Radically novel prostaglandins in animals and plants: the isoprostanes

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Animal prostaglandins and plant jasmonates are well-known enzymatically formed cyclopentanoic lipids that have regulatory functions and serve as inducible mediators of host defense reactions. A novel group of prostaglandin-like compounds, the isoprostanes, generated in animals and plants by a nonenzymatic, free radical-catalyzed process, are now suspected to be mediators of oxidant injury *in vivo*.

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Introduction

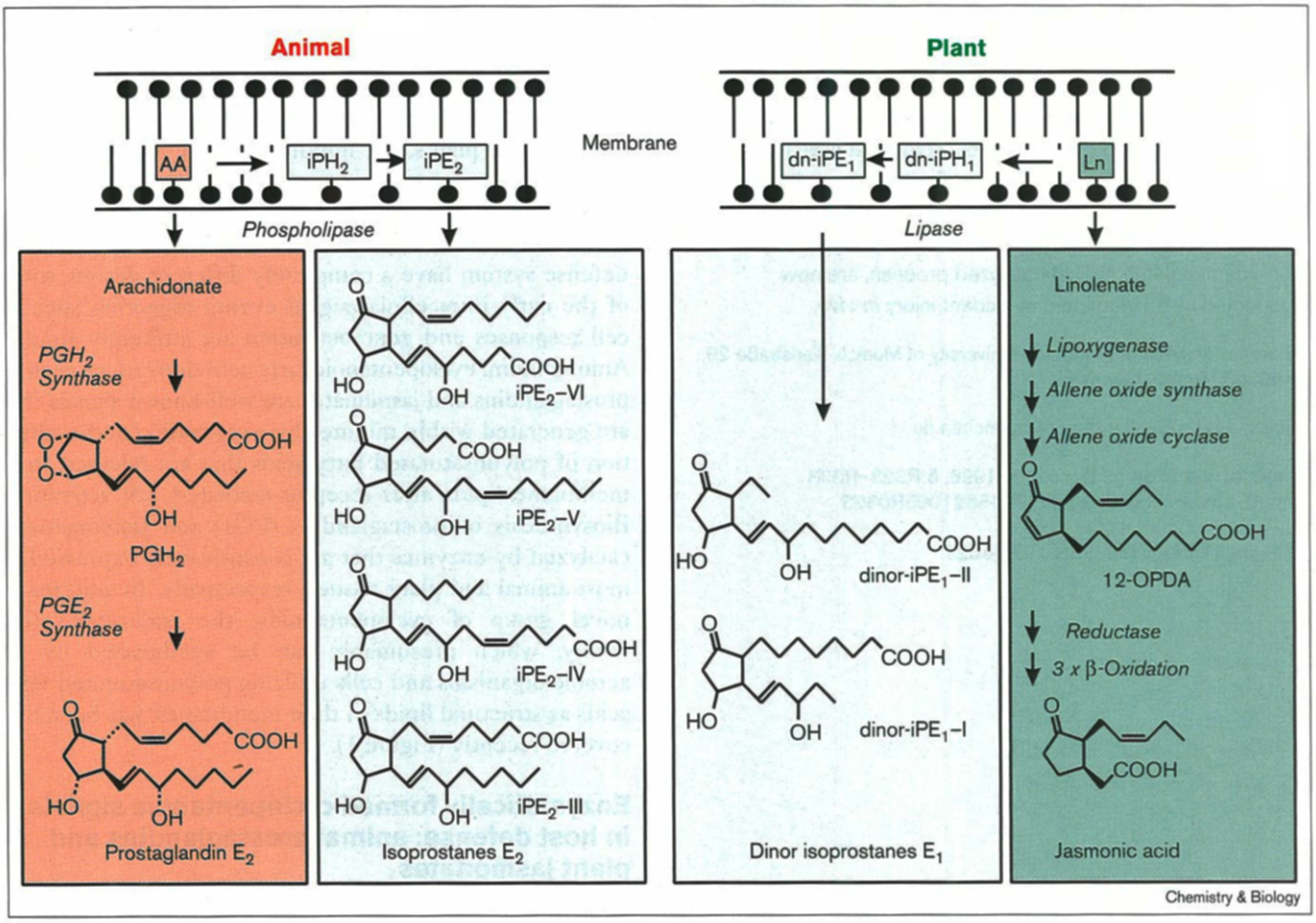
Animals and plants, for hundreds of million years, have evolved defensive strategies to protect themselves against pathogens, parasites and oxidant injury. Although the mammalian immune system and the plant chemical defense system have a completely different design, some of the early intracellular signal events triggering specific cell responses and gene activation are strikingly similar. Among them, cyclopentanoic fatty acid derivatives such as prostaglandins and jasmonates are well-known signals that are generated within minutes by oxygenation and cyclization of polyunsaturated fatty acids that are released from membrane lipids after receptor-mediated cell activation. Biosynthesis of prostaglandins (PGs) and jasmonates is catalyzed by enzymes that are constitutively expressed in most animal and plant tissues, respectively. In addition, a novel group of cyclopentanoids, the isoprostane (iP) family, which presumably can be synthesized by all aerobic organisms and cells utilizing polyunsaturated fatty acids as structural lipids in their membranes, has been discovered recently (Figure 1).

Enzymatically formed cyclopentanoic signals in host defense: animal prostaglandins and plant jasmonates

PGs, which are found in animals, are involved in a variety of physiological regulatory processes in kidney, bone, reproductive tissues, gut and blood vessels. But PG synthesis can also be triggered by inflammatory peptides, growth factors, cytokines, tumor promoters, microbial cell-wall fragments, cell injury and other stimuli; in these circumstances, PGs contribute to inflammatory responses (such as redness, edema, pain and heat sensations) [1]. PGs also modulate fever [2] and pain [3] in the nervous system. It is for this reason that anti-prostaglandin drugs such as aspirin, indomethacin and ibuprofen are used as standard anti-inflammatory therapeutics.

