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COMMUNICATIONS

Published on Web 12/23/2005

Convergent Oxygenation of Arachidonic Acid by 5-Lipoxygenase and Cyclooxygenase-2

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The formation of leukotrienes and prostaglandins represents two major pathways of conversion of arachidonic acid into lipid hormones with distinct (patho-)physiological roles.[1] Leukotrienes are best recognized for their role as potent bronchoconstrictors in asthma. The prostaglandins mediate tissue homeostasis and also the pathophysiology of pain, fever, inflammation, and cell proliferation in cancer.[1]

The leukotriene and prostaglandin pathways are initiated from the common substrate arachidonic acid by the action of 5-lipoxygenase (5-LOX) or cyclooxygenase (COX).[1] The initial 5-LOX product, 5*S*-hydroperoxyeicosatetraenoic acid (5*S*-HPETE), is either reduced to the hydroxy derivative 5*S*-HETE or transformed to the epoxide LTA_4, the precursor to all leukotrienes.[2] Both COX isoforms, COX-1 and COX-2, form the same bicyclic endoperoxide PGH_2 from arachidonic acid. In contrast to COX-1, COX-2 is able to oxygenate the ethanolamide and 2-glyceryl derivatives of arachidonic acid to the corresponding prostaglandin derivatives.[3] COX-1 and COX-2 are distinct in their tissue expression, regulation, and inhibition by anti-inflammatory agents.[4] PGH_2 is an unstable intermediate and subject to tissue-specific enzymatic transformation into the downstream effector prostaglandins. Thus, the initial oxygenation of arachidonic acid by 5-LOX or COX-2 to a peroxide intermediate (5*S*-HPETE or PGH_2) is recognized as the committed step toward the respective pathway. The possibility of a cross-pathway interaction has not heretofore been examined. In this study, we provide biochemical evidence for such a crossover of the 5-LOX and COX-2 pathways and describe the identification of a unique diendoperoxide product formed by the selective oxygenation of 5*S*-HETE by COX-2.

5*S*-HETE contains *cis* double bonds at positions 8, 11, and 14, the fundamental structural requirements for COX substrates.[3] The ability of the human COX-1 and COX-2 isozymes to oxygenate 5*S*-HETE or its enantiomer, 5*R*-HETE, was examined by measurement of oxygen uptake (see Supporting Information). We found that 5*S*-HETE (30 μM) was a very good substrate for COX-2 (270 nM), while 5*R*-HETE was only poorly oxygenated (Figure 1A). COX-1 showed no reaction at all with 5*S*-HETE or 5*R*-HETE (data not shown). Analysis of the reaction products of radiolabeled [^{14}C]5*S*-HETE with COX-2 using reversed-phase HPLC showed formation of one major product, **1** (Figure 1B; the two later eluting peaks were identified as dihydroxy derivatives of arachidonic acid). LC−MS analysis of **1** in the positive and negative ion modes revealed a molecular weight of 400 (see Supporting Information). The increase of 80 mass units over the molecular weight of the substrate 5*S*-HETE is equivalent to the incorporation of five atoms of oxygen. This can be rationalized by incorporation of three molecules of O_2 followed by removal of one oxygen atom by the peroxidase activity[3] of the COX enzyme.

