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Enzymic lipoxygenation of arachidonic acid: mechanism, inhibition, and role in eicosanoid biosynthesis

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Abstract: Studies on the lipoxygenation of arachidonic acid by various enzymes have been carried out using diverse approaches. A mechanistic picture of this reaction has been developed which both correlates known information and assists in the rational design of new lipoxygenase inhibitors. A working hypothesis is advanced for the biosynthesis of prostaglandins in mammalian systems. An entirely different mechanism is proposed for the biosynthesis of prostaglandins and related eicosanoids in marine organisms.

INTRODUCTION

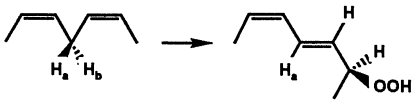
Enzymic lipoxygenation of polyunsaturated fatty acids, such as linoleic or arachidonic acids, transforms a *Z,Z*-1,4-diene subunit, R^1-CH=CHCH$_2$CH=CH-R^2, into a *Z,E*-pentadienyl hydroperoxide subunit, R^1-CH=CH-CH=CHCH(OOH)-R^2. The process is of unusual interest for a number of reasons. It is a critical and fundamental reaction in lipid biochemistry. It initiates the biosynthesis of biologically and medically important eicosanoids such as prostaglandins (PG's) and leukotrienes (LT's) from arachidonic acid. In addition the lipoxygenation reaction, which has never been simulated chemically, appears to proceed by a unique pathway that has no known mechanistic counterpart.

Lipoxygenation of polyunsaturated fatty acid can in principle occur at 2n-2 carbon sites where n is the number of *Z* double bonds separated by methylene along the molecular chain. In the case of arachidonic acid (**1**) the lipoxygenation (LO) reaction could occur at six carbon sites (carbons 5,8,9,11,12,15) and its versatility as a biosynthetic precursor stems partly from this fact. Currently known LO pathways for arachidonic acid include 5-LO (LT biosynthesis, potato lipoxygenase), 8-LO (coral, soybean lipoxygenase), 11-LO (PG's), 12-LO (blood platelets), 15-LO (reticulocytes, endothelial cells, soybean lipoxygenase) as summarized below.

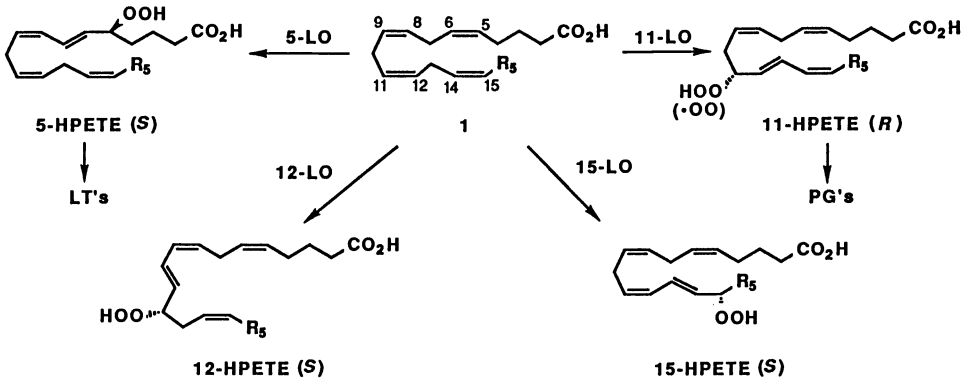
A major aim of research over the past several years has been to gain a better understanding of the LO reaction so as to facilitate the conceptually guided design of specific LO inhibitors. The well-known, non-steroidal anti-inflammatory agents such as aspirin, piroxicam and naproxen, function by virtue of their effectiveness as 11-LO (PG-synthase) inhibitors. Compounds which block the other LO pathways of arachidonate might be useful as medical research tools or therapeutic agents. The sections which follow deal with this topic and also with the related subject of the subsequent steps of eicosanoid biosynthesis. The approach taken integrates multistep chemical synthesis, stereochemistry, radiochemical structure determination, kinetics of enzymic reactions, and mechanistic reasoning.

1. 5- AND 15-LIPOXYGENASE SYSTEMS

The most readily available and widely studied lipoxygenase is that from soybean which is known to convert arachidonate to 15-*S*-HPETE by an antarafacial process (assuming a maximally extended conformer), which so



far seems to be common to the plant derived LO's.[2] The soybean LO, is a fairly stable enzyme, MW *ca.* 100,000, containing one non-heme iron per molecule.[3] In the native state the iron is in the high-spin Fe(II) form, but in the catalytically active enzyme iron is in the high-spin Fe(III) (d^5) state.[4] ESR experiments have allowed the detection of free radicals in air-free solutions of soybean LO and substrate, leading to a proposal that pentadienyl radicals are intermediates in the LO process.[5] The LO-produced hydroperoxide is capable of converting the native (colorless) Fe(II) form of soybean LO to the yellow Fe(III) form (UV absorbtion at 330 nm),[6] an explantion for the fact that the induction period observed for the LO reaction with soybean LO is abolished by addition of product hydroperoxide. The kinetic isotope effect for methylene deuterated substrate (kH/kD) in the LO reaction is *ca.* 9; a kinetic isotope effect is also observed in the activation of native enzyme by substrate and O_2.[6b] Soybean LO