The term prostaglandins refers strictly to eicosanoids that are generated from C20 fatty acids (predominantly arachidonic acid) by the action of prostaglandin H synthase (PGHS) and have a characteristic cyclopentane ring system (Figure 2). As polyunsaturated C20 fatty acids and PGHS are generally lacking in bacteria, yeasts and higher plants (the former can be found in algae, mosses, ferns and filamentous fungi, PGs are presumably restricted to the animal kingdom [4–6]. But plants do synthesize a group of structurally closely related cyclopentanoic acids such as 12-oxo-phytodienoic acid, jasmonic acid and their two methylene groups shorter dinor congeners (commonly referred to as jasmonates) [7]. Jasmonates are derived from

Figure 1



Biosynthesis of prostaglandin-like compounds in animals and plants. Animals and plants utilize their structural fatty acids in the membrane to form cyclopentanoic signal compounds. Prostaglandin H synthase (in animals only) and allene oxide synthase (in plants only) are the crucial enzymes involved in the formation of animal-specific prostaglandin and plant-specific jasmonate signals after liberation of the fatty acid from the membrane. Both animals and plants form isoprostanes via a nonenzymatic, free-radical-catalyzed pathway from esterified fatty acids of the membrane. In animals, arachidonate (AA) is the most common precursor that is utilized for biosynthesis of prostaglandins (PGs) and isoprostanes (iPs). Higher plants utilize α-linolenate (Ln) for the synthesis of 12-oxo-phytodienoic acid (12-OPDA) and jasmonic acid or dinor isoprostanes (dn-iPH). Prostaglandin H_2 (PGH_2), isoprostane H_2 (iPH_2) and dinor isoprostane H_1 (dn-iPH_1) are highly unstable, common intermediates not only for the formation of the E-ring structures shown, but for a variety of other ring types (see Figure 2) as well.

the main polyunsaturated plant fatty acids α-linolenic [8] and hexadecatrienoic acid [9] by the action of lipoxygenase and allene oxide synthase. α-Linolenic acid is only a minor fatty acid in mammals and allene oxide synthase does not occur in animals. Jasmonates are therefore characteristic plant mediators. Like prostaglandins in animals, jasmonates function as physiological regulators, although the processes are rather different, including flowering, tendril coiling, tuberisation, fruit ripening and storage [7].

One of the most striking similarities of prostaglandins and jasmonates uncovered recently is their essential function in the reproductive system. Prostaglandins F_2 and E_2 are essential for normal parturition and perinatal survival of mice pups [10,11], whereas mutant plants unable to synthesize jasmonates have sterile anthers and produce no seed [12,13]. Another exciting similarity recently became apparent when it was shown that jasmonates effectively function as mediators in host defense reactions in response to wounding, pathogen attack and UV radiation injury [7]. PGs have a similar function that has long been recognized. Jasmonates are potent transcriptional activators that induce *de novo* synthesis of a variety of defensive proteins [14] and accumulation of defensive low molecular weight compounds [15]. Mutant plants deficient in jasmonate biosynthesis are therefore sensitive to a number of pathogens and administering exogenous jasmonate can be protective [16–18]. Unfortunately, jasmonic acid has too many other effects to be used in agriculture as an inducer of endogenous plant

defense mechanisms. As structurally related jasmonate analogues display drastically different profiles of biological effects [19], thus, it might be possible to design analogues with more desirable biological characteristics.

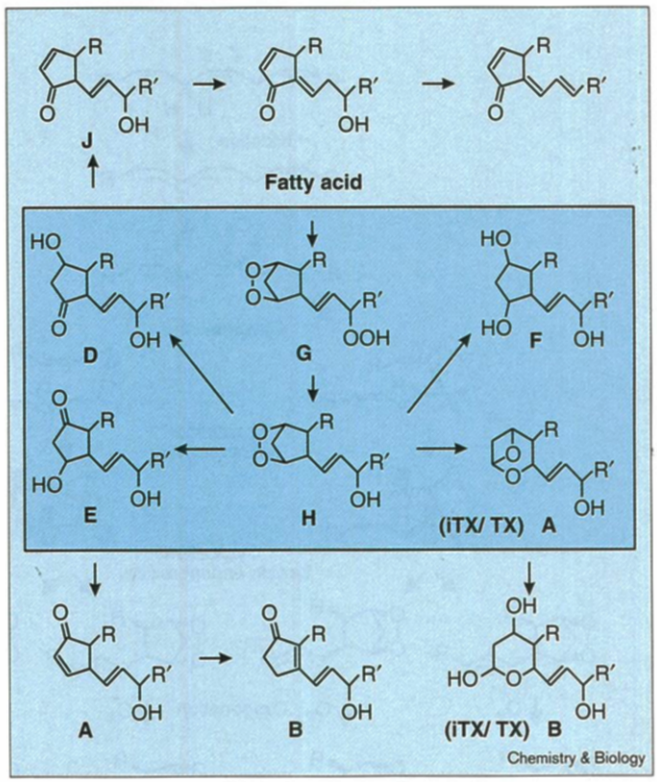
The isoprostanes: occurrence and mechanism of formation

