. Mannitol does not inhibit PGHS-1 cyclooxygenase activity with arachidonate (detected by oxidized heme intermediates or EPR) [41], suggesting that the hydroxyl radical is not important in cyclooxygenase activation.

Impaired peroxidase activity and cyclooxygenase activation

Modifications of the PGHS prosthetic group or amino acid residues that decrease the rate of reaction with peroxide impair cyclooxygenase activation, as expected from the branched chain mechanism (Fig. 2). Replacement of the heme with mangano protoporphyrin IX results in a markedly slower reaction with hydroperoxide to form the oxidized mangano intermediates and tyrosyl radical, and much less efficient activation of cyclooxygenase activity [19,42–44]. In PGHS-2, substitution of alanine for His207, the distal heme ligand, results in a dramatically slower acceleration of the cyclooxygenase; the long lags in the cyclooxygenase reaction can be shortened by addition of hydroperoxide or methyl imidazole, the latter presumably acting as a surrogate distal ligand [45]. Interestingly, the H207A mutant converts 15-HPETE preferentially to a 15-keto derivative instead of the 15-hydroxy derivative, suggesting increased one-electron reduction of this peroxide, but the 15-keto analogs of PGD_2 and PGE_2 are not detected during reaction of the mutant with arachidonate, indicating that PGG_2 itself is reduced by the conventional two-electron process [45]. Mutation of the PGHS-2 proximal heme ligand, His388, to a tyrosine also greatly reduces peroxidase catalysis [17]. This H388Y mutant retains cyclooxygenase activity, though the reaction kinetics have very long lag times reflecting slower activation; the lag times can be shortened by addition of peroxide. His386 is in the cyclooxygenase pocket and does not have direct interactions with the heme. Nonetheless, the PGHS-1 H386A mutant has no detectable peroxidase activity, accompanied by prolonged cyclooxygenase lag times indicative of impaired cyclooxygenase activation [46,47]. The observations that H386A has impaired heme binding and that binding of cyclooxygenase inhibitors restores much of the heme binding and peroxidase activity indicate that the H386A mutation has long range structural effects [47].

Different cyclooxygenase activation efficiencies in PGHS-1 and -2

Less GPx-1 is required to suppress cyclooxygenase activity in PGHS-1 than in PGHS-2 [48,49]. Quantitative analysis shows half-maximal cyclooxygenase activation at 2 nM PGG_2 in PGHS-2 and 20 nM PGG_2 in PGHS-1 [49]. This higher efficiency of cyclooxygenase activity in PGHS-2 provides a biochemical basis for the differential regulation of prostanoid synthesis by the two isoforms observed in vivo, where PGHS-1 and -2 are ascribed distinct pathophysiological roles [50–52]. Cyclooxygenase activation is also considerably more efficient in brook trout PGHS-2 than in PGHS-1 from the same species

(W. Liu and R. Kulmacz, unpublished observation). The sequences of the two trout proteins diverge considerably from their mammalian counterparts [53], but parallels in tissue distribution and cytokine inducibility [50,53–55] suggest that the functional specialization of the two PGHS isoforms in fish is homologous to that in the mammalian isoform pair. Thus, differential activation of PGHS-1 and -2 cyclooxygenase by peroxide may have evolved as a general regulatory feature in vertebrates.

PGHS-1 and -2 peroxidases have very similar rate constants for their initial reaction with lipid hydroperoxide, but the rate of intramolecular electron transfer to generate the Tyr385 radical, and the stability of the radical, are higher in PGHS-2 [56], providing a kinetic basis for the more efficient cyclooxygenase activation in PGHS-2. Mutations in Thr383 of human PGHS-2, a residue completely conserved in PGHS-2 from various species but appearing as histidine or glutamine in PGHS-1, result in increased cyclooxygenase sensitivity to suppression by GPx-1, ranking between wild type PGHS-2 and PGHS-1 [57]. Crucially, these Thr383 mutants have cyclooxygenase and peroxidase specific activities little different or even increased from those of the wild type, so the activation defect is very likely localized to the formation or stability of the Tyr385 radical. Thr383 is thus one of the structural determinants of the higher cyclooxygenase activation efficiency in PGHS-2.

GPx-1 is the GPx isozyme that is the predominant peroxide scavenger in most cells and has optimal activity against PGG_2 and the unesterified fatty acid hydroperoxides that are the most effective cyclooxygenase activators. Another GPx isozyme, GPx-4, has significant activity against fatty acid hydroperoxides esterified in phospholipids, and there is one report [58] that the relative sensitivities of PGHS-1 and -2 cyclooxygenases to inhibition by GPx-4 are the opposite of those seen with GPx-1 [48,49]. Phospholipid is not added to any of these in vitro assays, so the ability of GPx-4 to reduce phospholipid hydroperoxides should not be a factor. The inhibitory actions of GPx-1 are consistent with simple reduction of hydroperoxide rather than direct action on PGHS-1 or -2 because no inhibition occurs in the absence of glutathione and because GPx-1 can be replaced by an equivalent amount of PGHS-1 peroxidase (PGHS-1 treated with aspirin to selectively destroy cyclooxygenase activity) [48,49,59]. The oligomeric states of GPx-1 and -4 are quite different [60], and the possibility that GPx-4 has some direct interaction with PGHS-1 and/or -2 that alters the efficiency of PGG_2 interception has not been ruled out.

