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Review

Lipoxygenase and carotenoids: A co-oxidation story

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Lipoxygenases (LOXs), widely found in plants, fungi, and animals, are a large family of monomeric proteins with non-heme, non-sulphur, iron cofactor containing dioxygenases that catalyze the oxidation of polyunsaturated fatty acids such as linoleic, linolenic and arachidonic acid to yield hydroperoxides. Some LOX isoenzymes have the capacity to co-oxidise also the carotenoids. Carotenid biosynthesis occurs only in bacteria, fungi and plants where they have established functions that include their role as antenna in the light-harvesting proteins of photosynthesis, their ability to regulate light-energy conversion in photosynthesis, as well as the ability to protect the plant from reactive oxygen species, and coloration. In humans, some carotenoids (the provitamin A carotenoids: α-carotene, β-carotene, γ-carotene and the xanthophyll β-cryptoxanthin) are best known for their conversion into vitamin A. Lipoxygenase has negative food-related implications for color, off-flavour and antioxidant status of plant based foods. Up to now, β-carotene seems to attract more attention in developing strategies for food processing to prevent LOX-mediated deteriorations such as oxidation, rancidity, and off-flavor. More research is necessary for transferring the *in vitro* mechanistic studies on the LOX inhibition *in vivo*. Other carotenoids like lutein, zeaxanthin, β-cryptoxanthin in pure form as well as in natural extracts could be good candidates for LOX inhibition through their antioxidant action.

Key words: Lipoxygenase, carotenoids, oxidation.

INTRODUCTION

The existence of an enzyme "carotene oxidase" in soybeans, which catalyzes the oxidative destruction of carotene was reported by Bohn and Haas in 1928 (Bohn and Haas, 1928 cited by Christopher et al., 1970). Four years later, Andre and Hou (Andre and Hou, 1932 cited by Christopher et al., 1970) found that soybeans contained an enzyme, lipoxygenase (linoleate oxygen oxidoreductase), which they termed *"lipoxidase",* which catalyzed the peroxidation of certain unsaturated fatty acids.

In 1940 the observation was published that“lipoxydase” is identical to “carotene oxidase” (Sumner and Sumner, 1940). These early findings of lipoxygenase peroxidizing unsaturated fats and bleaching carotene were reported as the result of studies on the oxidation of crystalline carotene or carotene dissolved in unsaturated oil. Surprisingly, it was found that the carotene oxidase had an almost negligible bleaching action upon the crystalline carotene. On the contrary, when one employs carotene dissolved in a small quantity of fat, the bleaching is extremely rapid. With excessive quantities of fat, the rate of bleaching of the carotene diminishes, and it was concluded that the effect of added fat upon the rate of bleaching of carotene is probably due to a coupled oxidation (Sumner and Sumner, 1940).

Theorell et al. (1947) succeeded in crystallizing and characterizing lipoxygenase (LOX) from soybeans and

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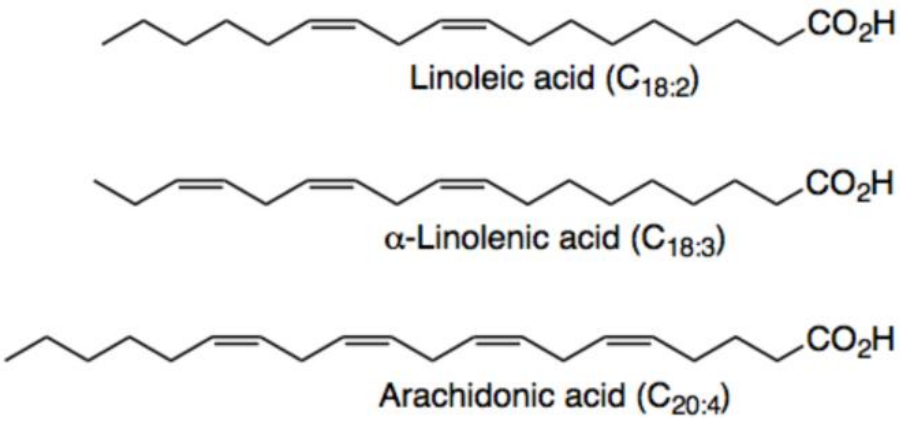

Figure 1. Lipoxygenase substrates, linoleic, α- linolenic and arachidonic acid.

since then among plant LOXs, soybean LOX-1 can be regarded as the mechanistic paradigm for these non-heme iron dioxygenases (Coffa et al., 2005; Minor et al., 1996; Fiorucci et al., 2008).