Animal isoprostanes

Until recently, PGHS was regarded as obligatory for prostanoid synthesis, but, in 1990, a series of prostaglandin-like compounds was reported to be produced in mammals by the free radical-catalyzed peroxidation of arachidonic acid independent of PGHS [20]. Isoprostanes have the same cyclopentane ring system and oxygen functionalities as PGs but differ from them by the *cis*-stereochemistry at the five-membered ring junction as compared with the *trans*-ring junction in all PGs. As formation of isoprostanes is nonenzymatic, the stereochemistry at each of the chiral centers of the molecules can be either R or S. In addition, the isoprostane carbon skeleton can be identical (iP regioisomer III) to that of the PGs or differ in the lengths of the sidechains (regioisomers IV–VI), see Figure 1. Thus, up to 64 isomers of, for instance, prostaglandin-G-like compounds may potentially be produced. Isoprostane formation *in vitro* was first observed over three decades ago [21–23] but was not thought to be relevant *in vivo*. We now know that isoprostanes occur ubiquitously in all mammalian tissues and extracellular fluids at concentrations that exceed those of PGs by more than an order of magnitude [24]. F_2-ring isoprostanes (iPF_2s), for instance, have been detected esterified in rat liver phospholipids (6 ng/g liver) and unesterified in fresh human plasma (35 pg/ml) and human urine (1.6 ng/mg creatinine) [25]. *In vivo*, these compounds increase dramatically during enhanced free radical formation, exert potent receptor-mediated biological activity and represent extremely sensitive and accurate markers of oxidative stress [26].

Interestingly, the production of isoprostanes from the precursor fatty acids (which are found almost completely esterified in the phospholipids and — in the case of plants — also in the glycolipids of membranes) appears to be by nonenzymatically oxygenation *in situ* [27]. Under physiological conditions, the level of free fatty acids in mammalian [28] and plant cells [29] is typically maintained below 0.25–1% of the total amount of fatty acids by an efficient esterification mechanism. Free fatty acids are therefore only a minor source for cellular isoprostane formation [25]. Unesterified isoprostanes, representing less than 2% of the cellular isoprostanes, are thought to be derived predominately from preformed isoprostanes that were released by phospholipases [25]. In contrast, in the enzymatic pathways of both mammals and plants, the fatty acid must first be liberated by (phospho-)lipases to become available to biosynthetic enzymes (Figure 1).

Figure 2



Enzymatic and nonenzymatic metabolism of G-ring prostaglandins/ isoprostanes. A- to J-ring prostaglandin/isoprostane as well as A- and B-ring isothromboxane/ thromboxane (iTX/TX) structures are derived from G-ring precursors. The primary enzymatic G-ring metabolites are shown in the box. All these products may also be formed nonenzymatically, however. In plain buffer, H-ring structures isomerize rapidly to E- and D-ring prostanoids and may be subject to dehydration/isomerization via an enol–enolate intermediate leading to A-, B- and J-ring compounds, respectively. In addition, J-ring structures are prone to undergo further isomerization and dehydration reactions [87]. In the physiological pH range, dehydration/ isomerization is a slow process, however [88]. The relatively stable F-ring can be formed *in vitro* and *in vivo* in the presence of natural reductants [20]. Thromboxane-A-like compounds are formed *in vitro* and *in vivo* presumably by a heavy-metal-catalyzed mechanism and are rapidly hydrolyzed by water to iTXB/TXB [38].

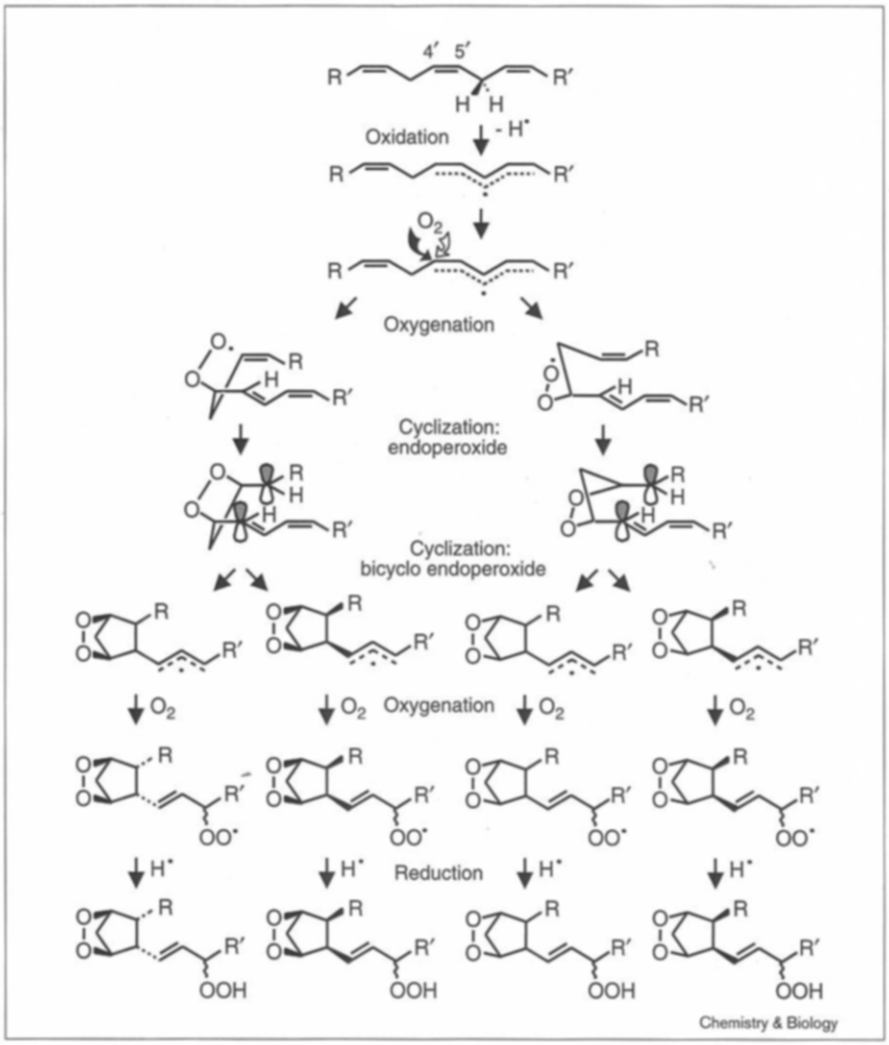
Unesterified (iso-)prostanoids and jasmonates, however, cannot be esterified into membrane lipids.