Tyrosyl radical dynamics

The tyrosyl radical is obviously at the heart of cyclooxygenase activation by peroxide. There has been extensive characterization of the tyrosyl radicals in both PGHS-1 and -2 [61]. A major challenge in these studies has been the fact that the tyrosyl radicals in the PGHS isoforms are transient, with several distinct types of tyrosyl radical EPR signals observed in each isoform. Most of these distinct EPR spectra can in theory arise from different electronic distribution or sidechain conformation of the Tyr385 radical [62] or from a tyrosyl radical on a residue other than Tyr385; each isoform has over two dozen tyrosine residues.

Some of this ambiguity has been removed in the case of PGHS-2, where mutagenic studies showed that the transition from the initially wide doublet tyrosyl radical to the later wide singlet EPR signal is due to migration of the unpaired electron from Tyr385 to Tyr504 in a fraction of the protein [63]. The narrow singlet signal from the Tyr504 radical adds to the wide doublet signal from the remaining Tyr385 radical to produce the observed wide singlet spectrum. Tyr504 lies on the distal side of the heme, some distance from the bound arachidonate (Fig. 3). The Tyr504 radical is thus incapable of oxidizing the fatty acid directly and is instead thought to act as a reservoir of oxidant that can be equilibrated with the Tyr385 radical [63]. It remains to be determined whether the EPR spectrum transition from wide doublet to wide singlet observed in PGHS-1 is due to conformational changes at Tyr385 or migration of the radical to another residue.

Substitution of a phenylalanine for Tyr504 in PGHS-2 restricts the peroxide-induced radical to Tyr385 without major changes in peroxidase or cyclooxygenase activities [63], making the Y504F mutant a useful starting point for detailed structure-function studies of the Tyr385 radical. One interesting aspect is the hydrogen bonding between Tyr385 and Tyr348, which is evident from crystal structures of both PGHS isoforms (Fig. 3) [23,24], and from EPR and ENDOR spectroscopy [64,65]. This hydrogen bonding is not absolutely required for cyclooxygenase catalysis because some mutations of Tyr348 in PGHS-1 and -2 retain activity [66–69]. However, results from a Y348F/Y504F double mutant suggest that hydrogen bonding with Tyr348 does considerably modulate the conformational flexibility and reactivity of the Tyr385 radical (C.E. Rogge, B. Ho, W. Liu, R.J. Kulmacz, A.-L. Tsai, unpublished observations).

Fatty acid structure and cyclooxygenase activation

Cyclooxygenase catalysis itself is an indispensable part of efficient feedback activation (Fig. 2), and impaired activation can be seen if the cyclooxygenase catalytic rate is slowed by decreased availability of arachidonate or by substitution of a fatty acid that is turned over more slowly. For example, the contribution of cyclooxygenase catalysis to the feedback activation loop can account for the cooperative response of PGHS-1 at low levels of arachidonate [44,70]. Another example is the reaction

of PGHS-1 with 20:5*n*-3, where the cyclooxygenase does not become fully activated [71]. Here, the cyclooxygenase product, PGG_3, is equivalent to PGG_2 as a peroxidase substrate but the rate of oxygenation of 20:5n-3 is too slow to sustain the feedback activation loop. The effect of a slow cyclooxygenase catalytic rate on feedback activation is also apparent in the ability of hydrogen peroxide to stimulate the reaction of PGHS-1 with several fatty acids other than arachidonate [61]. These sort of effects are not so apparent in PGHS-2 because of the more efficient formation of tyrosyl radical, but can be induced when the PGHS-2 peroxidase is impaired by reconstitution with mangano protoporphyrin IX instead of heme [44].

Peroxidase cosubstrates and cyclooxygenase activation

A variety of reducing compounds can serve as peroxidase cosubstrates in PGHS-1 and -2, promoting conversion of peroxide activators to the corresponding alcohols [72–74]. Reducing compounds also attenuate the accumulation of tyrosyl radical and accelerate its dissipation [12,75,76]. Thus, it is not surprising that many reducing compounds inhibit cyclooxygenase catalysis. What is surprising, though, is that many of the same reductants that inhibit cyclooxygenase at higher levels stimulate the cyclooxygenase at lower levels. This stimulatory effect is largely due to attenuation of several self-inactivating processes, leading to an increase in the number of cyclooxygenase catalytic turnovers rather than an increased rate of cyclooxygenase catalysis [77].