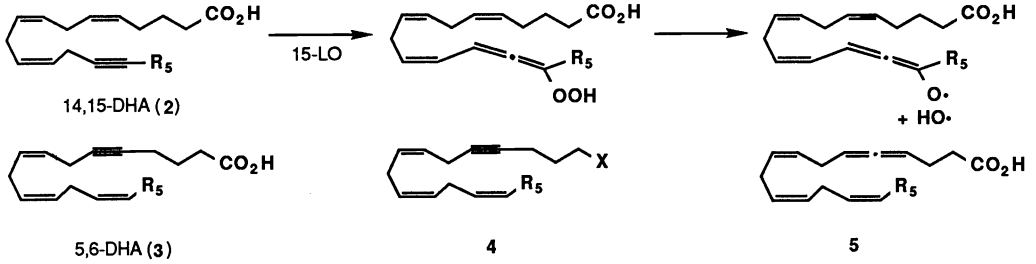
catalyzes other processes including: (1) further lipoxygenation of arachidonate to an 8,15-*bis*-hydroperoxide, (2) its own aerobic inactivation (total turnover numbers of *ca.* 10^4 are observed),[7] and (3) anaerobic conversion of 15*(S)*-HPETE to a 13*(R)*-hydroxy-15*(S)-trans*-14,15-epoxy-5,8,11*(Z)* eicosatetraenoic acid.[8] Soybean LO has lipophilic region(s) which enable it to bind fatty acids, one possible explanation of the observation that high levels of substrate depress the rate of the LO reaction.[9]

The 5-LO reaction of arachidonate is a more recent topic which is also more difficult because of the instability of the known 5-LO enzymes (sources: leukocytes,[10] rat basophilic leukemic cells (RBL-1),[11] and potato[12]) and their paucity. The 5-LO's from leukocytes and RBL-1 cells require Ca^{++} and ATP and appear to be stimulated or regulated by various factors.[10] Little is known regarding the catalytic site of the 5-LO's.

Because of importance of the 5-LO pathway leading to leukotrienes,[13,14] and the background of information on the soybean LO system, it seemed reasonable to carry out a parallel investigations with both systems.

The first approach to the development of new and specific LO inhibitors derived from a relatively simple idea which was based on reaction stoichiometry rather than mechanism. It was conjectured that 14,15-dehydroarachidonic acid (**2**,14,15-DHA) might serve as an acceptable substrate for the soybean 15-LO and that the vinylic hydroperoxide which might be produced would undergo homolysis to radicals capable of producing irreversible deactivation of the enzyme. Homolysis of a vinylic hydroperoxide ought to be facilitated by the extra stabilization of the resulting vinyloxy radical (estimated *ca.* 18 kcal./mole) for such decomposition. Accordingly 14,15-DHA was synthesized and its effect on the lipoxygenation of arachidonate was studied.[15] 14,15-DHA, even at concentrations below 1 μM,.was found to deactivate soybean 15-LO (Sigma, type 1 at 23° and pH 9.2) irreversibly in a time-dependent manner in the presence of O_2, but not in its absence. The efficiency of deactivation was *ca.* 0.4% (260 molecules of 14,15-DHA required to inactivate one molecule of enzyme). The three other possible monoacetylenic arachidonic acid analogs in which either the 5,6*(Z)*,8,9*(Z)*, or 11,12*(Z)*-olefinic linkage is replaced by a triple bond (5,6-DHA, 8,9-DHA or 11,12-DHA) were also synthesized[16] and studied as inhibitors of the soybean lipoxygenase. None of these DHA's caused irreversible inactivation of soybean LO in the presence of O_2 even at 100 μM. Only modest competive inhibition was observed with arachidonate as substrate, and indeed 5,6-DHA itself was found to serve as a substrate for the enzyme. An experiment in which the soybean LO was incubated with 2-tritiated 14,15-DHA in air produced inactive enzyme which could not be freed of tritium even after exhaustive extraction and reversed phase chromatographic fractionation.[15] The radioactivity remaining on the deactivated enzyme corresponded to that calculated for 0.87 molecules of tritiated 14,15-DHA per molecule of soybean LO. It thus appears possible that inhibitor and inactivated enzyme become covalently linked.

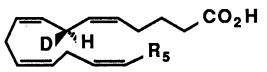
These results prompted a study of the effect of 5,6-DHA (**3**) on the 5-LO of RBL-1 cells which was carried out using a partially purified, but still relatively unstable, preparation. It was not surprising because of the results described above that 5,6-DHA inhibited the conversion of arachidonate to 5-LO products by the RBL-1 derived enzyme. The RBL-1 5-LO was irreversibly inactivated in the presence of 5,6-DHA, O_2 and Ca^{++} in a time-dependent manner and at a rate several hundred times faster than measured in appropriate control experiments.[17]



In the absence of Ca^{++} or O_2, 5,6-DHA did not cause deactivation of the 5-LO enzyme. These results coupled with the finding that the amide of arachidonic acid is converted to 5-HPETE amide by the 5-LO enzyme encouraged the study of a number of related compounds in which the carboxylic group of **3** was replaced by a polar surrogate as in **4**. The rates of inactivation of the analogs **4** relative to 5,6-DHA were found to be as follows for different X groups in **4**: $-PO_3H^-$, 0.53; $-SO_3^-$, 0.53; $-P(OCH_3)O_2^-$, 0.67; $-SO_2^-$, 1.14; $CONH_2$, 1.26; $(P{=}O)(OCH_3)_2$, 1.68; $S(=O)CH_3$, 2.26; $S(=O)_2CH_3$, 3.12.[18]