The first isoprostane formed by autoxidation, iPG, is an extremely reactive and versatile molecule that can readily be transformed to almost all known prostaglandin ring systems *in vitro* (Figure 2), many of which have already been shown to be biosynthesized nonenzymatically *in vivo*.

Plant isoprostanes

Analysis of the mechanism of isoprostane formation (see below) and experimental evidence suggest that formation of isoprostane-like compounds will occur when a fatty acid containing at least one triene unit meets molecular oxygen

Figure 3



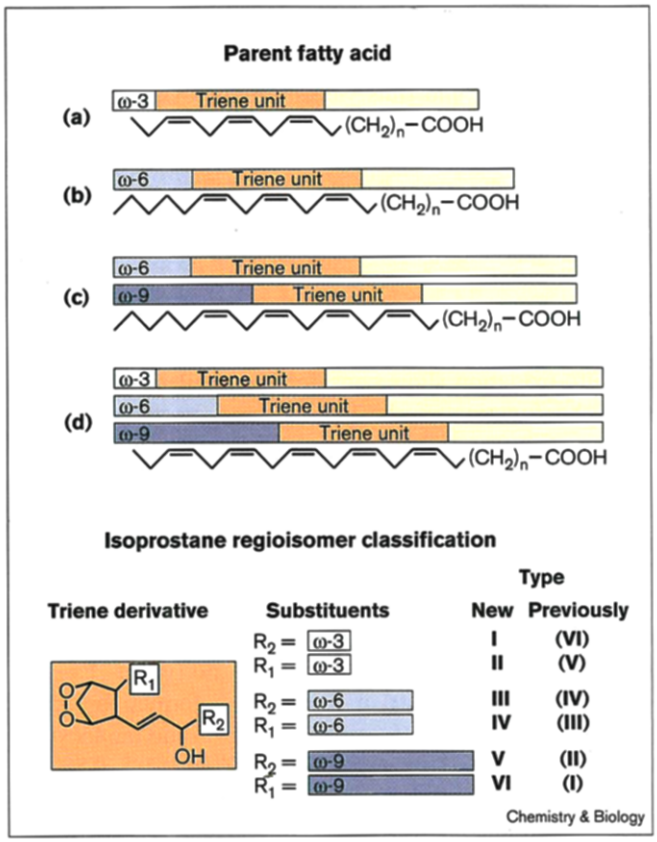
Isoprostane formation: the endoperoxide mechanism. Hydrogen abstraction from the bisallylic methylene group at carbon 6′ yields a pentadienyl radical that may be oxygenated at carbon 4′. The corresponding racemic peroxy radical may attack carbon 2′ yielding a racemic endoperoxy radical conformer which is stabilized in the geometry shown by delocalization into the radicaloid π orbital both from a β-peroxy oxygen lone pair and from the diene unit. Cyclization will involve rotation of *syn* π orbital lobes toward one another (disrotatory motion) and, as a consequence, the resulting bicyclo endoperoxide will possess almost exclusively *cis*-oriented appendages (either *exo*, *exo* or *endo*, *endo*) [34]. Attack of the sidechain radical by oxygen and reduction of the sidechain peroxy radical yields a PGG-like structure.

in the absence of an effective antioxidant. As mentioned previously, eicosanoids cannot be formed in higher plants. Instead, we have speculated that α-linolenic acid and hexadecatrienoic acid, which typically represent more than 40% and 10% of the total fatty acids in plant leaves [29], respectively, could substitute for arachidonate not only in the jasmonate but also in the isoprostane pathway. Isoprostanes derived from α-linolenate would differ from mammalian isoprostanes in that they are two methylene groups shorter and retain only one double bond from the precursor. Recently, we have shown that the postulated E_1-dinor isoprostanes (see [30] for a discussion of the nomenclature) can indeed be detected in cell cultures of four taxonomically different plant species in a concentration range of 5–60 ng/g of dry weight, which is more than one order of magnitude higher than normal jasmonate levels [31]. In addition, E_1- and F_1-dinor isoprostanes have also been detected in whole plants of several species, suggesting that dinor isoprostanes represent common plant analogues of the animal isoprostanes (S. Parchmann and M.J.M., unpublished observations).

The endoperoxide radical mechanism: nonenzymatic generation of iPG

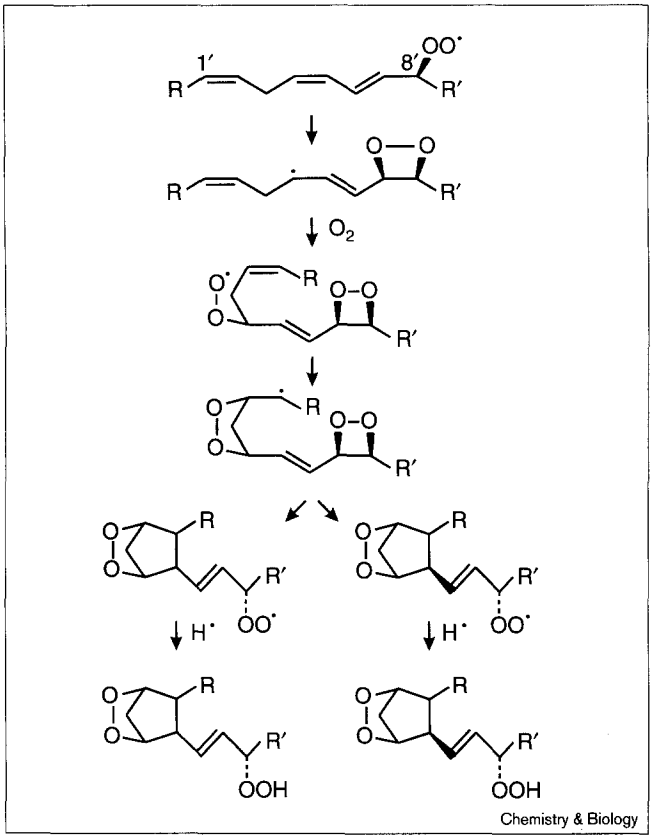
Reactive oxygen species are crucial in isoprostane formation. They can readily abstract a hydrogen atom from bisallylic methylene groups of polyunsaturated fatty acids, starting a radical reaction that leads to the formation of isoprostanes under aerobic conditions not only *in vitro* but also in animals and plants (Figure 3). As radical generation is nonenzymatic, all methylenes flanked by two *cis* double bonds can be involved in this reaction, although not necessarily to an equal extent [32]. After hydrogen abstraction, the pentadienyl radical can combine with O_2 to generate a racemic peroxyl radical that is prone to rearrange to a bicyclo endoperoxy radical, yielding equivalent amounts of α,α- and β,β-endoperoxy radicals. Analysis of bicyclo endoperoxides derived from