LIPOXYGENASE – DEFINITION, STRUCTURE, REACTION MECHANISM AND METABOLIC FUNCTIONS

Lipoxygenases (EC 1.13.11.12, linoleate:oxygen, oxidoreductases, LOXs) are a large family of monomeric proteins family with non-heme, non-sulphur, iron cofactor containing dioxygenases that catalyze the oxidation of polyunsaturated fatty acids (PUFA) as substrate with at least one 1*Z*, 4*Z*-pentadiene moiety such as linoleic, linolenic and arachidonic acid to yield hydroperoxides (Gardner, 1991) (Figure 1).

Lipoxygenases are found widely in plants, fungi, and animals (Grechkin, 1998). There are significant differences in size, sequence, and substrate preference between the plant and animal LOXs, but the overall folding and geometry of the non-heme iron-binding site are conserved (Kühn et al., 2005; Skrzypczak-Jankun et al., 2006). All LOXs are folded in a two-domain structure that is composed of a smaller β-barrel domain (N-terminal domain) and a larger α-helical catalytic domain (C-terminal domain) (Choi et al., 2008). The non-heme iron essential for activity is positioned deep in a large cavity that accommodates the substrate (Choi et al., 2008). The regio- and stereospecificities of the various LOX isozymes are believed to be determined by the shape and depth of the cavity as well as the binding orientation of the substrate in the cavity (Borngräber et al., 1999; Kühn, 2000; Coffa et al., 2005).

The initial step of LOX reaction is removal of a hydrogen atom from a methylene unit between double bonds in substrate fatty acids (Figure 2A). The resulting carbon radical is stabilized by electron delocalization through the double bonds. Then, a molecular oxygen is added to the carbon atom at +2 or –2 position from the original radical carbon, forming a peroxy radical as well as a conjugated *trans*,*cis*-diene chromophore. The peroxy radical is then hydrogenated to form a hydroperoxide. The initial hydrogen removal and the following oxygen addition occur in opposite (or antarafacial) sides related to the plane formed by the 1*Z*,4*Z*-pentadiene unit. In most LOX reactions, particularly those in plants, the resulting hydroperoxy groups are in *S*-configuration, while one mammalian LOX and some marine invertebrate LOXs produce *R*-hydroperoxides. Even in the reactions of such "*R*-LOXs", the antarafacial rule of hydrogen removal and oxygen addition is conserved (Chedea and Jisaka, 2011).

In cases of plant LOXs, including soybean LOXs, the usual substrates are C18-polyunsaturated fatty acids (linoleic and α-linolenic acids), and the products are their 9*S*- or 13*S*-hydroperoxides (Figure 2B). Most plant LOXs react with either one of the regio-specificity, while some with both. Therefore, based on the regio-specificty, plant LOXs are classified into 9-LOXs, 13-LOXs, or 9/13-LOXs (Chedea and Jisaka, 2011). Lipoxygenase from soybean seed is the best characterized among plant LOX constituting also structural and activity models for the lipoxygenase isoenzymes family (Chedea and Jisaka, 2011). Soybean seed lipoxygenase catalyses the hydroperoxidation of linoleic and linolenic acids, leading to the production of several reactive molecules that account for the grassy beany taste in soybean processed foods (Baysal and Demirdöven, 2007).

LOX isoenzymes of soybean seed are 94–97 kDa monomeric proteins with distinct isoelectric points ranging from about 5.7 to 6.4, and can be distinguished by optimum pH, substrate specificity, product formation and stability (Siedow, 1991). LOX-3 (857 amino acids; 96.5 kDa) exhibits its maximal activity over a broad pH range centred around pH 7.0 and displays a moderate preference for producing a 9-hydroperoxide product. It is the most active isoenzyme with respect to both carotenoid cooxidation and production of oxodienoic acids (Ramadoss et al., 1978).

