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Review

Molecular enzymology of lipoxygenases

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ABSTRACT

Lipoxygenases (LOXs) are lipid peroxidizing enzymes, implicated in the pathogenesis of inflammatory and hyperproliferative diseases, which represent potential targets for pharmacological intervention. Although soybean LOX1 was discovered more than 60 years ago, the structural biology of these enzymes was not studied until the mid 1990s. In 1993 the first crystal structure for a plant LOX was solved and following this protein biochemistry and molecular enzymology became major fields in LOX research. This review focuses on recent developments in molecular enzymology of LOXs and summarizes our current understanding of the structural basis of LOX catalysis. Various hypotheses explaining the reaction specificity of different isoforms are critically reviewed and their pros and cons briefly discussed. Moreover, we summarize the current knowledge of LOX evolution by profiling the existence of LOX-related genomic sequences in the three kingdoms of life. Such sequences are found in eukaryotes and bacteria but not in archaea. Although the biological role of LOXs in lower organisms is far from clear, sequence data suggests that this enzyme family might have evolved shortly after the appearance of atmospheric oxygen on earth.

Introduction

Lipoxygenases (LOXs)[1] are non-heme iron-containing dioxygenases [1–3] that catalyze the stereo-specific peroxidation of polyunsaturated fatty acids containing at least one 1-cis,4-cis-pentadiene system (Fig. 1). Fatty acid oxygenation by LOXs generally consists of four elementary reactions (hydrogen abstraction, radical rearrangement, oxygen insertion, peroxy radical reduction), usually proceeding in a sterically controlled manner (Fig. 1). Hydrogen abstraction, which constitutes the rate limiting step [4], follows a quantum-mechanical mechanism [5]. In soybean LOX1, H-atom transfer corresponds to a proton-coupled electron transfer [6,7]. The transferred electron does not localize on the proton, but tunnels directly from the substrate to the ferric iron in a concerted proton tunneling–electron tunneling process [6]. In the transition state the covalently linked Fe–O–H–C bridge lowers the energy barrier and provides an efficient pathway for this tunneling [6].

In most cells, the concentration of free fatty acids is limited and thus, LOX substrates have to be liberated from cellular stores by ester lipid hydrolyzing enzymes [8]. However, certain LOX-isoforms are capable of oxygenating polyunsaturated fatty acids that are incorporated in ester lipids located in biomembranes or lipoproteins [9–11]. The conventional nomenclature classifies animal LOXs with respect to their positional specificity of arachidonic acid oxygenation as 5-LOXs, 8-LOXs, 11-LOXs, 12-LOXs or 15-LOXs. This classification is not optimum since: (i) Arachidonic acid is not a good substrate for many non-mammalian LOXs and with other substrate fatty acids (e.g. linoleic acid) their reaction specificity can be quite different. (ii) Evolutionarily-related LOX-isoforms can exhibit distinct reaction specificities in contrast to those sharing a low degree of phylogenetic relatedness. Moreover, the rapidly growing availability of genomic sequences and our inability to predict the reaction specificity of arachidonic acid oxygenation from the primary structure of the enzymes leads to the confusing situation that most LOX-isoforms (for which we only have sequence information) cannot be classified according to a function-based enzyme nomenclature. Thus, a sequence-based classification procedure that considers the phylogenetic relatedness of the enzymes is desirable. Unfortunately, despite numerous attempts, no simple and unifying LOX nomenclature has been introduced that overcomes the above mentioned problems. The lack of comprehensive and straightforward classification criteria makes it difficult for non-expert scientists to follow the current developments in LOX research.