Figure 4



Architecture and nomenclature of isoprostane regioisomers. A triene unit is the principal building block for all isoprostane ring systems (PGH ring system in this example). The substitutes at the triene unit represent the methyl (blue) and the carboxyl (yellow) terminus of the fatty acid. The methyl terminal chain at each distinct triene unit can occupy the position R_1 or R_2 of the isoprostane, and, thus, two types of regioisomers can be produced per triene unit. As shown, trienoic, tetraenoic and pentaenoic fatty acids will form 2, 4 and 6 different regioisomers, respectively. Isoprostane regioisomers can be best classified according to the recently proposed nomenclature by Rokach *et al.* [30] (used throughout this article), which overcomes some important limitations of the previous nomenclature [89]. Representative examples are **(a)** α-linolenate (n = 6), **(b)** γ-linolenate (n = 3), **(c)** arachidonate (n = 2) and **(d)** eicosapentaenate (n = 2).

Figure 5



Isoprostane formation: the dioxetane mechanism. Cyclization of the 8′-hydroperoxy radical via the dioxetane mechanism will yield two regioisomers (R and R′ are interchangeable) each of which is comprised of only two isomers if an enantiomerically pure hydroperoxy radical is the starting material. For instance, Corey and Wang [36] observed cyclization of the 15(*S*)-hydroperoxy radical of arachidonate to yield PGG_2 (natural chirality) and the 12-*iso*-PGG_2 epimer in a ratio of 1:3 in 43% yield.

γ- and α-linolenate, and arachidonate revealed that the two sidechains are configured almost exclusively *cis* with respect to the cyclopentane ring [23,33,34]. Next, the bicyclo endoperoxy radical can trap an oxygen on either face of the sidechain, yielding racemic hydroperoxy bicyclo endoperoxy radicals. At this stage, termination of the radical chain reaction by hydrogen abstraction from a suitable donor molecule such as a polyunsaturated fatty acid or glutathione will yield iPG (Figure 3).

The mechanism outlined above is principally applicable to polyunsaturated fatty acids that contain one or more triene units. Each triene unit has two mechanistically equivalent but distinct substituents and will therefore form two different regioisomers (Figure 4), each of which is theoretically composed of eight racemic diastereomers (including the minor sidechain *trans*-substituted isomers, not shown in Figure 3). For example, linolenate can form 32 stereoisomers and arachidonate will yield 64 stereoisomers. Although synthesis of bicyclo endoperoxides from dienoic fatty acids appears theoretically feasible, as yet no indication of the formation of such compounds from, for example, linoleic acid has been obtained [35].

The dioxetane mechanism: an alternative pathway for isoprostane formation

Recently, Corey and Wang [36] have shown that isoprostanes can not only be formed via the endoperoxide mechanism described above, but also by an alternative free-radical-catalyzed pathway *in vitro*. The alternative dioxetane mechanism starts from the 1′- and 8′-hydroperoxy radicals (Figure 5) rather than from the 4′- and

5′-hydroperoxy radicals (Figure 3) that are required for the endoperoxide mechanism. This route will also lead to the same two regioisomers per triene subunit as the endoperoxide mechanism and, presumably, also operates in animals and plants. Interestingly, this pathway is much more stereoselective than the endoperoxide mechanism and yields two regioisomers, each of which is comprised of two racemic diastereomers, one of which has the sidechain *cis* substitution at the cyclopentane ring, whereas the other one has the *trans* configuration. This finding casts doubt upon the commonly held belief that autoxidation of polyunsaturated fatty acids yields almost exclusively *cis* substituted prostaglandin-like compounds. Earlier studies have dealt predominately with 13(*S*)-hydroperoxy α-linolenic acid, 9(*S*)-hydroperoxy γ-linolenic acid or chemical model compounds as starting material that cannot undergo the dioxetane mechanism and thus forms predominately *cis* substituted prostaglandin-like compounds [23,33,34]. The relative contribution of these two pathways in isoprostane formation *in vitro* and *in vivo* remains to be evaluated, however.

The highly unstable isoprostane iPG can be converted nonenzymatically to D-, E- and F-ring isoprostanes and isothromboxane B *in vivo*

In mammalian cells, the PGHS products PGG and PGH are the common precursors for PGD, PGE, PGF, PGI and thromboxane A, reactions catalyzed by prostaglandin D, E, F, I and thromboxane A synthases, respectively. The spectrum of products formed (Figure 2, boxed structures) in a specific cell type will critically depend on the presence of one or more of these enzymes. All of these prostaglandin ring systems, except for PGI, have been shown, however, to be produced by nonenzymatic reactions from the common PGG- or PGH-ring system (Figure 2). As the G- and H-ring endoperoxide structures are extremely labile compounds with a half-life of ~5 min in aqueous medium [37], they can isomerize rapidly to PGE- and PGD-like compounds, both *in vitro* and *in vivo* [38,39].