Cell cytosol is an abundant source of cosubstrates for PGHS peroxidase, and uric acid has been identified as one such cosubstrate [78]; in contrast, the prominent intracellular reductant, glutathione, is a poor peroxidase cosubstrate for both isoforms [34,73,74]. Purified PGHS preparations themselves contain endogenous reductants that cannot be removed by treatment with mild oxidant (ferricyanide) and which amount to some 10 equivalents per monomer [28]; these endogenous reductants are probably responsible for the spontaneous decay of the tyrosyl radicals [12,18,79] but have yet to be identified or detected directly.

PGHS-1 cyclooxygenase catalysis becomes remarkably more resistant to inhibition by GPx-1 if reducing cosubstrates are not added [80]. This resistance is probably due to elevation in the fraction of enzyme with tyrosyl radical and is unlikely to be observed in vivo because reducing cosubstrates that promote cyclooxygenase suppression by GPx-1 are abundant in cytosol [81].

Peroxides and cyclooxygenase inhibitor actions

The effectiveness of some PGHS-1 cyclooxygenase inhibitors, including acetaminophen, has long been recognized to be sensitive to the level of hydroperoxide activator [82]; this has more recently been confirmed for both PGHS isoforms [74,83,84]. The effect of these inhibitors on the cyclooxygenase can be understood in terms of radical quenching agents that return the tyrosyl radical to ground state and require the enzyme to react with additional peroxide to regenerate the tyrosyl radical [85]. Peroxides antagonize cyclooxygenase inhibition by these agents, which may account for their ineffectiveness against inflammatory conditions where oxidant levels tend to be elevated [83,86]. Acetaminophen is a much weaker inhibitor of cyclooxygenase in PGHS-2 than in PGHS-1 [74], consistent with the lower peroxide activator requirement in PGHS-2 [48,49]. Interestingly, cyclooxygenase inhibition by acetaminophen or salicylate can be antagonized not just by peroxide but also by increases in arachidonate levels [83,84]; a similar synergistic interaction between arachidonate and peroxide is seen in the cooperative substrate response of PGHS-1 [44]. Acetaminophen has been suggested to be a specific inhibitor of a splice variant of PGHS-1, termed "cox-3" [87]. However, the mRNA splicing in many species, including humans, involves frameshifting so that the cox-3 protein lacks essential cyclooxygenase and peroxidase catalytic components and thus cannot contribute to prostanoid production or explain the tissue specificity of acetaminophen pharmacology [88].

Peroxide activation of other fatty acid dioxygenases in the myeloperoxidase family

PGH synthases comprise a subfamily of myeloperoxidase-related proteins [89]. Two other myeloperoxidase family hemoproteins, fungal linoleate diol synthase (LDS) [90] and plant alpha dioxygenase (PADOX) [91], are also known to have fatty acid dioxygenase activity. LDS shows many characteristics similar to those of PGHS-1 and -2, including conservation of the tyrosine residue corresponding to Tyr385 [90] and oxygenase kinetics with a lag time that is decreased by added peroxide and increased by GPx-1 [92]. Further, aerobic reaction of LDS with fatty acid or hydroperoxide produces absorbance spectrum changes consistent with oxidized peroxidase intermediates and a wide doublet radical EPR signal near $g = 2$, similar to the tyrosyl radicals of ribonucleotide reductase R2 subunit and PGHS [92]. The LDS oxygenase activity thus appears to be activated by peroxidase-dependent formation of a tyrosyl radical, just as in PGHS. In PADOX, the tyrosine corresponding to Tyr385 in PGHS is conserved and required for oxygenase activity [93–95]. Addition of hydroperoxide shortens the lag in PADOX oxygenase kinetics [94] and GPx-1 is a potent inhibitor of the oxygenase (W. Liu and R. Kulmacz, unpublished observation), consistent with feedback activation by the

hydroperoxy fatty acid product. However, PADOX has no detectable steady-state peroxidase activity [93–95], and there is very little change in the heme absorption spectrum or the EPR spectrum during reaction of PADOX with fatty acid or hydroperoxide [95]. The heme in PADOX is not readily bound by small ligands such as cyanide, indicating that access to the distal heme pocket is restricted and explaining the lack of detectable peroxidase activity [95]. The mechanism by which peroxide activates PADOX oxygenase thus remains an interesting puzzle. Further analysis of the similarities and differences among the myeloperoxidase family members should provide valuable insights into how the heme peroxidase mechanism can be harnessed to accomplish fatty acid oxygenase catalysis.

Future directions

Investigations by many researchers over the last 35 years have established the basic mechanism by which peroxides generate the Tyr385 radical and thus activate cyclooxygenase activity in PGHS-1 and -2. However, our understanding of how this powerful oxidant is managed and utilized by the protein environments in the two isoforms is still at a rudimentary stage. Many interesting puzzles remain to be unravelled, including how endogenous and exogenous cosubstrates interact with the Tyr385 radical and what functional advantage may be gained by radical migration to Tyr504.