The allenic 4,5-dehydroarachidonic acid **5** was also synthesized and tested as a 5-LO inhibitor. As anticipated from the results with 5,6-DHA, **5**, which upon lipoxygenation would be converted to an unstable vinylic hydroperoxide, caused irreversible time - and O_2-dependent deactivation of the RBL-1 5-LO enzyme.[19]

It was possible to show experimentally that the irreversible deactivation of the RBL-1 5-LO enzyme by 5,6-DHA occurs as a result of hydroperoxide formation in the following way. First 7*(R)*-deuterioarachidonic acid (**6**) was synthesized by a multistep process from tartaric acid and then subjected to the 5-LO reaction.[20] The 5*(S)*-HPETE obtained therefrom was shown to retain the deuterium label using both RBL-1 and potato 5-LO enzymes.[20] This established the antarafacial stereochemistry for these reactions. 7*(S)*-deuterio-5,6-DHA was synthesized by a process parallel to that used for **6** and then studied kinetically as an irreversible inhibitor of the RBL-1 5-LO enzyme.[18] Irreversible deactivation was observed to occur with this deuterated 5,6-DHA at a rate at least one-seventh that for 5,6-DHA itself.[18] The occurrence of this stereospecific kinetic isotope effect coupled with the requirement of O_2 for deactivation shows that stereospecific lipoxygenation is involved in the aerobic inactivation of the RBL 5-LO by 5,6-DHA.



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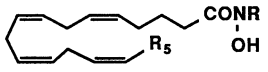

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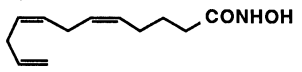

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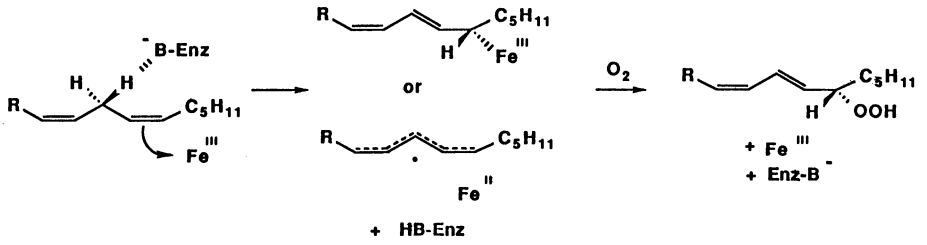
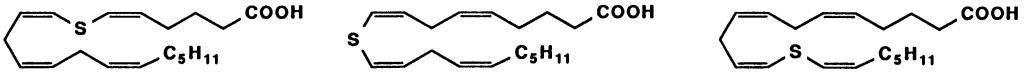
The correspondence of the results obtained for the soybean 15-LO and 14,15-DHA with those found for the RBL 5-LO and 5,6-DHA suggested that the basic catalytic mechanisms might be similar and therefore that Fe(III) might be part of the catalytic unit in the RBL 5-LO. Since arachidonic amide can serve as a substrate for the 5-LO it is clear that the 5-LO can accept a non-ionized substrate. Further since the Fe(III) center is likely to be proximate to the 5,6-double bond and the carboxylic group of arachidonic acid, it seemed of interest to test inhibitors in which a strong Fe(III) binding ligand was affixed to C(1) of arachidonate. The obvious candidate for such a ligand was the hydroxamic acid function. The N-hydroxyarachidonamides **7**, R=H, CH_3 and *t*-Bu, were all found to be excellent competitive inhibitors of the RBL 5-LO with *Ki* values of 0.13, 0.04, and 0.11 μM, respectively.[21] That the full eicosanoid chain is not required for inhibition was demonstrated by the finding that truncated hydroxamate analogs such as **8** (*Ki* = 1.9 μM) also were good inhibitors of the 5-LO. These results support the proposition that the catalytic process is similar for the soybean 15-LO and RBL 5-LO enzymes and that both involve Fe(III) as a critical element.

In order to test this possibility further and also to help design other classes of enzyme activated inhibitors it was desirable to develop mechanistic working hypotheses for the LO reaction. Two reasonable possibilities are shown in Scheme I. In each of these the removal of hydrogen is postulated to occur by proton transfer to a basic group on the enzyme with strong assistance arising from proximity of Fe(III) (more specifically a vacant coordination site) to the carbon which eventually is oxygenated. The critical assistance to deprotonation could be provided either by carbon-iron sigma bonding or electron transfer to iron, the former producing a σ-organoiron intermediate and the latter a free radical. The process is then completed by reaction with O_2 to generate LO product and catalytically active enzyme. A modified version of the mechanism in which the cleavage of the C-H bond occurs by hydrogen atom transfer to an enzymic free radical such as RS• or ArO• is also possible. Such a radical-mediated process is illustrated by the hypothetical sequence: Fe(III) + Enz-S^- → Fe(II) + Enz-S•; Enz-S• + RH → Enz-SH + R•; R• + Fe(II) + O_2 → ROO^- + Fe(III). The large value of the kinetic isotope effect for lipoxygenation (kH/kD = *ca.* 9) argues slightly in favor of a proton abstraction (Scheme 1) rather than a hydrogen atom abstraction mechanism,[22] but certainly does not allow a conclusion.