Only small quantities of iPFs are formed from iPGs in plain buffer *in vitro*, but iPFs are found in similar concentrations to iPDs and iPEs *in vivo* [38]. A mechanism has been proposed that involves the reduction of either iPG or iPH to iPFs by naturally occurring reductants such as glutathione, hematin, lipoic acid, polyunsaturated fatty acids or glutathione peroxidase [20]. Isothromboxane (iTX)-like compounds are not formed from iPG or iPH unless iron is present in a complex such as hemin *in vitro* [40]. iTXs occur in mammals, however, in similar concentrations to the isoprostanes described above, but the exact mechanism of formation *in vivo* has not yet been clarified [38]. As the biosynthesis of isoprostanes proceeds predominately within the membrane it is assumed that the contribution of enzymes to the formation of iPG or iPH metabolites *in vivo* is negligible.

Endoperoxide radical mechanism (almost perfectly) under steric control: the prostaglandin H synthases

The enzymatic biosynthesis of prostaglandins in animals also proceeds via a radical mechanism that is under the tight control of PGHS, and thus yields, predominately, a single stereoisomer (Figure 6). Two PGHS isozymes, PGHS-1 and PGHS-2, have been purified as homodimers with a subunit molecular mass of about 72 kDa. The enzyme contains heme (one per subunit) that is required for its two catalytic activities — as a cyclooxygenase and a peroxidase [6].

Despite the complex chemistry involved, the steps in the cyclooxygenase reaction and those in nonenzymatic fatty acid auto-oxidation show considerable similarities. Apparently, the function of the enzyme appears to be rather simple — fixation of the conformation of the substrate and stereospecific hydrogen removal. Additionally, the enzyme favours the correct radical rearrangements and shields the intermediate radicals to prevent side reactions.

Neither PGHS-1 nor PGHS-2 acts in a strictly stereospecific manner, however. Both isozymes form small amounts of the isoprostane 8-*iso*-$PGF_{2\alpha}$ (but no other F_2-isoprostanes). The amounts of 8-*iso*-$PGF_{2\alpha}$ formed by activated human platelets (containing PGHS-1) and monocytes (containing PGHS-2) are only ~0.05–0.1% and ~0.5–0.8% of the amount of thromboxane formed, respectively [41,42]. Enzymatic formation of 8-*iso*-$PGF_{2\alpha}$ is commonly believed to contribute only insignificant amounts to overall 8-*iso*-$PGF_{2\alpha}$ biosynthesis *in vivo*, however. So 8-*iso*-$PGF_{2\alpha}$ can still be used as an index of oxidant stress (see below).

Significance and putative function of isoprostanes

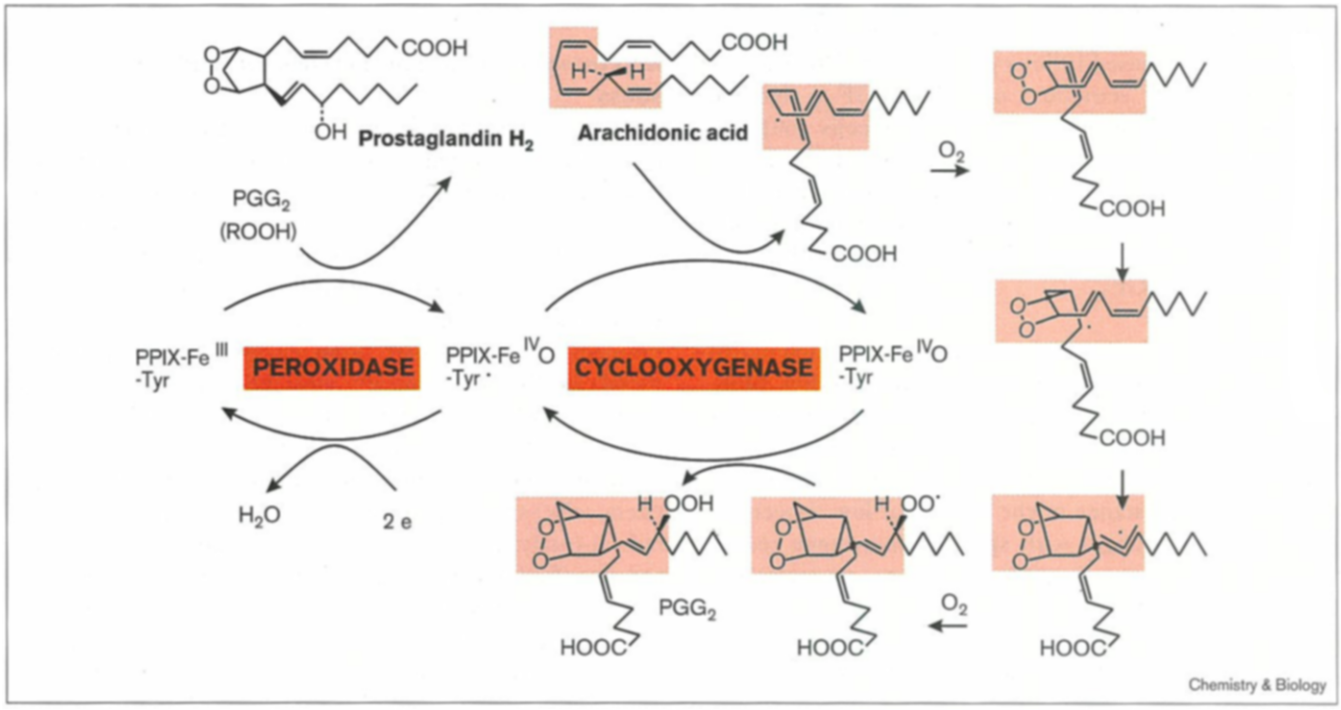
Isoprostanes have stimulated great interest, as potential mediators of oxidant injury, as agents that might disturb the physical properties and normal function of cell membranes, and as markers of oxidative stress. So far, studies in these areas have been limited exclusively to C20 isoprostanes in mammals and nothing is yet known about the biological significance of the recently discovered dinor isoprostanes in plants.

