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Energetics of the biosynthesis of cyclopentenones from unsaturated fatty acids

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Dedicated to the memory of Professor J. Grimaldi (1940–2013) who discovered in 1969 the cyclization of vinyl allene oxides

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ABSTRACT

Polyunsaturated fatty acids like linolenic acid or arachidonic acid upon enzymatic hydroxylation (lipoxygenases) can lead to corresponding hydroperoxides. Their dehydration gave rise to vinyl allene oxides, which cyclized into cyclopentenones, precursors of jasmonic acid or prostanoids.

We have studied the energetics of these cascade reactions to determine their thermodynamic parameters and reveal the key steps. The formation of hydroperoxydes from polyunsaturated fatty acids and triplet dioxygen appeared as a surprisingly slightly exergonic reaction (~6–15 kcal mol^{-1}). In contrast, the cumulated following reactions were highly exergonic (~95–100 kcal mol^{-1}). Cyclization of the (*E*)-vinyl allene oxides occurred with an activation barrier of ~23 kcal mol^{-1}, while cyclization of the (*Z*)-isomer required two steps: first isomerization into vinylcyclopropanone (TS: ~20 kcal mol^{-1}), and then isomerization of the latter into cyclopentenone (TS: ~18 kcal mol^{-1}).

1. Introduction

Polyunsaturated fatty acids like linolenic acid or arachidonic acid[1] can lead to cyclopentenones as jasmonic acid or prostanoids upon enzymatic hydroxylation. In plants, jasmonic acid and its derivates (plant oxylipins) are obtained from 13*S*-hydroperoxide of α-linolenic acid, and are associated with diverse physiological functions mainly wound response and pathogenesis.[2] In corals, *Plexaura homomalla* is renowned for its high content of prostaglandin esters, which contribute to 2–3% of the coral dry weight. These marine invertebrates, as well as starfish,[3] afford prostanoids from the 8*R*-hydroperoxide of arachidonic acid (Scheme 1).[4]

We have studied the energetics of these cascade reactions to determine their thermodynamic parameters and reveal the key steps. Lipoxygenases (LOXs) are an ubiquitous family of enzymes that catalyze the oxidation of unsaturated fatty acids, and are found in cyanobacteria, fungi, algae, plants, and mammalian cells. Lipoxygenases are non-heme iron dioxygenases that catalyze the stereo- and regio-specific formation of fatty acid hydroperoxides from polyunsaturated fatty acids, which are commonly found in plants and animals.[5] Soybean lipoxygenase-1 (SBL-1) is often used as prototype for this class of enzymes. In the presence of molecular oxygen, 13-lipoxygenase (EC 1.13.11.12) reacts with the α-linolenate anion (α-LA) **1** to give 13(*S*)-hydroperoxy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid (LOOH) **4**.

The pathway of jasmonic acid biosynthesis is shown in Scheme 1. Jasmonic acid and its octadecanoid precursors are synthesized from **1**, which is found in great extent in plastidial membranes.[6]

Crystallographic determination of the active site of the SBL-1 has shown the presence of one atom of iron per molecule of protein.[7] The octahedral coordination sphere of the iron atom includes a molecule of water and the side chains of His^{499}, His^{504}, His^{690}, and Asn^{694} as well as the terminal carboxylate of Ile^{839}.[8] In the generally accepted mechanism, the Fe(III)-bound OH abstracts the 11-pro-*S* hydrogen atom from bound linolenate anion, yielding a C9–C13 delocalized pentadienyl radical **2**. The H-transfer step occurred via a proton-coupled electron transfer (PCET) process.[9,10,14a] This step is followed by the attack of molecular oxygen at the substrate, antarafacial to the iron center, as shown in Scheme 2, as the iron center hinders one face of the radical **2**. Then, rotation of the peroxyl intermediate **3** from an antarafacial to a suprafacial arrangement followed by a second PCET process gave rise to hydroperoxide **4**.[11]

