Chapter 20

Lipoxygenases, Apoptosis, and the Role of Antioxidants

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Summary

Lipoxygenases are a family of enzymes that dioxygenate unsaturated fatty acids, thus initiating lipoperoxidation of membranes and the synthesis of signaling molecules, or inducing structural and metabolic changes in the cell. This activity is the basis for the critical role of lipoxygenases in a number of pathophysiological conditions, in both animals and plants. In the past few years, a pro-apoptotic effect of lipoxygenase has been reported in different cells and tissues, leading to cell death along unrelated apoptotic pathways. However, anti-apoptotic effects of lipoxygenases have also been reported, often based on the use of enzyme inhibitors. In the present review, the characteristics of the lipoxygenase family and the role of lipoxygenase activation in apoptosis of animal and plant cells are discussed, suggesting a common signal transduction pathway in cell death conserved through the evolution of both kingdoms. In addition, the inhibition of lipoxygenases by antioxidants and its consequences on apoptosis will be presented.

I. Introduction

Lipoxygenases (linoleate:oxygen oxidoreductase, EC 1.13.11.12; LOXs) are a family of monomeric non-heme, non-sulfur iron dioxygenases that catalyze the conversion of polyunsaturated fatty acids into conjugated hydroperoxides. The unsaturated fatty acids, which are essential in humans, are absent in most bacteria and thus LOXs are also absent in typical prokaryotes. LOXs are widely expressed in animal and plant cells, sometimes at high level, and their activity may initiate the synthesis of a signaling molecule or may induce structural or metabolic changes in the cell. Mammalian lipoxygenases have been implicated in the pathogenesis of several inflammatory conditions such as arthritis, psoriasis, and bronchial asthma (Kühn and Borngraber, 1999). They are also thought to have a role in atherosclerosis (Cathcart and Folcik, 2000), brain aging (Manev et al., 2000), HIV infection (Maccarrone et al., 2000a), kidney disease (Maccarrone et al., 1999a; Montero and Badr, 2000), and terminal differentiation of keratinocytes (Heidt et al., 2000). In plants, lipoxygenases play a role in germination, and participate in the synthesis of traumatin and jasmonic acid and in the response to abiotic stress (Grechkin, 1998; Feussner and Wasternack, 2002; Weichert et al., 2002). Remarkably, several of the above-mentioned conditions are associated with apoptosis (programmed cell death, PCD) in both animals (Han et al., 1995; Lizard et al., 1996; Martín-Malo et al., 2000; Fumelli et al., 2000)

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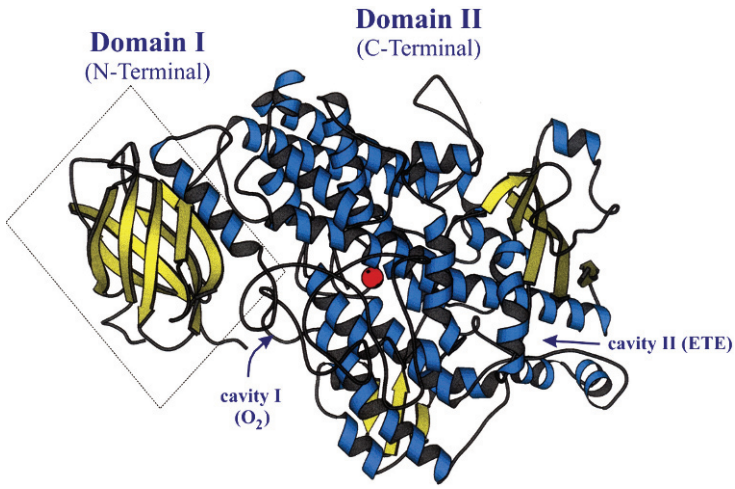