Isoprostanes have potent, receptor-mediated biological activities in mammals

Only a few isoprostanes are now available as pure isomers obtained by total synthesis, including 8-*iso*-$PGF_{2\alpha}$ and 8-*iso*-PGE_2, which are two of the most abundant isoprostanes of arachidonate autoxidation.

The availability of these compounds has allowed extensive testing of their biological activities. Many studies have shown that isoprostanes are potent vasoconstrictors in a variety of tissues. For instance, 8-*iso*-$PGF_{2\alpha}$ [43,44] and 8-*iso*-PGE_2 [45] are extremely potent constrictors of the afferent renal arteriole, leading to a drop in glomerular

Figure 6



Prostaglandin synthesis: proposed reaction mechanism of PGH synthases. The reaction starts with a two-electron reduction of a hydroperoxide, which is necessary to activate the enzyme by oxidation of the protoporphyrin IX (PPIX) with iron in the ferric form (hematin). Upon reaction with the hydroperoxide, hematin is oxidized and a tyrosyl radical is formed that can stereospecifically abstract the 13 pro-(*S*) hydrogen from arachidonic acid. This, in turn, leads to the formation of a carbon radical that undergoes a similar reaction sequence as in autoxidation. Because of the spatial orientation of arachidonate in the active site, the oxygenation and cyclization steps occur in a stereo-specific fashion. The radical chain reaction within the substrate is terminated by its reduction to PGG_2 and regeneration of the tyrosyl radical of the enzyme. The tyrosyl radical must be reduced by natural reductants such as uric acid or glutathione before the peroxidase can act. The cyclooxygenase and peroxidase activities of PGHS appear to be associated with spatially distinct yet interactive sites on the enzyme. The hydroperoxy group of PGG_2 can be reduced to the corresponding hydroxy group by oxidation of hematin to an oxo-ferryl intermediate, which is thought to abstract an electron from Tyr385, thereby preparing the enzyme for a new cycle of arachidonate turnover [6,90].

capillary pressure and filtration rate in the low nanomolar range. 8-*iso*-$PGF_{2\alpha}$ is also a potent vasoconstrictor in liver [46], heart [47,48], lung [49] and the eye retina [50]. The potency of 8-*iso*-prostaglandin E_2 in the guinea pig heart [48] is almost identical to 8-*iso*-$PGF_{2\alpha}$. These findings were surprising, because the prostaglandins PGE and PGF, the cognate ligands of PGE and PGF receptors, generally have opposite effects on smooth muscle cells of the vasculature — PGE_2 is a vasodilator and $PGF_{2\alpha}$ is a vasoconstrictor.

Interestingly, all the effects of 8-*iso*-$PGF_{2\alpha}$ and 8-*iso*-PGE_2 in the aforementioned studies were almost completely abrogated by thromboxane A_2 receptor antagonists, suggesting that these isoprostanes might act via a common thromboxane receptor. Although 8-*iso*-$PGF_{2\alpha}$ does functionally interact with the thromboxane A_2 receptor [51], binding characteristics and functional studies suggest that its physiological plasma concentrations are far below the micromolar concentrations required to affect platelet responses mediated by the thromboxane A_2 receptor *in vivo* [52].

In contrast, 8-*iso*-$PGF_{2\alpha}$ and 8-*iso*-PGE_2 are much more potent than thromboxane A_2 receptor agonists in inducing a functional response on vascular smooth muscle cells [45,53], suggesting that other mechanisms mediate these effects *in vivo*. Low (K_d = 0.94 μM) and high (K_d = 32 nM) affinity binding sites were found for 8-*iso*-$PGF_{2\alpha}$ that have been attributed to binding of 8-*iso*-$PGF_{2\alpha}$ to the thromboxane A_2 receptor and to a hypothetical high-affinity isoprostane receptor that is also sensitive to thromboxane A_2 receptor antagonists, respectively [54,55]. In addition, a novel mechanism for the vasoconstrictor action of 8-*iso*-$PGF_{2\alpha}$ on retinal blood vessels was reported recently. In the eye, 8-*iso*-$PGF_{2\alpha}$ may contract retinal vessels with an EC_{50} of about 6 nM via stimulation of endothelin release and PGHS-generated thromboxane formation [50]. 8-*iso*-$PGF_{2\alpha}$ also has a pronounced effect on smooth muscles other than those of the vasculature and might cause bronchoconstriction [56–58], as well as contraction of peripheral lymphatics [59].

Much less information is available on the biological activities of other isoprostanes. For instance, 12-*iso*-$PGF_{2\alpha}$ was

shown to functionally activate the ocular $PGF_{2\alpha}$ receptor, albeit less potently (EC_{50} = 5 μM) than $PGF_{2\alpha}$ (EC_{50} = 10 nM) [60], whereas several other F_2-isoprostanes, including 8-*iso*-$PGF_{2\alpha}$, caused little or no activation. Other established biological activities of 12-*iso*-$PGF_{2\alpha}$ include stimulation of mitogenesis in NIH 3T3 cells [60] and hypertrophy of cardiomyocytes [61]. Interestingly, 12-*iso*-$PGF_{2\alpha}$ was a weak agonist of the heart myocyte $PGF_{2\alpha}$ receptor but a potent inducer of myocyte hypertrophy (IC_{50} ~150–200 nM) via a different signaling pathway to that activated by $PGF_{2\alpha}$.