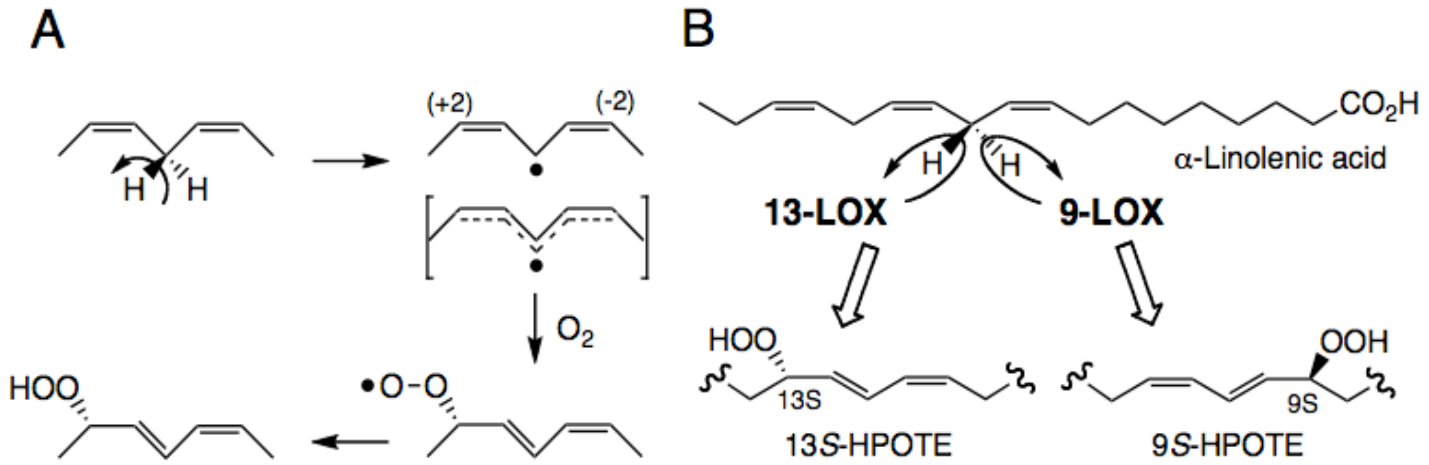

Figure 2. LOX reaction showing the principal steps of LOX reaction (Panel A), and the actual reactions of plant LOXs and α-linolenic acid (Panel B). HPOTE: Hydroperoxyoctadecatrienoic acid (Chedea and Jisaka, 2011).

Arachidonic acid metabolism by LOX in human plate-lets was demonstrated in 1974 (Hamberg and Samuelsson, 1974). Rabbit reticulocyte LOX was described for the first time in 1975 (Schewe et al., 1975). There are 6 functional LOXs in humans: 5-LOX; 12/15-LOX (15-LOX-1); platelet -type 12-LOX; 12R-LOX; epidermis-type 15-LOX (15-LOX-2); and epidermis-ALOX3. Each of these LOX genes expresses a catalytically active enzyme except epidermis-ALOX3, which encodes an enzyme that has hydroperoxidase activity. Thus, the main LOX enzymes with fatty acid oxygenase activity found in humans are 5-LOX, 15-LOX-1, 15-LOX-2, platelet-type 12-LOX and 12R-LOX (Bhattacharya et al., 2009).

LOX products play important roles in the development of acute inflammation but they have also been implicated in inflammatory resolution (Ivanov et al., 2010). Available data also suggest that lipoxygenases contribute to *in vivo* metabolism of endobiotics and xenobiotics in mammals (Kulkarni, 2001). Recent reviews describe the role of lipoxygenase in cancer (Bhattacharya et al., 2009; Pidgeon et al., 2007; Moreno, 2009), inflammation (Duroudier et al., 2009; Hersberger, 2010) and vascular biology (Chawengsub et al., 2009; Mochizuki and Kwon, 2008) and for an extensive presentation of the role of eicosanoids in prevention and management of diseases the reader is referred to the review of Szefel et al. (2011).