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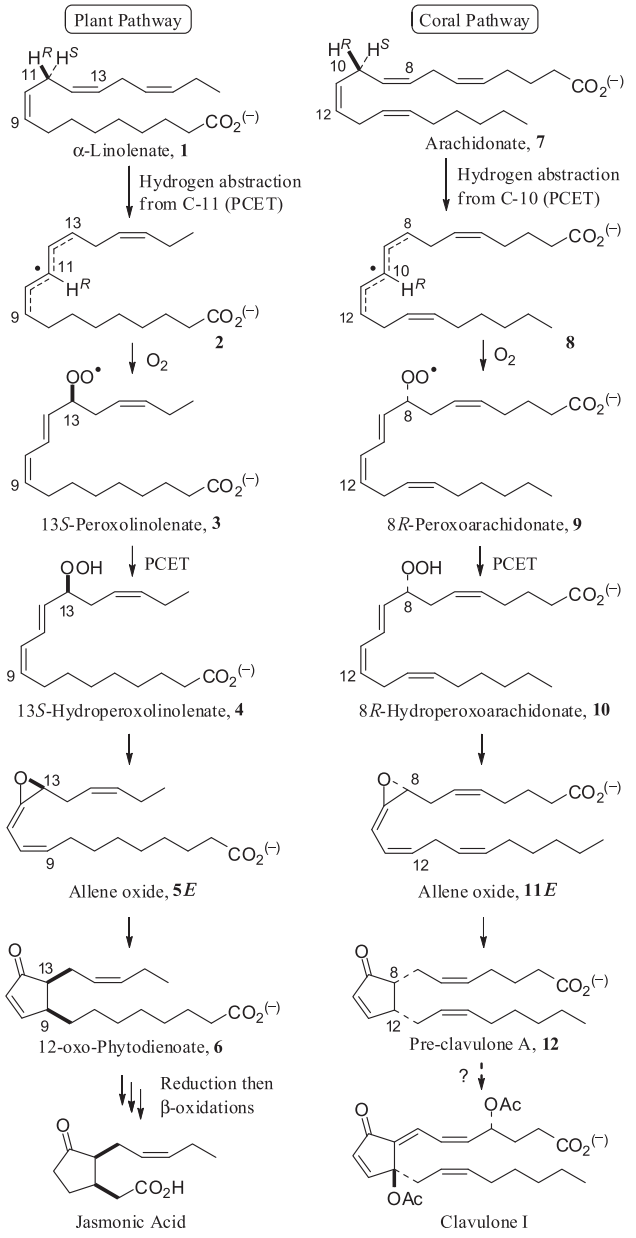
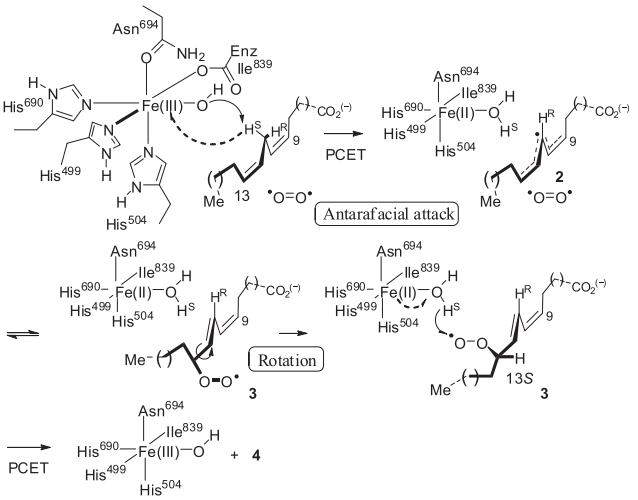


Scheme 1. Biosynthetic pathways to cyclopentenone products in plants and corals.[18]



Scheme 2. The general accepted mechanism of 13-lipoxygenase.