Acknowledgments

Thanks are due to Dr. Ah-Lim Tsai and Dr. Corina E. Rogge for a critical reading of the manuscript. Research in the author's laboratory is supported by National Institutes of Health Grant GM 52170.

References

[1] B. Samuelsson, M. Goldyne, E. Granstrom, M. Hamberg, S. Hammarstrom, C. Malmsten, Prostaglandins and thromboxanes, Annu. Rev. Biochem. 47 (1978) 997–1029.
[2] T. Miyamoto, N. Ogino, S. Yamamoto, O. Hayaishi, Purification of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes, J. Biol. Chem. 251 (1976) 2629–2636.
[3] M. Hemler, W.E.M. Lands, Purification of the cyclooxygenase that forms prostaglandins. Demonstration of two forms of iron in the holoenzyme, J. Biol. Chem. 251 (1976) 5575–5579.
[4] F.J. Van der Ouderaa, M. Buytenhek, D.H. Nugteren, D.A. Van Dorp, Purification and characterisation of prostaglandin endoperoxide synthetase from sheep vesicular glands, Biochim. Biophys. Acta 487 (1977) 315–331.
[5] W.L. Smith, W.E.M. Lands, Oxygenation of polyunsaturated fatty acids during prostaglandin biosynthesis by sheep vesicular gland, Biochemistry 11 (1972) 3276–3285.
[6] M.E. Hemler, G. Graff, W.E.M. Lands, Accelerative autoactivation of prostaglandin biosynthesis by PGG2, Biochem. Biophys. Res. Commun. 85 (1978) 1325–1331.
[7] M.E. Hemler, H.W. Cook, W.E.M. Lands, Prostaglandin biosynthesis can be triggered by lipid peroxides, Arch. Biochem. Biophys. 193 (1979) 340–345.
[8] H.W. Cook, W.E.M. Lands, Mechanism for suppression of cellular biosynthesis of prostaglandins, Nature 260 (1976) 630–632.
[9] M. Hamberg, B. Samuelsson, On the mechanism of the biosynthesis of prostaglandins E-1 and F-1-alpha, J. Biol. Chem. 242 (1967) 5336–5343.
[10] A.M. Lambeir, C.M. Markey, H.B. Dunford, L.J. Marnett, Spectral properties of the higher oxidation states of prostaglandin H synthase, J. Biol. Chem. 260 (1985) 14894–14896.
[11] R.J. Kulmacz, W.E.M. Lands, Quantitative similarities in the several actions of cyanide on prostaglandin H synthase, Prostaglandins 29 (1985) 175–190.
[12] R. Karthein, R. Dietz, W. Nastainczyk, H.H. Ruf, Higher oxidation states of prostaglandin H synthase, EPR study of a transient tyrosyl radical in the enzyme during the peroxidase reaction, Eur. J. Biochem. 171 (1988) 313–320.
[13] R. Dietz, W. Nastainczyk, H.H. Ruf, Higher oxidation states of prostaglandin H synthase. Rapid electronic spectroscopy detected two spectral intermediates during the peroxidase reaction with prostaglandin G2, Eur. J. Biochem. 171 (1988) 321–328.
[14] A.L. Tsai, L.C. Hsi, R.J. Kulmacz, G. Palmer, W.L. Smith, Characterization of the tyrosyl radicals in ovine prostaglandin H synthase-1 by isotope replacement and site-directed mutagenesis, J. Biol. Chem. 269 (1994) 5085–5091.
[15] M.S. Tang, R.A. Copeland, T.M. Penning, Detection of an Fe2+-protoporphyrin-IX intermediate during aspirin-treated prostaglandin H2 synthase II catalysis of arachidonic acid to 15-HETE, Biochemistry 36 (1997) 7527–7534.
[16] A.L. Tsai, R.J. Kulmacz, J.S. Wang, Y. Wang, H.E. Van Wart, G. Palmer, Heme coordination of prostaglandin H synthase, J. Biol. Chem. 268 (1993) 8554–8563.
[17] D.C. Goodwin, S.W. Rowlinson, L.J. Marnett, Substitution of tyrosine for the proximal histidine ligand to the heme of prostaglandin endoperoxide synthase 2: implications for the mechanism of cyclooxygenase activation and catalysis, Biochemistry 39 (2000) 5422–5432.
[18] R.J. Kulmacz, Y. Ren, A.L. Tsai, G. Palmer, Prostaglandin H synthase: spectroscopic studies of the interaction with hydroperoxides and with indomethacin, Biochemistry 29 (1990) 8760–8771.
[19] R.J. Kulmacz, G. Palmer, C. Wei, A.L. Tsai, Reaction and free radical kinetics of prostaglandin H synthase with manganese protoporphyrin IX as the prosthetic group, Biochemistry 33 (1994) 5428–5439.
[20] G. Xiao, A.L. Tsai, G. Palmer, W.C. Boyar, P.J. Marshall, R.J. Kulmacz, Analysis of hydroperoxide-induced tyrosyl radicals and lipoxygenase activity in aspirin-treated human prostaglandin H synthase-2, Biochemistry 36 (1997) 1836–1845.
[21] T. Shimokawa, R.J. Kulmacz, D.L. DeWitt, W.L. Smith, Tyrosine 385 of prostaglandin endoperoxide synthase is required for cyclooxygenase catalysis, J. Biol. Chem. 265 (1990) 20073–20076.
[22] D.C. Goodwin, M.R. Gunther, L.C. Hsi, B.C. Crews, T.E. Eling, R.P. Mason, L.J. Marnett, Nitric oxide trapping of tyrosyl radicals generated during prostaglandin endoperoxide synthase turnover. Detection of the radical derivative of tyrosine 385, J. Biol. Chem. 273 (1998) 8903–8909.
[23] D. Picot, P.J. Loll, R.M. Garavito, The X-ray crystal structure of the membrane protein prostaglandin H2 synthase-1, Nature 367 (1994) 243–249.
[24] R.G. Kurumbail, A.M. Stevens, J.K. Gierse, J.J. McDonald, R.A. Stegeman, J.Y. Pak, D. Gildehaus, J.M. Miyashiro, T.D. Penning, K. Seibert, P.C. Isakson, W.C. Stallings, Structural basis for