CAROTENOIDS

There are over 600 fully characterized, naturally occurring molecular species belonging to the class of carotenoids. Carotenid biosynthesis occurs only in bacteria, fungi and plants where they have established functions that include their role as antenna in the light-harvesting proteins of photosynthesis, their ability to regulate light-energy conversion in photosynthesis, their ability to protect the plant from reactive oxygen species, and coloration. If these were the only known functions/properties of carotenoids in the natural world, continuous research in the field would be adequate; but these molecules are also part of the diet in higher species, and in animals and humans, carotenoids assume a completely different set of important function/actions (Mayne, 2010).

In humans, some carotenoids (the provitamin A carotenoids: α-carotene, β-carotene, γ-carotene and the xanthophyll β-cryptoxanthin) are best known for converting enzymatically into vitamin A; diseases resulting from vitamin A deficiency remain among the most significant nutritional challenges worldwide. Also, the role that carotenoids play in protecting those tissues that are the most heavily exposed to light (example photoprotection of the skin, protection of the central retina) is perhaps most evident, while other potential roles for carotenoids in the prevention of chronic diseases (cancer, cardiovascular disease) are still being investigated (example lycopene). Because carotenoids are widely consumed and their consumption is a modifiable health behaviour (via diets or supplements), health benefits for chronic disease prevention, if real, could be very significant for public health (Mayne, 2010).

The unique structure of carotenoids determines their potential biological functions and actions (Britton, 1995). Most carotenoids can be derived from a 40-carbon basal structure, which includes a system of conjugated double bonds. The central chain may carry cyclic end-groups which can be substituted with oxygen-containing functional groups. Based on their composition, carotenoids are divided in two classes, carotenes containing only carbon and hydrogen atoms, and oxocarotenoids (xanthophylls) which carry at least one oxygen atom (Stahl and Sies, 2003). The pattern of conjugated double bonds in the polyene backbone of carotenoids determines their light