Scheme I



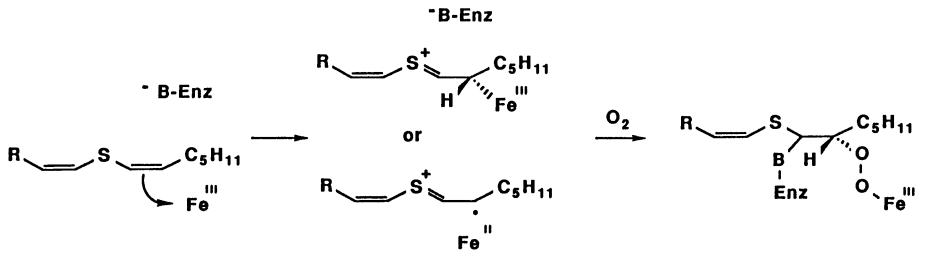
Stimulated by these mechanistic possibilities, the synthesis and study of the three arachidonic acid analogs in which each doubly allylic methylene group is replaced by sulfur was undertaken. 7-Thia-, 10-thia-, and 13-thiaarachidonic acids (**9**,**10**,**11**) were synthesized by unambiguous stereospecific routes.[23,24] 7-Thiaarachidonic acid (**9**) serves as a substrate for soybean 15-LO under standard conditions (pH 9.2, 0.2 M sodium borate buffer at 22°) and is converted to the 15-hydroperoxide, UV max at 234 nm. Similarly, 10-thiaarachidonic acid (**10**) is cleanly transformed by soybean 15-LO to the 15-hydroperoxide which displays characteristic UV absorption at 286 nm due to the chromophore S-CH=CH-CH=CH.[25] In contrast, 13-thiaarachidonic acid (**11**) is not a substrate for this 15-LO but is instead an irreversible inhibitor of the lipoxygenation of arachidonic acid.[25] Kinetic studies of this inhibition revealed an O_2 and time dependence characteristic of enzyme-induced self inactivation. Rates of enzyme inactivation were measured by incubating **11** at concentrations of 0 to 25 μM with soybean 15-LO (0.7 μg/ml, 6 nM) at pH 9.2 and 22° in air for varying times (0-10 min), adding arachidonic acid and measuring the change in UV absorbance at 236 nm to determine initial velocity of the lipoxygenation of arachidonic acid.[6,7] An apparent rate constant was determined by plotting log (percent remaining activity) vs preincubation time. Double reciprocal plots of apparent k vs conc. of inactivator **11** were linear in the range 0.3-2.4 μM. The reciprocal of the y-intercept provided the rate constant for inactivation, $ki = 0.41$ min^{-1} and the negative reciprocal of the x-intercept gave the binding constant for **11**, $Ki = 0.8$ μM. At 1 atm of O_2 the rate of inactivation was approximately double the rate in air, whereas under an atmosphere of argon, inactivation was barely observable at 10 min. Activity of soybean 15-LO inhibited by incubation with **11** could not be restored by dialysis. The Ki value of 0.8 μM indicated that **11** is more tightly bound to SB-LO than is arachidonic acid (Km = 13 μM).[25]



9 **10** **11**

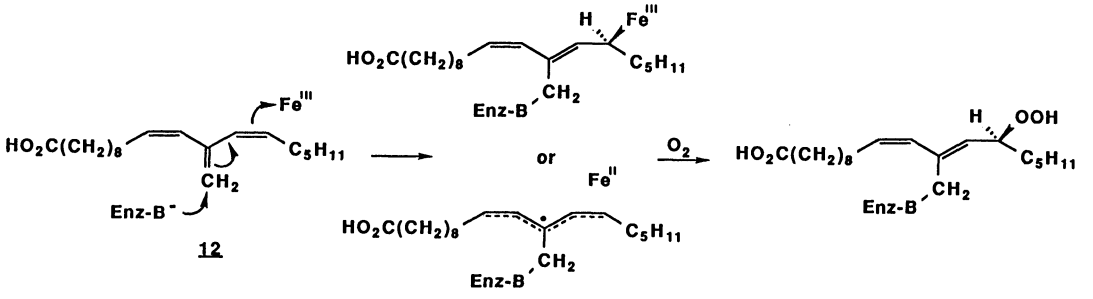
The sulfoxide of **11**, prepared by periodate oxidation, was not a substrate for soybean 15-LO and did not cause irreversible inactivation, but behaved simply as a competitive inhibitor, $Ki = 66$ μM.[25] These data on the inhibition of soybean 15-LO by **11** and the corresponding sulfoxide are most simply understood in terms of the normal lipoxygenase pathway outlined in Scheme I. For the thiaarachidonate **11**, which lacks an abstractable hydrogen but is π-electron rich, the analogous reaction mode is that expressed in Scheme II. According to this mechanism the inhibition of soybean 15-LO should be irreversible as a consequence of covalent attachment of the enzyme via the proton acceptor function to C(14) of the thia acid **11**. The inability of the sulfoxide of **11** to effect irreversible deactivation is consistent with the greatly reduced availability of electrons in the 14,15-double bond of the sulfoxide.[25] The contrasting behavior of **11** and the corresponding sulfoxide, which is easily understood in terms of the mechanisms summarized in Scheme II, argues strongly against the alternative pathways for lipoxygenation in which hydrogen abstraction is effected by a radical such as RS•, as mentioned above. Such a catalytically active radical should be trapped effectively both by **11** and the corresponding sulfoxide, contrary to the experimental findings.[25]

Scheme II



The RBL-1 5-LO enzyme was found to suffer time-dependent irreversible deactivation when incubated aerobically with 7-thiaarachidonate (**9**).[23] Control experiments showed that the 5-LO enzyme was completely stable in air over time periods more than sufficient for complete deactivation by **9**. The rate of deactivation of the 5-LO was greatly reduced when the air atmosphere was replaced by argon. The sulfoxide corresponding to **9** and the corresponding sulfone did not cause irreversible inactivation of the 5-LO enzyme, but instead, served as reversible competitive inhibitors.[23] The behavior of the 7-thia acid **9** with respect to the RBL-1 5-LO enzyme thus parallels exactly the observations made for the 13-thia acid **11** with the soybean 15-LO. The same mechanistic arguments therefore apply both to the 5-LO and 15-LO systems.