selective inhibition of cyclooxygenase-2 by anti-inflammatory agents, Nature 384 (1996) 644–648.
[25] C. Luong, A. Miller, J. Barnett, J. Chow, C. Ramesha, M.F. Browner, Flexibility of the NSAID binding site in the structure of human cyclooxygenase-2, Nat. Struct. Biol. 3 (1996) 927–933.
[26] M. Bakovic, H.B. Dunford, Intimate relation between cyclooxygenase and peroxidase activities of prostaglandin H synthase. Peroxidase reaction of ferulic acid and its influence on the reaction of arachidonic acid, Biochemistry 33 (1994) 6475–6482.
[27] C. Wei, R.J. Kulmacz, A.L. Tsai, Comparison of branched-chain and tightly coupled reaction mechanisms for prostaglandin H synthase, Biochemistry 34 (1995) 8499–8512.
[28] A.L. Tsai, G. Wu, R.J. Kulmacz, Stoichiometry of the interaction of prostaglandin H synthase with substrates, Biochemistry 36 (1997) 13085–13094.
[29] V. Koshkin, H.B. Dunford, Coupling of the peroxidase and cyclooxygenase reactions of prostaglandin H synthase, Biochim. Biophys. Acta 1430 (1999) 341–348.
[30] B. Bambai, R.J. Kulmacz, Prostaglandin H synthase. Effects of peroxidase cosubstrates on cyclooxygenase velocity, J. Biol. Chem. 275 (2000) 27608–27614.
[31] G. Wu, C. Wei, R.J. Kulmacz, Y. Osawa, A.L. Tsai, A mechanistic study of self-inactivation of the peroxidase activity in prostaglandin H synthase-1, J. Biol. Chem. 274 (1999) 9231–9237.
[32] G. Wu, J.L. Vuletich, R.J. Kulmacz, Y. Osawa, A.L. Tsai, Peroxidase self-inactivation in prostaglandin H synthase-1 pretreated with cyclooxygenase inhibitors or substituted with mangano protoporphyrin IX, J. Biol. Chem. 276 (2001) 19879–19888.
[33] I. Song, T.M. Ball, W.L. Smith, Different suicide inactivation processes for the peroxidase and cyclooxygenase activities of prostaglandin endoperoxide H synthase-1, Biochem. Biophys. Res. Commun. 289 (2001) 869–875.
[34] S. Ohki, N. Ogino, S. Yamamoto, O. Hayaishi, Prostaglandin hydroperoxidase, an integral part of prostaglandin endoperoxide synthetase from bovine vesicular gland microsomes, J. Biol. Chem. 254 (1979) 829–836.
[35] R.J. Kulmacz, J.F. Miller Jr., R.B. Pendleton, W.E.M. Lands, Cyclooxygenase initiation assay for hydroperoxides, Methods. Enzymol. 186 (1990) 431–438.
[36] R.B. Pendleton, Hydroperoxide stimulation of prostaglandin endoperoxide synthase, Ph.D. Thesis, University of Illinois at Urbana-Champaign, 1990.
[37] L.J. Marnett, P. Wlodawer, B. Samuelsson, Co-oxygenation of organic substrates by the prostaglandin synthetase of sheep vesicular gland, J. Biol. Chem. 250 (1975) 8510–8517.
[38] D.C. Goodwin, L.M. Landino, L.J. Marnett, Effects of nitric oxide and nitric oxide-derived species on prostaglandin endoperoxide synthase and prostaglandin biosynthesis, FASEB J. 13 (1999) 1121–1136.
[39] L.M. Landino, B.C. Crews, M.D. Timmons, J.D. Morrow, L.J. Marnett, Peroxynitrite, the coupling product of nitric oxide and superoxide, activates prostaglandin biosynthesis, Proc. Natl. Acad Sci. USA 93 (1996) 15069–15074.
[40] W. Nastainczyk, D. Schuhn, V. Ullrich, Spectral intermediates of prostaglandin hydroperoxidase, Eur. J. Biochem 144 (1984) 381–385.
[41] P.J. O'Brien, The mechanism of oxygen activation involved in the prostaglandin synthetase mechanism, Prog. Lipid. Res. 20 (1981) 295–298.
[42] S. Strieder, K. Schaible, H.J. Scherer, R. Dietz, H.H. Ruf, Prostaglandin endoperoxide synthase substituted with manganese protoporphyrin IX. Formation of a higher oxidation state and its relation to cyclooxygenase reaction, J. Biol. Chem. 267 (1992) 13870–13878.
[43] R. Odenwaller, K.R. Maddipati, L.J. Marnett, Detection of a higher oxidation state of manganese-prostaglandin endoperoxide synthase, J. Biol. Chem. 267 (1992) 13863–13869.