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[1] *Abbreviation used:* LOX(s), lipoxygenase(s); 15-H(p)ETE, (5Z,8Z,11Z,13E)-15-hydro(pero)xyeicosa-5,8,11,13-tetraenoic acid; 12-H(p)ETE, (5Z,8Z,10E,14Z)-12-hydro(pero)xyeicosa-5,8,10,14-tetraenoic acid; 11-H(p)ETE, (5Z,8Z,12E,14Z)-11-hydro(pero)xyeicosa-5,8,12,14-tetraenoic acid; 8-H(p)ETE, (5Z,9E,11Z,14Z)-8-hydro(pero)xyeicosa-5,9,11,14-tetraenoic acid; 5-H(p)ETE, (6E,8Z,11Z,14Z)-5-hydro(pero)xyeicosa-6,8,11,14-tetraenoic acid; 13-H(p)ODE, (9Z,11E)-13-hydro(pero)xyoctadeca-9,11-dienoic acid; SAXS, small angle X-ray scattering.

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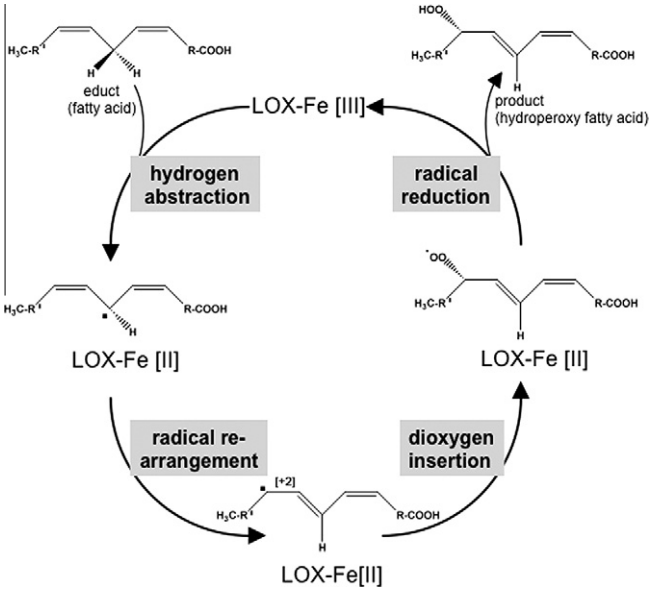

Fig. 1. Detailed mechanism of the LOX-reaction. LOX catalyzed oxygenation of fatty acids consists of four consecutive elementary reactions, the stereochemistry of which are tightly controlled. (i) Stereoselective hydrogen abstraction from a bisallylic methylene: The hydrogen atom is removed as proton and the resulting electron is picked up by the ferric non-heme iron that is reduced to the ferrous form. (ii) Radical rearrangement: During this elementary reaction the radical electron is dislocated either in the direction of the methyl end of the fatty acid ([+2] rearrangement) or in the direction of the carboxylate ([−2] rearrangement). (iii) Oxygen insertion: Molecular dioxygen is introduced antarafacially (from opposite direction of the plane determined by the double bond system) related to hydrogen abstraction. If the hydrogen located above the double bond plane is removed, dioxygen is introduced from below this plane. (iv) peroxy radical reduction: The peroxy radical formed via oxygen insertion is reduced by an electron from the ferrous non-heme iron converting the radical to the corresponding anion. Thereby the iron is reoxidized to its ferric form. Finally, the peroxy anion is protonated.

Another confusing problem, which LOX research shares with other areas in molecular enzymology, is isoform-multiplicity within a particular species and functional heterogeneity of the different isoenzymes. In soybeans, up to 13 different LOX-isoforms have been identified, while the rice genome contains more than 20 different LOX genes. The human genome contains six functional LOX genes, five of which are found clustered on chromosome 17 with only the 5-LOX gene on chromosome 10. In contrast, the murine genome contains seven functional LOX genes (the gene for the murine epidermis 12S-LOX is a functionless pseudogene in the human LOX gene cluster), located in a syntenic region on the mouse chromosome 11. Here again, only 5-LOX is located on a different chromosome (chromosome 6).