Apparently, there are several mechanisms by which multiple species of isoprostanes induce vasoconstriction, bronchoconstriction and mitogenesis. Some isoprostanes appear to react incidentally with prostaglandin receptors (often in the micromolar range) but there is evidence that some of the isoprostanes might interact at low concentrations (nanomolar range) with specific isoprostane receptor(s). None of these putative isoprostane receptors has been cloned, however.

Isoprostanes might cause physical damage and dysfunction of membranes

Free radicals cause lipid peroxidation that might result in the alteration of the physical and functional properties of cellular membranes, such as plasma leakage and modification of transport properties. Under physiological conditions, the respiratory chain, the photosynthetic apparatus of plants and cytochrome P450 enzymes are thought to generate the vast majority of reactive oxygen species. It might therefore be expected that isoprostanes would be found in the corresponding membranes of mitochondria, chloroplasts, endoplasmic reticulum and nuclei. In addition, autoxidation processes, the oxidative burst of leukocytes and elicited plant cells, and chemical oxidants presumably lead to an accumulation of isoprostanes in the cell membrane. The subcellular distribution of isoprostanes and their effect on specialized membranes is not known, however.

As isoprostane-containing phospholipids are extremely kinked molecules, they are expected to have profound effects on the fluidity and integrity of membranes [25]. *In vitro* studies on artificial membranes suggested that structural and functional membrane alterations occur when the oxidation of the membrane phospholipids exceeds 3–5% [62]. Such a degree of oxidation has been reported for mitochondrial membranes of rabbit reticulocytes that are degraded during the maturation of red blood cells [63]. In tissues with enhanced free radical production, such as in atheriosclerotic lesions, a hydroperoxylinoleate/linoleate ratio of 0.1–1% and an isoprostane/arachidonate ratio of about 15 ppm was found, suggesting that isoprostane-containing phospholipids are only minor components of a much larger pool of oxidized lipids [62]. The possible effect of elevated isoprostane-containing phospholipid levels on the physical properties of membranes remains to be demonstrated *in vivo*.

Isoprostanes are accurate and sensitive markers of oxidant stress

Although isoprostanes have been used as markers of oxidant injury for only a few years, an increasing number of studies has demonstrated that they represent extremely sensitive and reliable markers of oxidative stress [64], correlating well with other classical oxidative stress indices [65]. As common markers of oxidative stress such as lipid peroxides, malondialdehyde, alkanes and aldehydes are derived, at least in part, from enzymatic processes, are metabolically or chemically unstable and are, in some cases, volatile, they have often been unreliable as a measure of free radical injury *in vivo* [66]. F_2-isoprostanes overcome most of these limitations because they are chemically stable end products generated by a free-radical-catalyzed process from specific fatty acids. As they are esterified in membranes, they represent a long-lasting marker of oxidant injury and enable the site of endogenous lipid peroxidation to be identified [26]. Furthermore, urinary isoprostanes and isoprostane metabolites represent an ideal noninvasive index of systemic oxidative stress.

In most studies, the isoprostane 8-*iso*-$PGF_{2\alpha}$ has served as a marker for isoprostane formation and oxidative stress *in vitro* and *in vivo*, but $iPF_{2\alpha}$-VI would be an attractive alternative because it is more abundant in urine [67]. F_4-isoprostanes generated from docosahexaenoic acid could be suitable markers in special tissues such as brain, which is particularly enriched with this fatty acid [68,69]. Isoprostanes can be measured in plasma, in urine or after hydrolysis of tissue phospholipids by radio- and enzyme-immunoassay or — more specifically — using GC–MS [70].

Increased plasma and urinary isoprostanes have been measured after intoxication and in several syndromes suspected to be associated with excessive generation of free radicals, including poisoning with paraquat [71] and CCl_4 [72], halothan treatment [73], smoking [74], alcohol-induced liver disease [75], hepatic cirrhosis [76], coronary [77] and liver [78] reperfusion after ischemia, and atherosclerosis (in atherosclerotic lesions) [62]. Elevated isoprostane levels were also measured in patients with hepatorenal syndrome [79], scleroderma [80] and hypercholesterolemia [81]. In addition, isoprostane analysis greatly exceeds the value of other standard approaches to assess the effectiveness of antioxidants *in vitro* and *in vivo* and might help to establish reliable dose–response relationships of antioxidant treatments in clinical trials [82–86].

As plants do not produce eicosanoids, dinor isoprostanes derived from α-linolenic acid could, potentially, be used as an index of oxidative stress status in plants or as a measure of oxidative degradation of plant products.

Isoprostanes – a perspective for future research

The discovery of isoprostanes as products of free-radical-induced lipid peroxidation has opened up new avenues of research regarding the role of oxidant injury in human and plant physiology. Not only has it been shown that isoprostanes increase during several human disorders associated with enhanced free-radical generation but also that several isoprostanes exert potent biological effects such as vasoconstriction, bronchoconstriction and mitogenesis. Detailed physiological investigations into how these events might be linked *in vivo* have only just begun, however.

Reactive oxygen species have also been implicated in oxidative damage of plant organs and as mediators of plant systemic acquired resistance against pathogens. The principal inducible defenses against reactive oxygen species in plants include detoxifying enzymes (e.g. catalase and superoxide dismutase), low molecular weight secondary products with antioxidant activity and activation of pathogenesis related genes. Little is known about how reactive oxygen species trigger these responses. Plant dinor isoprostanes might, by analogy to the mammalian pathway, play a functional role but this still remains to be elucidated. It will be exciting to see if isoprostanes have any general functions in response to oxidative stress in both plants and animals and to elucidate their full spectrum of biological activities.

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