[44] W. Chen, T.R. Pawelek, R.J. Kulmacz, Hydroperoxide dependence and cooperative cyclooxygenase kinetics in prostaglandin H synthase-1 and -2, J. Biol. Chem. 274 (1999) 20301–20306.
[45] L.M. Landino, B.C. Crews, J.K. Gierse, S.D. Hauser, L.J. Marnett, Mutational analysis of the role of the distal histidine and glutamine residues of prostaglandin-endoperoxide synthase-2 in peroxidase catalysis,hydroperoxide reduction, and cyclooxygenase activation, J. Biol. Chem. 272 (1997) 21565–21574.
[46] T. Shimokawa, W.L. Smith, Essential histidines of prostaglandin endoperoxide synthase. His-309 is involved in heme binding, J. Biol. Chem. 266 (1991) 6168–6173.
[47] S.A. Seibold, T. Ball, L.C. Hsi, D.A. Mills, R.D. Abeysinghe, R. Micielli, C.J. Rieke, R.I. Cukier, W.L. Smith, Histidine 386 and its role in cyclooxygenase and peroxidase catalysis by prostaglandin-endoperoxide H synthases, J. Biol. Chem. 278 (2003) 46163–46170.
[48] J.H. Capdevila, J.D. Morrow, Y.Y. Belosludtsev, D.R. Beauchamp, R.N. DuBois, J.R. Falck, The catalytic outcomes of the constitutive and the mitogen inducible isoforms of prostaglandin H2 synthase are markedly affected by glutathione and glutathione peroxidase(s), Biochemistry 34 (1995) 3325–3337.
[49] R.J. Kulmacz, L.H. Wang, Comparison of hydroperoxide initiator requirements for the cyclooxygenase activities of prostaglandin H synthase-1 and -2, J. Biol. Chem. 270 (1995) 24019–24023.
[50] H.R. Herschman, Prostaglandin synthase 2, Biochim. Biophys. Acta 1299 (1996) 125–140.
[51] R.J. Kulmacz, Cellular regulation of prostaglandin H synthase catalysis, FEBS Lett. 430 (1998) 154–157.
[52] W.L. Smith, Cyclooxygenases, peroxide tone and the allure of fish oil, Curr. Opin. Cell. Biol. 17 (2005) 174–182.
[53] S.B. Roberts, D.M. Langenau, F.W. Goetz, Cloning and characterization of prostaglandin endoperoxide synthase-1 and -2 from the brook trout ovary, Mol. Cell. Endocrinol. 160 (2000) 89–97.
[54] T. Grosser, S. Yusuff, E. Cheskis, M.A. Pack, G.A. FitzGerald, Developmental expression of functional cyclooxygenases in zebrafish, Proc. Natl. Acad Sci. USA 99 (2002) 8418–8423.
[55] J. Zou, N.F. Neumann, J.W. Holland, M. Belosevic, C. Cunningham, C.J. Secombes, A.F. Rowley, Fish macrophages express a cyclo-oxygenase-2 homologue after activation, Biochem. J 340 (Pt1) (1999) 153–159.
[56] G. Lu, A.L. Tsai, H.E. Van Wart, R.J. Kulmacz, Comparison of the peroxidase reaction kinetics of prostaglandin H synthase-1 and -2, J. Biol. Chem. 274 (1999) 16162–16167.
[57] B. Bambai, C.E. Rogge, B. Stec, R.J. Kulmacz, Role of Asn-382 and Thr-383 in activation and inactivation of human prostaglandin H synthase cyclooxygenase catalysis, J. Biol. Chem. 279 (2004) 4084–4092.
[58] H.S. Huang, C.J. Chen, H. Suzuki, S. Yamamoto, W.C. Chang, Inhibitory effect of phospholipid hydroperoxide glutathione peroxidase on the activity of lipoxygenases and cyclooxygenases, Prostaglandins Other Lipid Mediat. 58 (1999) 65–75.
[59] R.J. Kulmacz, J.F. Miller Jr., W.E.M. Lands, Prostaglandin H synthase: an example of enzymic symbiosis, Biochem. Biophys. Res. Commun. 130 (1985) 918–923.
[60] F. Ursini, M. Maiorino, C. Gregolin, The selenoenzyme phospholipid hydroperoxide glutathione peroxidase, Biochim. Biophys. Acta 839 (1985) 62–70.
[61] R.J. Kulmacz, W.A. van der Donk, A.L. Tsai, Comparison of the properties of prostaglandin H synthase-1 and -2, Prog. Lipid. Res. 42 (2003) 377–404.
[62] B.A. Barry, M.K. el-Deeb, P.O. Sandusky, G.T. Babcock, Tyrosine radicals in photosystem II and related model compounds. Characterization by isotopic labeling and EPR spectroscopy, J. Biol. Chem. 265 (1990) 20139–20143.
[63] C.E. Rogge, W. Liu, G. Wu, L.H. Wang, R.J. Kulmacz, A.L. Tsai, Identification of Tyr504 as an alternative tyrosyl radical site in human prostaglandin H synthase-2, Biochemistry 43 (2004) 1560–1568.