Fig. 1. Schematic diagram of the three-dimensional structure of soybean (*Glycine max*) lipoxygenase-1, showing the small N-terminal domain I (rectangular box) and the large C-terminal domain II. The iron-containing active site is located in domain II, and can be reached by molecular oxygen (O_2) through cavity I and by arachidonic acid (eicosatetraenoic acid, ETE) through cavity II. β-Sandwiches are represented in yellow, α-helices in blue, random coils in gray, and iron is the red sphere. The three-dimensional structure was modelled through the RASMOL program, using the lipoxygenase-1 sequence (PDB accession number: 2SBL).

and plants (Greenberg, 1996; Jones and Dangl, 1996; Wang H. et al., 1996; Wang M. et al., 1996; Koukalová et al., 1997).

LOXs from animal and plant tissues have been sequenced, purified, and characterized, and have been shown to form a closely related family with no similarities to other known sequences. The phylogenetic tree shows that plant and animal enzymes are separate branches, each forming several subgroups (Brash, 1999). When arachidonic (eicosatetraenoic, C20:4; ETE) acid is the substrate, different LOX isozymes can add a hydroperoxy group at carbons 5, 12, or 15, and are therefore designated 5-, 12-, or 15-lipoxygenases. Linoleic (octadecadienoic, C18:2; OD) acid and linolenic (octadecatrienoic, C18:3; OT) acid are also substrates of LOXs. Soybean (*Glycine max* (L.) Merrill) lipoxygenase-1 (LOX-1) is a 15-lipoxygenase widely used as a prototype for studying the homologous family of lipoxygenases from tissues of different species, both in structural (Boyington et al., 1993; Minor et al., 1996; Gan et al.; 1996; Sudharshan and Appu Rao, 1999; Sudharshan et al., 2000) and kinetic (Glickman and Klinman, 1995; Jonsson et al., 1996; Maccarrone et al., 2001a, 2001b; Di Venere et al., 2003) investigations. The primary sequence (Shibata et al., 1987) and three dimensional structure (Boyington et al., 1993; Minor et al., 1996) of LOX-1 have been determined, showing that it is a prolate ellipsoid of 90 by 65 by 60 Å, with 839 amino acid residues and a molecular mass of 93840 Da. LOX-1 is made of two domains: a 146-residue β-barrel at the N-terminal (domain I) and a 693-residue helical bundle at the C-terminal (domain II). The iron-containing active site is in the center of domain II, liganded to four conserved histidines and to the carboxyl group of the C-terminal conserved isoleucine. It can be reached through the two cavities (I and II) shown in Fig. 1.

Cavity I presents an ideal path for the access of molecular oxygen to iron, whereas cavity II can accommodate arachidonic acid or even slightly larger fatty acids (Boyington et al., 1993). Mammalian lipoxygenases lack the N-terminal domain present in LOX-1 and related plant lipoxygenases, thus showing smaller molecular mass (75 - 80 kDa compared to 94 -104 kDa in plants). It has been suggested that the N-terminal domain in LOX-1 makes only a loose contact with the C-terminal domain (Boyington et al., 1993), and that it may be dispensable for plant lipoxygenases (Minor et al., 1996), because all of the amino acid side chains responsible for catalysis are located in

Abbreviations: ERK – extracellular regulated kinase; ETE – eicosatetraenoic (arachidonic) acid; FLAP – 5-lipoxygenase activating protein; HR – hypersensitive response; LOX – lipoxygenase; LRP – lentil root protoplast; MAPK – mitogen-activated protein kinase; NDGA – nordihydroguaiaretic acid; NF-kB – nuclear factor-kB; NSAID, – nonsteroidal anti-inflammatory drug; OD – octadecadienoic (linoleic) acid; OT – octadecatrienoic (linolenic) acid; PCD – programmed cell death; PLA_2 – phospholipase A_2; PPAR – peroxisomal proliferator-activated receptor; ROS – reactive oxygen species; SPD – spermidine; SPN – spermine; TGF – transforming growth factor; TNF – tumor necrosis factor.