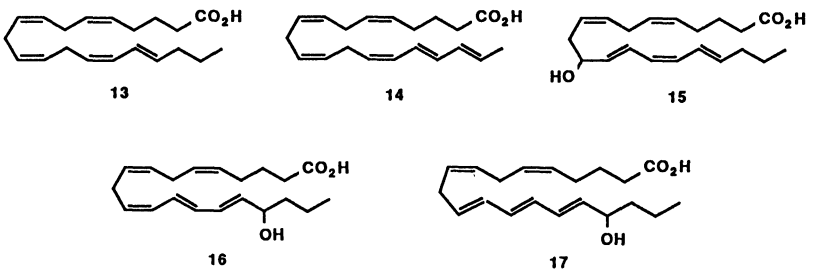
Scheme III



If the mechanism of lipoxygenation is as outlined in Scheme I, it would appear reasonable that the 13-methylidene analog of norlinoleic acid **12** could serve as an inactivator of the soybean 15-LO. The trienoic acid **12** was readily synthesized (from commercially available undeca-10-ynoic acid) and evaluated with the 15-LO enzyme.[26] Incubation of **12** with soybean 15-LO resulted in time- and dioxygen-dependent irreversible inactivation of the enzyme. Activity could not be restored by dialysis. The *K*i and *k*i values found for **12** from kinetic studies were 35 μM and 0.82 min.$^{-1}$ For comparison *K*i and *k*i values for deactivation of the soybean 15-LO by the 13-thia acid **11** were found to be 0.8 μM and 0.4 min.$^{-1}$. The sequence of events summarized in Scheme III is consistent with all of our observations.[26]

In Scheme I two alternative mechanistic pathways for enzymic lipoxygenation[25,26] are presented which differ with regard to whether the reactive intermediate in the dioxygenation process is a pentadienyl radical or an organoiron compound. Consideration of these alternatives brings to the fore several extremely interesting questions. If the LO product is formed by way of a free radical how is tight enzymic control of the position specificity and stereospecificity achieved? Why does the radical not collapse to a σ-organoiron intermediate? Is molecular dioxygen present before the radical is formed or does the radical have to "wait" for its arrival? On the other hand if the carbon-dioxygen bonding step involves a σ-organoiron intermediate, does the replacement of σ-iron by oxygen occur by stereochemical inversion or retention; in one or two steps; by electron transfer or oxidative addition to O_2? How stable is the organoiron intermediate relative to homolytic decomposition to form the pentadienyl radical? The fact that the dioxygenation occurs after the rate limiting step makes these questions all the more interesting and difficult to answer.

In order to gain an insight as to which of these pathways, organoiron or radical, corresponds to the enzymic process, the dehydro arachidonic acid **13** (16,17-DHA) and the bisdehydro arachidonic acid **14** (16,17-,18,19-*bis*-DHA) were synthesized and tested as substrates and inhibitors of the soybean 15-LO.[27] It was found that 16,17-DHA (**13**) serves as a substrate for the soybean enzyme which transforms it into a mixture of products (25 μM **13**, 23°C, pH 9.2 borate buffer, air atmosphere, 5 nM soybean 15-LO) which were identified after reduction as **15**, **16** and **17** in a ratio of 2:85:13 (HPLC analysis). The UV spectra of **15** (258, 268 (max), 278 nm), **16** (262, 272 (max), 282 nm), and **17** (258, 268 (max), 279 nm) show that each possesses a conjugated triene chromophore. None of the "normal" 15-hydroperoxide product, which would have a conjugated diene chromophore and be readily detected by HPLC and UV analysis, was observed. The structure of the major product **16** was determined unequivocally by UV, PMR, and mass spectral studies and it was shown to be *racemic* by conversion to the (l)-menthyloxycarbonyl derivative, ozonolysis, and esterification (CH_2N_2) followed by gas chromatographic comparison with the (l)-menthyloxycarbonyl derivative of methyl *(R)*-2-hydroxypentanoate.[27] It is interesting that the major product **16** is *racemic* . Also noteworthy is the observation that at 4°C or under higher pressures of O_2 (50 atm vs 0.2 atm of O_2 in air) the formation of the minor products is almost completely suppressed. A simple explanation of these findings might be that an organoiron intermediate is the precursor of the major product **16** and that minor products **15** and **17** arise by homolysis of that intermediate to a free radical which is trapped by O_2 at carbons **11** and **17**. It is clearly less attractive to suppose that all three products (**15**, **16**, and **17**) are derived from a common heptadienyl radical precursor.