[64] P. Dorlet, S.A. Seibold, G.T. Babcock, G.J. Gerfen, W.L. Smith, A.L. Tsai, S. Un, High-field EPR study of tyrosyl radicals in prostaglandin H(2) synthase-1, Biochemistry 41 (2002) 6107–6114.
[65] J.C. Wilson, G. Wu, A.L. Tsai, G.J. Gerfen, Determination of the structural environment of the tyrosyl radical in prostaglandin H2 synthase-1: a high frequency ENDOR/EPR study, J. Am. Chem. Soc. 127 (2005) 1618–1619.
[66] L.C. Hsi, C.W. Hoganson, G.T. Babcock, R.M. Garavito, W.L. Smith, An examination of the source of the tyrosyl radical in ovine prostaglandin endoperoxide synthase-1, Biochem. Biophys. Res. Commun. 207 (1995) 652–660.
[67] G. Hochgesang, S. Rowlinson, L. Marnett, Tyrosine-385 is critical for acetylation of cyclooxygenase-2 by aspirin, J. Am. Chem. Soc. 122 (2000) 6514–6515.
[68] E.D. Thuresson, K.M. Lakkides, C.J. Rieke, Y. Sun, B.A. Wingerd, R. Micielli, A.M. Mulichak, M.G. Malkowski, R.M. Garavito, W.L. Smith, Prostaglandin endoperoxide H synthase-1: the functions of cyclooxygenase active site residues in the binding, positioning, and oxygenation of arachidonic acid, J. Biol. Chem. 276 (2001) 10347–10357.
[69] E.D. Thuresson, M.G. Malkowski, K.M. Lakkides, C.J. Rieke, A.M. Mulichak, S.L. Ginell, R.M. Garavito, W.L. Smith, Mutational and X-ray crystallographic analysis of the interaction of dihomo-gamma -linolenic acid with prostaglandin endoperoxide H synthases, J. Biol. Chem. 276 (2001) 10358–10365.
[70] D.C. Swinney, A.Y. Mak, J. Barnett, C.S. Ramesha, Differential allosteric regulation of prostaglandin H synthase 1 and 2 by arachidonic acid, J. Biol. Chem. 272 (1997) 12393–12398.
[71] R.J. Kulmacz, R.B. Pendleton, W.E.M. Lands, Interaction between peroxidase and cyclooxygenase activities in prostaglandin-endoperoxide synthase. Interpretation of reaction kinetics, J. Biol. Chem. 269 (1994) 5527–5536.
[72] L.J. Marnett, K.R. Maddipati, Prostaglandin H synthase, in: J. Everse, M.B. Grisham, K.E. Everse (Eds.), Peroxidases in Chemistry and Biology, Vol. 1, CRC Press, Boca Raton, 1991, pp. 293–334.
[73] C.M. Markey, A. Alward, P.E. Weller, L.J. Marnett, Quantitative studies of hydroperoxide reduction by prostaglandin H synthase. Reducing substrate specificity and the relationship of peroxidase to cyclooxygenase activities, J. Biol. Chem. 262 (1987) 6266–6279.
[74] M. Ouellet, M.D. Percival, Mechanism of acetaminophen inhibition of cyclooxygenase isoforms, Arch. Biochem. Biophys. 387 (2001) 273–280.
[75] G. Lassmann, R. Odenwaller, J.F. Curtis, J.A. DeGray, R.P. Mason, L.J. Marnett, T.E. Eling, Electron spin resonance investigation of tyrosyl radicals of prostaglandin H synthase. Relation to enzyme catalysis, J. Biol. Chem. 266 (1991) 20045–20055.
[76] A.L. Tsai, G. Palmer, R.J. Kulmacz, Prostaglandin H synthase. Kinetics of tyrosyl radical formation and of cyclooxygenase catalysis, J. Biol. Chem. 267 (1992) 17753–17759.
[77] C.A. Rouzer, L.J. Marnett, Mechanism of free radical oxygenation of polyunsaturated fatty acids by cyclooxygenases, Chem. Rev. 103 (2003) 2239–2304.
[78] N. Ogino, S. Yamamoto, O. Hayaishi, T. Tokuyama, Isolation of an activator for prostaglandin hydroperoxidase from bovine vesicular gland cytosol and its identification as uric acid, Biochem. Biophys. Res. Commun. 87 (1979) 184–191.