The hydrogen abstraction step is rate-limiting and an abnormally large primary H/D isotopic effect of 81 has been observed[12] suggesting that this step proceeds via a tunneling mechanism.[13,14] The resulting 13-hydroperoxide, i.e., 13(*S*)-hydroperoxy-9(*Z*),11(*E*),15(*Z*)-octadecatrienoic acid (13-HPOT) **4**, is further dehydrated with the help of allene oxide synthase (AOS) (EC 4.2.1.92),[15] providing the unstable intermediate 12,13-epoxy-9(*Z*),11,15(*Z*)-octadecatrienoic acid (12,13-EOT) **5*Z*** or **5*E***. Allene oxide cyclase (AOC) (EC 5.3.99.6) catalyzes the reaction within an octadecanoid pathway, which guarantees enantiomeric specificity, by converting 12,13-EOT to 12-oxo-10,15(*Z*)-phytodienoic acid (OPDA) **6**.[16] Formation of the allene oxide structure by an enzymatic reaction was discovered in 1987.[17,34a]

The allene oxide synthases are of two structurally-unrelated types. In plants, a subfamily of cytochrome P450, designated as *CYP74A*, uses the hydroperoxides of linoleic and linolenic acids **4** as substrates.[19] Both the 9- and the 13-hydroperoxides are converted to allene oxides and subsequently give rise to plant signaling molecules.[20] The crystal structure of a *CYP74A2*,[21] and its of *Arabidopsis thaliana*[22] have been determined. OPDA **6** is then transferred from the chloroplast to the peroxisome where it is further metabolized by reduction of the Δ10-double bond catalyzed by oxo-phytodienoic acid reductase, yielding 3-oxo-2(2′(*Z*)-pentenyl)-cyclopenta-1-octanoic acid (OPC-8:0).[23] It is generally agreed that OPC-8:0 undergoes three consecutive β-oxidations, which results in the production of bioactive jasmonic acid with (3*R*,7*S*) absolute configuration.[24] The isolation and structural elucidation of allene oxides prepared from the 13*S*-hydroperoxides of linoleic and linolenic acids by using a very active enzyme preparation from flawseed have been reported.[25] In the same way, allene oxide synthase from the cyanobacterium *Acaryochloris marina* has been identified and its products from polyunsaturated fatty acids characterized.[26]

The lipoxygenase, arachidonate 8-lipoxygenase (EC 1.13.11.40) from the coral *Pseudoplexaura porosa* converts exogenous arachidonic acid into (5*Z*,9*E*,11*Z*,14*Z*)-(8*R*)-8-hydroperoxyeicosa-5,9,11,14-tetraenoate (8-HPETE) **10**.[27] The structure of the 8*R*-lipoxygenase from *P. homomalla* reveals a U-shaped channel that would allow the substrate to access to the catalytic non-heme iron. The arachidonate anion can slide into the channel and wrap around the arched helix, positioning the central carbon of a (1*Z*,4*Z*)-pentadiene system above the metallic center.[28] The iron is chelated by His^{385}, His^{390}, His^{571}, Asn^{575}, and Ile^{694} (via its C-terminus). The first step of the mechanism has been proposed to involve abstraction of the *pro-S* hydrogen atom from C10 to generate a pentadienyl radical spanning C8—C12. For arachidonic acid, with coral 8*R*-lipoxygenase, a clear relationship between the iron-catalyzed hydrogen abstraction and the oxygenation on the opposite face of the reacting substrate has been observed.[29]

Coral allene oxide synthase (AOS), a hemoprotein, is the N-terminal domain of a naturally occurring fusion protein with an 8*R*-lipoxygenase activity. Brash and co-workers have further characterized two domains of this fusion protein, a lipoxygenase domain that transforms arachidonic acid to its 8*R*-hydroperoxide, and the allene oxide synthase (AOS) that forms the 8,9-epoxy allene oxide, a key intermediate in cyclopentenone biosynthesis.[30] AOS, whose crystal structure has been reported, has been found to be a tyrosinate-ligated heme enzyme **A**, which homolytically cleaves the peroxide bond to yield an alkoxy radical **B**.[31] Cyclization of the alkoxy radical results in formation of an epoxide allylic radical **C**, that is, then subsequently oxidized via an electron transfer into an oxiryl carbinyl cation **D**. The last step is a deprotonation to generate the allene oxide moiety **E** (Scheme 3).[32]