13 14 15

16 17

Kinetic studies[27] with 16,17-dehydroarachidonic acid (**13**) reveal values of Km = 3.4 μM and Vmax (turnover number) = 44 sec^{-1} which compare with those for arachidonic acid, Km = 10 μM and Vmax 250 $sec.^{-1}$ In parallel experiments under the same conditions the total turnover number (total maximum conversion of fatty acid to LO product per molecule of soybean 15-LO) for **13** was always considerably less than for arachidonate. For example at 10 μM fatty acid and 0.5 nM enzyme at 23° in air total turnover numbers of 1200 for **13** and 13,000 for arachidonic acid were determined. Deactivation of soybean 15-LO during lipoxygenation does not seem to be due to a time-dependent reaction involving the product hydroperoxide as indicated by the following experiments. It is known that the soybean LO is unaffected by the presence of sodium borohydride which serves to reduce 15-HPETE to 15-HETE very rapidly.[28] Measurement of total turnover numbers for arachidonate and **13** showed that neither was changed significantly by the presence of sodium borohydride. Thus enzyme deactivation by **13** is more efficient than for arachidonate. This result is also more simply explained in terms of a 15-LO pathway in which product is formed by combination of dioxygen with an organoiron intermediate rather than a radical. For such a mechanism enzyme self-inactivation would be understandable as a result of homolysis of the organoiron intermediate to generate a radical which is capable of destroying catalytic function, clearly the organoiron compound resulting from **13** would be more prone to homolysis than that from arachidonate and a lower total turnover would be expected for **13**. The formation of (±)-**16** the major enzymic product from the reaction of **13** may be interpreted on the basis of a σ-organoiron intermediate either as attack by O_2 at C(17) on either a C(15)-σ-iron intermediate or a C(17)-σ-iron intermediate; the step in which stereospecificity is lost cannot be identified.

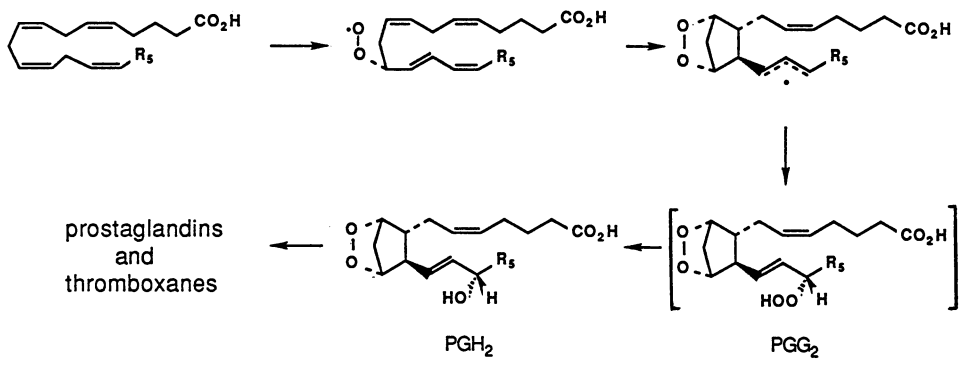
The bisdehydroarachidonic analog **14** was found not to be substrate for the soybean 15-LO enzyme. The reason for this is that **14** is both a rapidly acting and strong competitve inhibitor of the enzyme (*Ki* 0.2 μM measured as an inhibitor of arachidonic acid oxidation at time approaching zero) and a time-dependent, irreversible deactivator.

Whether the mechanism of incorporation of dioxygen into LO products involves capture of a pentadienyl radical or a σ-pentadienyliron precursor is obviously far from settled. However, the evidence thus far seems to weigh in favor of the latter possibility. The case for the intermediacy of a σ-organoiron derivative also deserves attention because this mechanism provides the clearest explanation for (1) facilitation of the initial deprotonation step (Lewis acid catalysis) and (2) stereocontrol and stereospecificity in the oxygenation step. The postulate of an σ-organoiron intermediate is not necessarily inconsistent with the observation of free radicals in LO systems.[3,5] Homolysis of σ-pentadienyliron structures may be so facile that the formation of a sufficient number of radicals to be detected by electron spin resonance is possible.

2. DETAILED HYPOTHESIS FOR THE BIOSYNTHESIS OF PROSTAGLANDINS AND THROMBOXANES FROM ARACHIDONATE

The biosynthesis of prostaglandins from arachidonate ranks as one of the most impressive of nature's achievements in molecular modification and one of the least likely to be simulated chemically in the near future. A single enzyme in combination with a heme cofactor (e.g. protoporphyrin IX or hematin) transforms arachidonate to PGG_2 (or bound equivalent) and thence to PGH_2. The prostaglandin H synthase functions both as a cyclooxygenase to form the PGG_2 structure and as a peroxidase to generate PGH_2 by reduction of the O-O linkage of the 15-hydroperoxy group.[28] Scheme IV outlines the mechanistic rationale for the biosynthesis of PGG_2 which is commonly presented in the literature. In this section we address the following question: Is it possible to formulate a more detailed and rational working hypothesis for the PGH synthase catalyzed pathway based on the ideas developed for the lipoxygenation process in the foregoing section and the facts now available regarding the PGH synthase itself? A major issue is the matter of how the enzyme maintains control over the biosynthesis with regard to position and stereoselectivity. There is no inherent preference for the stereochemical pathway shown in Scheme IV with regard to the arrangement of appendages on the dioxabicyclo[2.2.1]heptane (endoperoxide) nucleus. In fact attempts at chemical simulation of the radical pathway for endoperoxide formation have produced exclusively product in which the two appendages on the nucleus are *cis* to one another.[29] The control of stereochemistry at C(11) and C(15) also require strong direction for the enzyme.