[79] R.J. Kulmacz, A.L. Tsai, G. Palmer, Heme spin states and peroxide-induced radical species in prostaglandin H synthase, J. Biol. Chem. 262 (1987) 10524–10531.
[80] M.E. Hemler, W.E.M. Lands, Evidence for a peroxide-initiated free radical mechanism of prostaglandin biosynthesis, J. Biol. Chem. 255 (1980) 6253–6261.
[81] P.J. Marshall, R.J. Kulmacz, W.E.M. Lands, Constraints on prostaglandin biosynthesis in tissues, J. Biol. Chem. 262 (1987) 3510–3517.
[82] A.M. Hanel, W.E.M. Lands, Modification of anti-inflammatory drug effectiveness by ambient lipid peroxides, Biochem. Pharmacol. 31 (1982) 3307–3311.
[83] O. Boutaud, D.M. Aronoff, J.H. Richardson, L.J. Marnett, J.A. Oates, Determinants of the cellular specificity of acetaminophen as an inhibitor of prostaglandin H(2) synthases, Proc. Natl. Acad Sci. USA 99 (2002) 7130–7135.
[84] D.M. Aronoff, O. Boutaud, L.J. Marnett, J.A. Oates, Inhibition of prostaglandin H2 synthases by salicylate is dependent on the oxidative state of the enzymes, J. Pharmacol. Exp. Ther 304 (2003) 589–595.
[85] W.E.M. Lands, A. Hanel, Inhibitors and activators of prostaglandin biosynthesis, in: C. Pace-Asciak, E. Granstrom (Eds.), Prostaglandins and Related Substances, Elsevier, Amsterdam, 1983, pp. 203–223.
[86] W.E.M. Lands, Actions of anti-inflammatory drugs, Trends Pharmacol. Sci. 2 (1981) 78–80.
[87] N.V. Chandrasekharan, H. Dai, K.L. Roos, N.K. Evanson, J. Tomsik, T.S. Elton, D.L. Simmons, COX-3, a cyclooxygenase-1 variant inhibited by acetaminophen and other analgesic/antipyretic drugs: cloning, structure, and expression, Proc. Natl. Acad Sci. USA 99 (2002) 13926–13931.
[88] B. Kis, J.A. Snipes, D.W. Busija, Acetaminophen and the COX-3 Puzzle: Sorting out Facts, Fictions and Uncertainties, J. Pharmacol. Exp. Ther. (2005).
[89] H. Daiyasu, H. Toh, Molecular evolution of the myeloperoxidase family, J. Mol. Evol. 51 (2000) 433–445.
[90] L. Hornsten, C. Su, A.E. Osbourn, P. Garosi, U. Hellman, C. Wernstedt, E.H. Oliw, Cloning of linoleate diol synthase reveals homology with prostaglandin H synthases, J. Biol. Chem. 274 (1999) 28219–28224.
[91] M. Hamberg, A. Sanz, C. Castresana, alpha-oxidation of fatty acids in higher plants. Identification of a pathogen-inducible oxygenase (piox) as an alpha-dioxygenase and biosynthesis of 2-hydroperoxylinolenic acid, J. Biol. Chem. 274 (1999) 24503–24513.
[92] C. Su, M. Sahlin, E.H. Oliw, A protein radical and ferryl intermediates are generated by linoleate diol synthase, a ferric hemeprotein with dioxygenase and hydroperoxide isomerase activities, J. Biol. Chem. 273 (1998) 20744–20751.
[93] A. Sanz, J.I. Moreno, C. Castresana, PIOX, a new pathogen-induced oxygenase with homology to animal cyclooxygenase, Plant. Cell. 10 (1998) 1523–1537.
[94] T. Koeduka, K. Matsui, Y. Akakabe, T. Kajiwara, Catalytic properties of rice alpha-oxygenase. A comparison with mammalian prostaglandin H synthases, J. Biol. Chem. 277 (2002) 22648–22655.
[95] W. Liu, C.E. Rogge, B. Bambai, G. Palmer, A.L. Tsai, R.J. Kulmacz, Characterization of the heme environment in Arabidopsis thaliana fatty acid alpha-dioxygenase-1, J. Biol. Chem. 279 (2004) 29805–29815.
[96] M.G. Malkowski, S.L. Ginell, W.L. Smith, R.M. Garavito, The productive conformation of arachidonic acid bound to prostaglandin synthase, Science 289 (2000) 1933–1937.