A Pubmed search with the keyword "lipoxygenase" gives about 14,000 hits with more than 500 new articles published annually. Thus, it is impossible to reference all of these papers in our review. Furthermore, this article is intended to cover thematic priorities, which have either emerged recently or have not been reviewed in the past. Since we will focus on structural and evolutionary aspects, a detailed discussion of the biological function of LOXs goes beyond the scope of this review. However, we wish to point out two aspects of LOX biology: (i) 30 years ago leukotrienes generated by 5-LOX were identified as potent pro-inflammatory mediators [12] [13]. Since then, additional pro-inflammatory products have been discovered [14]. On the other hand, anti-inflammatory and/or pro-resolving lipids generated by mammalian isoforms have also been identified, including lipoxins [15,16], resolvins [17], protectins [18,19] and maresins [20]. Thus, LOX products play important roles in the development of acute inflammation but they have also been implicated in inflammatory resolution. (ii) Mice deficient in 12R-LOX develop normally during pregnancy but die immediately after birth due to excessive dehydration [21,22]. Although the molecular mechanisms of postpartum mortality are unknown, the enzyme was implicated in the formation of the epidermal water barrier. Genetic polymorphisms of the corresponding human gene have been related to ichthyosis [23], a disease characterized by dry, thickened, scaly or flaky skin.

To obtain more information on different aspects of LOX biology the reader is referred to other reviews, which address their role in cancer [24–26], vascular biology [27,28], and inflammation [29,30].

The structural basis of LOX catalysis

Mammalian lipoxygenases consist of a single polypetide chain that folds into a two-domain structure but in lower organisms fusion proteins may occur

The complete crystal structures of several plant and animal LOXs are available (Table 1), along with a partial set of X-ray coordinates for the human platelet-type 12-LOX. X-ray data is also available for enzyme–ligand complexes of plant LOXs (Table 1).

Most LOXs consist of a single polypeptide chain that folds into a two-domain structure; a small N-terminal β-barrel domain and a larger mostly helical catalytic domain. The rabbit 12/15-LOX (Fig. 2A) is of cylindric shape (height of 10 nm) with an eliptic ground square (longer diameter 6.1 nm, shorter one of 4.5 nm). The structure of coral 8R-LOX (Fig. 2B) is closely related to the rabbit enzyme also resembling a cylinder (diameter 6 nm, height 10 nm) [48]. In contrast, the soybean LOX1 (Fig. 2C) is ellipsoid (9 nm × 6.5 nm × 6 nm) [36].

In lower organisms, LOXs may occur as fusion proteins, in which the LOX-domain is linked to another catalytic domain that plays a role in the secondary metabolism of hydroperoxy fatty acids. The first LOX-fusion protein was discovered in the coral *Plexaura homomalla* [50]. Here, the LOX-domain that produces 8R-HpETE is linked to a heme-containing peroxidase domain, which converts the fatty acid peroxide to an allene oxide. This unstable intermediate may further be converted to cyclopentenone eicosanoids. The fusion protein was cloned and the two subenzymes were separately expressed and characterized [51,52]. Their crystal structures were also solved [34,48,53]. Although the degree of amino acid conservation between the LOX-domain of this fusion protein and the rabbit 12/15-LOX was not particularly impressive (30%), the 3D-structures of the two LOX-isoforms are rather similar. The low-resolution structure of the whole fusion protein indicated that the allene oxide synthase domain interacts non-covalently with both LOX sub-domains while the putative calcium-binding sites and the membrane interacting Trp residues (see "The small N-terminal LOX-domain is important for membrane binding and regulates the catalytic activity") are not shielded but remain surface exposed [49]. Membrane binding of the fusion protein induces alterations in the spatial orientation of the different sub-domains (interdomain movement), as indicated by the appearance of a new proteolytic cleavage site [49].

Another LOX-fusion protein, sharing 84% sequence identity with the *P. homomalla* enzyme, was detected in the coral *Gersemia fruticosa* [54] suggesting a broader distribution of these enzymes in octocorals. In addition, allene oxide synthase/LOX-fusion proteins have been discovered in the cyanobacteria *Anabaena PCC 7120* and *Acaryochloris marina* [55,56]. In contrast to the coral enzymes, in which the fusion proteins contain complete LOXs, the cyanobacteria isoforms lack the N-terminal LOX-domain and the catalytic LOX-domain is truncated (but active). Although the biological role of these fusion proteins is unclear, they have been implicated in the biosynthesis of lipid signaling molecules [50,57]. In all LOX-fu-