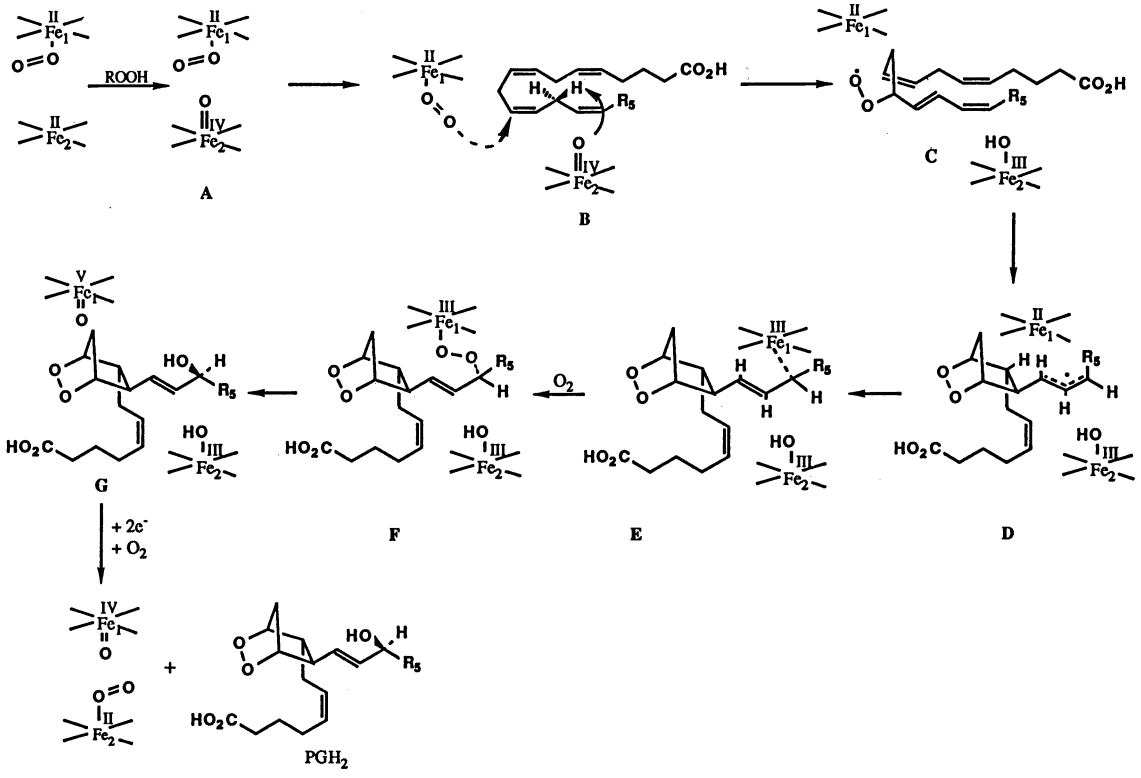
Scheme IV



The following information is now available regarding the structure and function of the PGH synthase. The initial removal of H from C(13) and attachment of oxygen at C(11) (to give the 11*(R)*-configuration) occur with antarafacial stereochemistry[30] relative to the extended chain conformation. PGH synthase from seminal vesicles, where it occurs bound to membrane, has been purified and found to be a homodimer of subunits having MW *ca.* 70,000,[28a,33] each of which contains two molecules of tightly bound iron protoporphyrin IX as an essential cofactor.[28c,31b,c] The native ferrous enzyme is catalytically inactive. It is activated by PGG_2 or other ("activator") lipid hydroperoxides. PGH synthase is not inhibited by carbon monoxide.[32] The enzyme is inhibited by non-steroidal anti-inflammatory agents such as aspirin, phenylbutazone, piroxicam, naproxen, and indomethacin, as well as by various phenolic antioxidants. Although heme-containing enzyme functions both as a PG endoperoxide synthase and a peroxidase, enzyme containing manganese protoporphyrin in place of heme shows the former catalytic activity but not the latter.[32] Aspirin acetylates a serine hydroxyl of the enzyme with consequent loss of cyclooxygenase but not peroxidase activity.[33] PGH synthase is converted on a millisecond time scale by lipid hydroperoxides to higher valent iron species which may correspond to the catalytically active enzyme.[34] Cooxidation reactions of a variety of oxidizable molecules have been observed during prostaglandin biosynthesis[35,36] and appear to involve oxo-ferryl species. During *in vitro* PG biosynthesis the reduction of such peroxidase produced oxo-ferryl species is effected by the inclusion of a reducing agent such as tryptophan, epinephrine, or hydroquinone.[28c]

Taking into account the data available on PGH synthase and the findings outlined above on the lipoxygenation process it is possible to construct a number of plausible mechanistic schemes for PG biosynthesis. One such possibility is outlined in Scheme V. It is based on the speculation that two hemes (of the four present in PGH synthase homodimer) may be involved in the removal of hydrogen and delivery of oxygen to arachidonate. Starting from a resting state in which Fe_1 and Fe_2 are ferrous, activation by hydroperoxide could produce state A in which one iron (Fe_2) is oxidized to the Fe(IV)-porphyrin ↔ Fe(III)-porphyrin radical cation state and the other iron (Fe_1) is ferrous (perhaps coordinated to dioxygen). Binding of arachidonate then follows with removal of hydrogen and delivery of O_2 (or Fe_1 followed by O_2) as in B so as to lead to C. Cyclization of the conformer shown in C then produces D which by a rotation brings the back face of the allylic radical into bonding with Fe_1 to produce a 15*(S)*-σ-organoiron species E. Oxidative addition of O_2 at C(15) of E leads to the peroxidase-like intermediates F and then G. In G, Fe_1 is in the oxoferryl state (most likely Fe(IV)•porphyrin radical cation) which results as a consequence of PGH_2 formation from the 15-hydroperoxy state. One electron reduction of Fe_1 and of Fe_2 in G converts these to the original catalytically active form of the enzyme assuming Fe_1 and Fe_2 to occupy equivalent sites (which implies that they reside in different subunits rather than in the same subunit), allowing catalysis to continue without the need for activation by ROOH. On the other hand, further reduction of Fe_1 in G