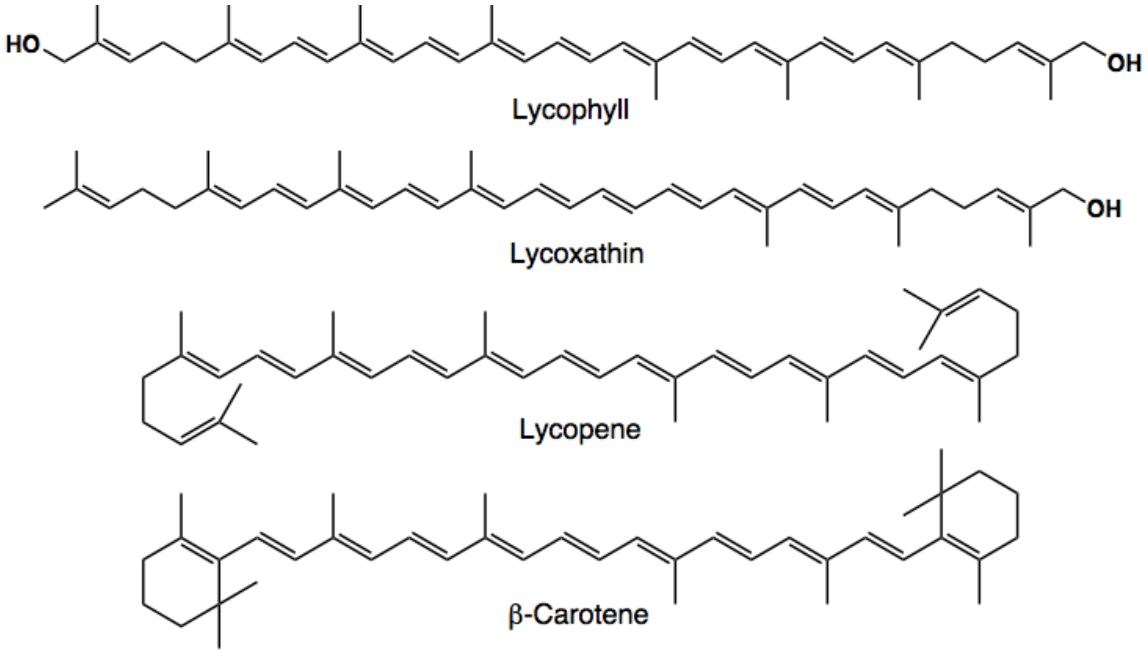

Figure 3. Carotenoids oxidized by lipoxygenase.

absorbing properties and influences the antioxidant activity of carotenoids (Stahl and Sies, 2003).

CAROTENOIDS' OXIDATION AND INHIBITION OF LIPOXYGENASE

Lipoxygenase not only has food-related applications in bread making (Casey, 1997) and aroma production (Whitehead et al., 1995); but also has negative implications for color, off-flavour and antioxidant status of plant based foods (Casey et al., 1996). In cereal products, vitamin decrease occurs after technological treatments (Fratianni et al., 2012). Since lipophilic vitamins are distributed in a tissue specific manner in cereal kernels, the most critical phase is milling, since it removes the outer layers (germ and aleurone) where most vitamins (tocopherols, β-carotene and zeaxanthin) are located (Panfili et al., 2004). During secondary technological processes a further loss could be due to several physical–chemical factors since tocols, carotenoids and retinols are sensitive to light, heat, air, and active surfaces. Moreover they could be degraded by both direct and lipoxygenase-mediated oxidation (Leenhardt et al., 2006; Wennermark and Jägerstad, 1992).

Studying the soya-lipoxygenase-catalyzed degradation of carotenoids from tomato, Biacs and Daood (2000) found that β-carotene was the most sensitive component, followed by lycoxanthin and lycopene (Figure 3). Their results also implied that β-carotene can actively perform its antioxidant function during the course of lipid oxidation. It seems that oxidative degradation and, accordingly, antioxidant activity of each carotenoid depends on the rate of its interaction with the peroxyl radical produced through the lipoxygenase pathway (Biacs and Daood, 2000) and thus is able to inhibit lipoxygenase. The inhibition of the hydroperoxide formation by carotenoids has been attributed to their lipid peroxyl radical-trapping ability (Burton and Ingold, 1984).

In vitro, lycopene is a substrate of soybean lipoxygenase. The presence of this enzyme also significantly increased the production of lycopene oxidative metabolites (dos Anjos Ferreira et al., 2004; Biacs and Daood, 2000). It was reported that during the co-oxidation of β-carotene by LOX-mediated hydroperoxidation reactions, inhibition of LOX activity takes place also (Lomnitski et al., 1993; Trono et al., 1999; Pastore et al., 2000). The activity of soybean lipoxygenase-1 was inhibited by β-carotene which inhibits the chain reaction at the beginning stage of linoleic acid hydroperoxidation (Serpen and Gökmen, 2006). Besides soybean lipoxygenase (Ikedioby and Snyder, 1977; Hildebrand and Hymowitz, 1982) carotene oxidation during lipoxygenase-mediated linoleic acid oxidation has been reported in various studies for the enzymes extracted from potato (Aziz et al., 1999), pea (Yoon and Klein, 1979; Gökmen et al., 2002), wheat (Pastore et al., 2000), olive (Jaren-Galan et al., 1999) and pepper (Jaren-Galan and Minguez-Mosquera, 1997). Soybean lipoxygenase-1 and recombinant pea lipoxygenase-2 and lipoxygenase-3, oxidizing β-carotene, yield apocarotenal, epoxycarotenal, apocarotenone and epoxycarotenone (Wu et al., 1999). Through molecular modelling, Hazai et al. (2006) predicted that lycopene and lycophyll bind with high affinity in the superficial cleft at the interface of the β-barrel and the catalytic domain of