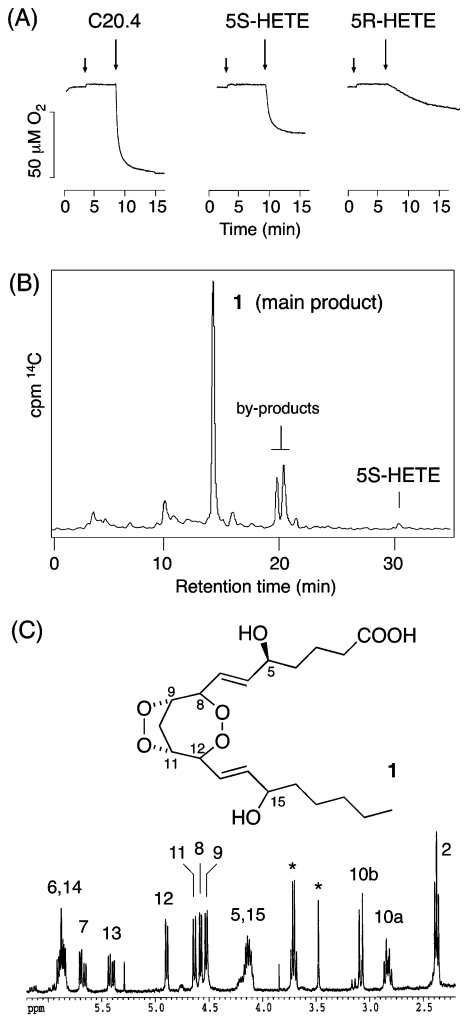

Figure 1. Reaction of COX-2 with 5*S*-HETE. (A) Oxygen electrode assays using arachidonic acid (left panel), 5*S*-HETE (middle), and 5*R*-HETE (right). Oxygen uptake was recorded upon addition of COX-2 (short arrow) and substrate (long arrow). (B) Reversed-phase HPLC analysis of the reaction products of [^{14}C]5*S*-HETE with COX-2. (C) ^{1}H NMR spectrum (6.5−2 ppm) and structure of **1**. The signals marked with an asterisk are from residual solvent in the sample.

A first insight into the structure of **1** was derived from its UV spectrum exhibiting only end absorbance, indicating the absence of any conjugated diene or other prominent UV chromophore. The substrate 5*S*-HETE contains a conjugated 6,8-diene, and the usual first reaction in cyclooxygenase catalysis generates an 11-peroxyl with a conjugated 12,14-diene. As both UV chromophores are absent in the product (and given the addition of three molecules of O_2), the evidence points to a series of oxygenations that eliminate both the 6,8- and 12,14-conjugated dienes, and implying the

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10.1021/ja056517y CCC: $33.50

occurrence of a reaction across the carbon chains during biosynthesis of **1**.

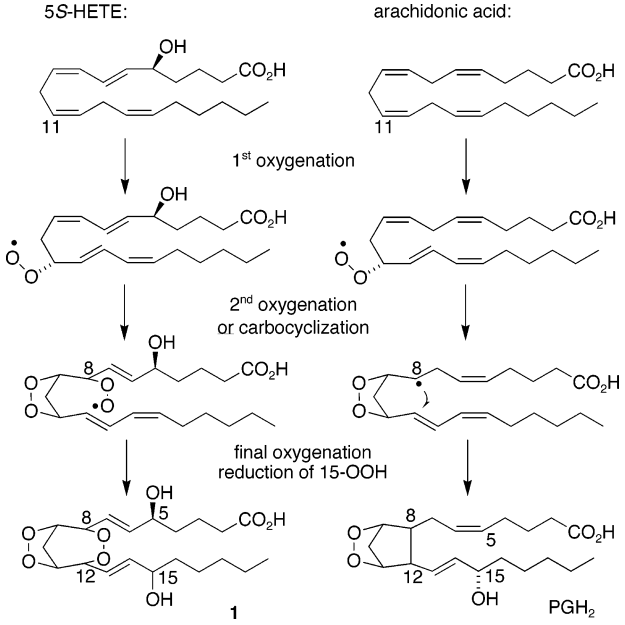
The proton NMR spectrum of **1** displayed a notably symmetrical pattern of signals that were assigned based on chemical shift and correlation in homo- and heteronuclear 2D experiments (Figure 1C and Supporting Information). Two partially superimposed sets of signals could be assigned to 5-hydroxy-6,7-*trans*-ene and 15-hydroxy-13,14-*trans*-ene moieties. Both geminal hydroxy protons (H5 and H15) were coupled to aliphatic methylene protons representing H4 through H2 and the omega carbon chain (H16−H20), respectively. Having thus accounted for the olefinic and geminal hydroxyl protons in the ^{1}H NMR spectrum, the remaining downfield resonances are a very striking group of four doublets at ∼4.5−4.9 ppm. The chemical shift of the four protons indicated a similar type of oxygen substitution for each corresponding carbon. The ^{1}H as well as the ^{13}C chemical shifts fit well with the values reported for the endoperoxide-bearing carbons 9 and 11 of PGH_2,[5] implicating for **1** an endoperoxide bridge from C9 to C11 and, in addition, a second endoperoxide from C8 to C12. H8 and H12 were readily assigned based on their coupling to the adjacent double bond. H9 and H11 were distinguished through H−C coupling in the HMBC and HSQC experiments (heteronuclear multiple-bond correlation and heteronuclear single-quantum correlation). That all four signals are doublets is attributed to a lack of coupling between the 8−9 and 11−12 protons as each pair is fixed at a dihedral angle of about 90° in the seven-membered ring (see Supporting Information). The significant downfield shift observed for H12 is explicable by its axial configuration; it is on the same face of the molecule as the 9,11-endoperoxide group, and this proximity to the second oxygen bridge causes enhanced deshielding. The remaining proton signals are the fifth doublet at 3.08 ppm (H10b) that couples solely to H10a at 2.83 ppm; again, H10b is at an unfavorable dihedral angle to its nominal neighbors, H9 and H11, whereas more complex multiplicities (*ddd*) are observed for H10a.