Scheme V

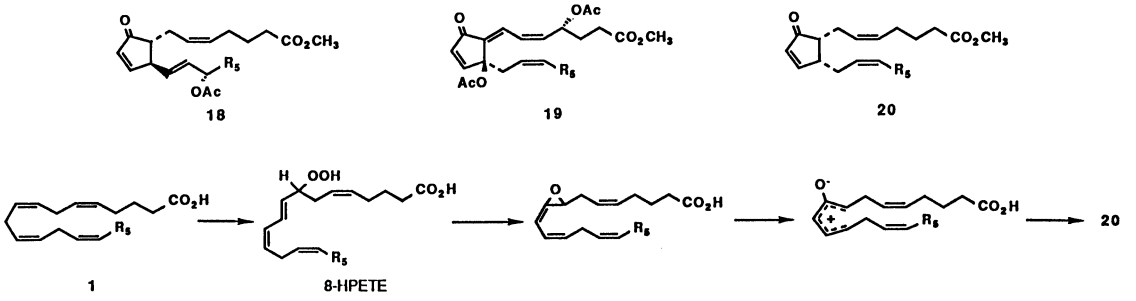


(a cooxidation process) could deactivate the catalytic center such as to require oxidative activation by ROOH. Although this hypothesis is speculative, and doubtless not correct at the level of fine detail, it provides a satisfying picture which may be of heuristic value. A clear depiction of how stereochemistry may be controlled and how cyclooxygenase and peroxidase activities of the PGH_2 synthase are linked emerges from this model. The general pathway outline in Scheme V also is in accord with current views on peroxidase mechanisms[37] and other redox processes involving iron porphyrins.[38] Present knowledge would suggest the likelihood that an imidazole ligand is attached to each iron as a sixth ligand. Scheme V does not include the binding interactions between enzyme, heme units and substrate which are obviously critical. No role is assigned to the remaining two heme units, although it is possible that these may be involved in election transfer to maintain the catalytic irons, Fe_1 and Fe_2, at the proper oxidation levels.

3. BIOSYNTHESIS OF MARINE EICOSANOIDS

The Caribbean gorgonian *Plexaura homomalla* is uniquely a rich source of prostaglandin A_2 methyl ester acetate (**18**) (*ca.* 1.8% dry weight).[39] Evidence has been presented that this PGA_2 derivative is synthesized by the coral from arachidonic acid[40] by a pathway which differs from the mammalian route.[41] However, practical problems of obtaining fresh coral for day-to-day work and the instability of coral enzyme preparations have hindered further research on this interesting biosynthetic problem. In the meantime another group of structurally related eicosanoids, the clavulones, exemplified by clavulone I (**19**) has been isolated and characterized from the Pacific coral *Clavularia viridis*.[42] Using coral collected off Ishigaki Island and preserved at -78° it was possible to obtain an enzyme preparation which metabolized tritiated arachidonate.[43] Although no labelled clavulones could be detected, a new substance was produced which was determined by radiochemical-structural methods to be the *cis*-disubstituted cyclopentenone **20** (absolute configuration unassigned).[43] This substance, termed preclavulone-A since it is a likely predecessor of the clavulones, underwent epimerization to the more stable *trans*-disubstituted cyclopentenone, the methyl ester of which was compared with synthetic material.[43] It was also found[44] that an acetone powder prepared from *C. viridis* converts arachidonate to 8-HPETE which is further transformed by a homogenate made from the coral to preclavulone A (**20**). These experiments point to the pathway of biosynthesis for **20** which is shown in Scheme VI. A key step in this process is the antifacial pericyclic closure of the pentadienyl cation directly to **20**, a reaction analogous to that proposed for the biosynthesis of the plant hormone *cis*-jasmonic acid.[45]

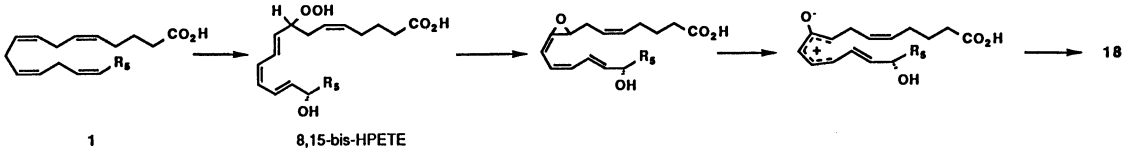
Scheme VI



A biomimetic and general synthesis of 4,5-disubstituted cyclopent-2-en-1-ones such as **20** by way of epoxy allenes, as depicted in Scheme VI, has recently been demonstrated in our laboratories.

Since the biosynthesis of clavulones now appears to involve a cationic process rather than a free radical pathway analogous to mammalian PG biosynthesis, it becomes obvious that the biosynthesis of PGA_2 methyl ester acetate by *P. homomalla*, known for some time to be different from the mammalian route,[41] could also be cationic. A simple mechanistic possibility now under study is outlined in Scheme VII. If verified, it would constitute a dramatic example of two totally different natural biosynthetic pathways to the same class of compounds. It should be mentioned that another marine eicosanoid, hybridalactone, also appears to be biosynthesized by a cationic mechanism.[46]

Scheme VII



The above survey of lipoxygenation and other reactions involved in eicosanoid biosynthesis reflects both the complexity and beauty of nature as revealed through organic chemical studies. Clearly, much remains to be learned. The study of this field will continue to challenge and reward the chemical sciences for some time to come.[47]

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