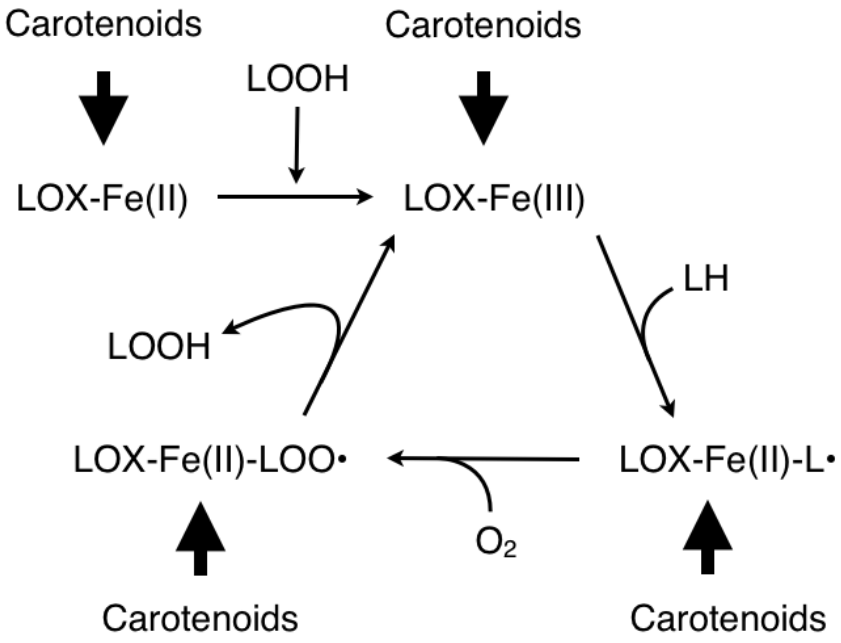

Figure 4. Possible interactions of carotenoids with lipoxygenase pathway.

5-LOX (the “cleavage site”) suggesting potential direct competitive inhibition of 5-LOX activity by these molecules after *in vivo* supplementation, particularly in the case of the dial metabolite. Subsequent evaluation of the direct interaction of the optically inactive stereoisomer of disodium disuccinate astaxanthin (Cardax™, meso-dAST) with human 5-LOX using spectroscopic techniques, and molecular docking calculations using 15-LOX as a surrogate for 5-LOX (for which XRC data has not been reported), suggested that the meso-compound was capable of interaction with, and binding to, the solvent-exposed surface of the enzyme (Lockwood et al., 2006). There is no clear data yet to evaluate any clear molecular interaction mechanism of carotenoids and their intermediates in LOX reaction. Summarizing the present knowledge we can say that:

1. Carotenoids (example lycopene, lycophyll, disodium disuccinate astaxanthin) could interact with LOX directly.
2. The interaction could yield allosteric effects as well as interaction with intermediates.
3. Therefore, all the members in the reaction cycle (Figure 4) may be the targets of carotenoids.

CONCLUSIONS

The enzyme LOX is worth special attention because of its possible repercussion on the final quality and colour of foodstuffs. LOX oxidises the carotenoids and chlorophyll, process known also as co-oxidation, forming hydroperoxides. As carotenoids are an important class of antioxidants, their destruction through oxidation decreases the power of this defence molecular system. On the other hand, the balance will be in the favour of the pro-oxidant hydroperoxides and so the damage increases. This oxidative behaviour has a negative effect also on the human health; the inhibition of lipoxygenase has important medical applications in cancer and inflammation. Up to now β-carotene seems to attract more attention in developing strategies for food processing to prevent LOX-mediated deteriorations such as oxidation, rancidity, and off-flavor. Lycopene and lycophyll were also studied as inhibitors of 5-LOX after *in vivo* supplementation. More research is necessary for transferring the *in vitro* mechanistic studies on the LOX inhibition *in vivo*. Other carotenoids like lutein, zeaxanthin, β-cryptoxanthin in pure form as well as in natural extracts could be good candidates for antioxidant action and so, LOX inhibition. Even though more difficult to test, the raw carotenoidic extracts keeping as much as possible the original matrix for antioxidant food supplements could prevent or lower the harmful LOX action in food and human tissues.

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