Thus, **1** contains the same 9,11-endoperoxide and 15-hydroxide as PGH_2, but the five-membered carbon ring is extended to a seven-membered ring by inclusion of a second endoperoxide from C8 to C12. Therefore, **1** is a bicyclic diendoperoxide, 15-hydroxy derivative of 5*S*-HETE. The absolute configuration of the chiral centers on the ring can be predicted based on the through-space coupling of the protons and the course of biosynthesis as 5*S*,15*S*-dihydroxy-9*S*,11*R*-8*S*,12*S*-diendoperoxy-6*E*,13*E*-eicosadienoic acid (see Supporting Information).

The formation of **1** by COX-2 is compatible with the mechanism of formation of PGH_2[3] (Scheme 1). Following hydrogen abstraction at C13 of arachidonic acid or 5*S*-HETE, the first oxygenation occurs in the 11*R* configuration followed by peroxyl radical cyclization to C9 and transfer of the radical to C8. With arachidonic acid as substrate, the C8 radical closes the five-membered prostaglandin ring to C12 followed by the second oxygenation in the 15*S* configuration. With 5*S*-HETE as a substrate, C8 is first oxygenated to a peroxyl radical that then cyclizes to C-12, followed by a final (third) oxygenation at C15. The 15-hydroperoxide is then reduced at the peroxidase active site of COX-2 to the hydroxy product. Thus, with 5*S*-HETE, COX-2 catalyzes a triple oxygenation giving rise to the unique endoperoxide bridge from C8 to C12 instead of the carbon−carbon bond of PGH_2.

A significant role for the 5-LOX and COX-2 cross-pathway interaction can be inferred. Concomitant expression of 5-LOX and COX-2 is a characteristic feature of many cells, tissues, and disease

Scheme 1. Proposed Mechanism of Formation of **1** by COX-2



conditions.[6,7] Prominent examples are macrophages, monocytes, and dendritic cells,[8,9] sites of inflammation in atherosclerosis[7,9] and asthma,[10,11] and the neoplastic tissues of breast and pancreatic cancer.[6,12] Furthermore, 5-LOX and COX-2 share similar subcellular localization at the nuclear envelope of activated cells.[4,8] COX-2 has biological functions that sets it apart from COX-1, and therefore the isoform-specific metabolism of 5-HETE by COX-2 and not COX-1 could be functionally relevant. The physiological complexity is further enhanced by the possibility of enzymatic transformation of the 9,11-endoperoxide bridge of **1** as occurs with PGH_2. Rearrangement of **1** could give rise to a series of products representing a new class of highly oxygenated lipid mediators within the eicosanoid family.

Acknowledgment. This work was supported by NIH Grant GM53638. We thank Drs. Brian Bachmann, Alan Brash, Thomas Harris, and Ned Porter for helpful discussions on the product identification and the mechanism of formation.

Supporting Information Available: Experimental details and characterization of **1** by LC−MS and NMR. This material is available free of charge via the Internet at http://pubs.acs.org.

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JA056517Y