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Review

Mammalian Carotenoid-oxygenases: Key players for carotenoid function and homeostasis ☆

Glenn P. Lobo, Jaume Amengual, Grzegorz Palczewski, Darwin Babino, Johannes von Lintig *

Case Western Reserve University, School of Medicine, Department of Pharmacology, Cleveland, OH 44106, USA

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ABSTRACT

Humans depend on a dietary intake of lipids to maintain optimal health. Among various classes of dietary lipids, the physiological importance of carotenoids is still controversially discussed. On one hand, it is well established that carotenoids, such as β,β-carotene, are a major source for vitamin A that plays critical roles for vision and many aspects of cell physiology. On the other hand, large clinical trials have failed to show clear health benefits of carotenoids supplementation and even suggest adverse health effects in individuals at risk of disease. In recent years, key molecular players for carotenoid metabolism have been identified, including an evolutionarily well conserved family of carotenoid-oxygenases. Studies in knockout mouse models for these enzymes revealed that carotenoid metabolism is a highly regulated process and that this regulation already takes place at the level of intestinal absorption. These studies also provided evidence that β,β-carotene conversion can influence retinoid-dependent processes in the mouse embryo and in adult tissues. Moreover, these analyses provide an explanation for adverse health effects of carotenoids by showing that a pathological accumulation of these compounds can induce oxidative stress in mitochondria and cell signaling pathways related to disease. Advancing knowledge about carotenoid metabolism will contribute to a better understanding of the biochemical and physiological roles of these important micronutrients in health and disease. This article is part of a Special Issue entitled Retinoid and Lipid Metabolism.

Carotenoids are tetraterpenoids (C_{40}) containing up to 15 conjugated double bonds. According to their chemical properties these isoprenoid pigments are divided into two classes; pure hydrocarbons such as β,β-carotene and lycopene are called carotenes, whereas their oxygenated derivatives such as zeaxanthin and lutein are called xanthophylls (Fig. 1). Carotenogenic organisms include all photosynthetic plants, protists and bacteria, as well as some heterotrophic bacteria and some fungi. Carotenoids are accessory pigments in the antennae of chloroplasts, where they augment the light-harvesting capacity by absorbing light in the blue-green range of the visible spectrum (450–550 nm) and transferring the energy to chlorophylls. Carotenoids are also involved in photoprotection in plants and are known antioxidants [1]. Other carotenoid roles derive from the exploitation of their intense and attractive coloration. Hence, carotenoids are important in the pigmentation of flowers and fruits to attract animals for pollination and seed dispersal. In animals, carotenoids contribute in the pigmentation of invertebrates, birds and fishes. As a coloration of male birds, carotenoids have been shown to be an important trait for sexual attractiveness [2,3]. Human tissues also retain considerable amounts of carotenoids that may play a role in preventing oxidative damage. Additionally, the central part of the primate retina, the macula lutea, owes its yellow color to high levels of the xanthophylls, lutein and zeaxanthin. These macular pigments have been suggested to filter short-wavelength visible light, to lessen chromatic aberration and to prevent the onset of age-related macular degeneration [4,5].

Living organisms transform carotenoids to generate unique series of metabolites [6]. These cleavage products of carotenoids, apocarotenoids, are important signaling molecules and chromophores of photopigments in all kingdoms of living organisms. In higher plants, carotenoid-derived abscisic acid (ABA) and strigolactones influence processes, as diverse as seed dormancy, morphogenesis and environmental adaptation. Cleavage of carotenoids in plants also yields other derivatives (e.g., β-ionone, α-ionone and saffranal) which are important for coloration and chemoattraction (or repulsion) (for review see, [7]). Similarly, most animals metabolize carotenoids to diterpenoid molecules such

Abbreviations: C/EBPα, CCAAT/enhancer-binding protein α; BCMO1, β,β-carotene-15,15′-monooxygenase 1; BCDO2, β,β-carotene-9,10-dioxygenase 2; CCE, carotenoid cleaving enzyme; CD36 (SCARB3), Cluster of Differentiation 36; FABP4 (aP2), fatty acid-binding protein 4; FHR, fenretinide; HPLC, high-performance liquid chromatography; iWAT, inguinal white adipose tissue; PPAR, peroxisome proliferator-activated receptor; SR-BI, scavenger receptor class B type I; RAL, all-*trans*-retinal; RA, all-*trans*-retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; RetSat, retinol saturase; RPE65, retinal pigment epithelium 65 kDa protein; RE, retinyl esters; ROS, reactive oxygen species; TG, triacylglycerol; VAD, vitamin A-deficient diet; VAS, vitamin A-sufficient diet; WT, wild type

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* Corresponding author at: Department of Pharmacology (W333), Case Western Reserve University, 10900 Euclid Avenue, Cleveland, Ohio 44160, USA. Tel.: +1 216 368 3528; fax: +1 216 368 1300.
E-mail address: Johannes.vonlintig@case.edu (J. von Lintig).

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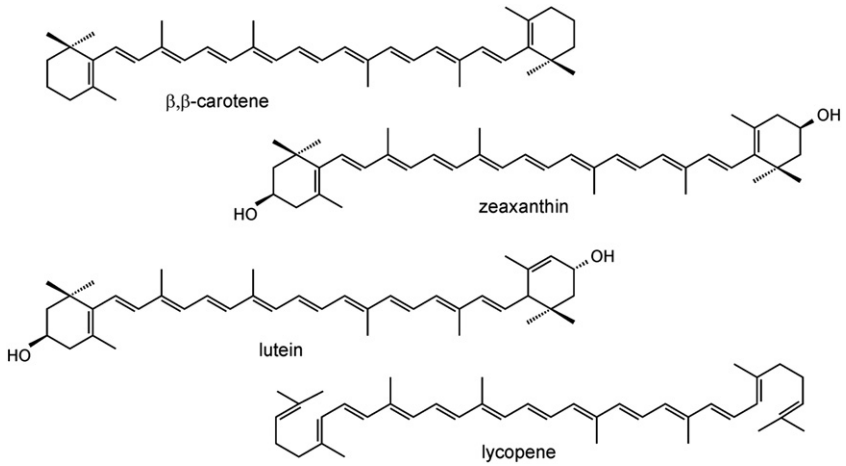

Fig. 1. Chemical structure of major carotenoids in the human blood.

as retinaldehyde, the visual chromophore [11], and all-*trans*-retinoic acid (RA), a hormone-like compound that regulates gene expression [12]. Retinaldehyde serves as a photosensory pigment of mammalian cone and rod visual pigments. A similar function is found in green algae [8] and it also is involved in light-dependent proton pumping in *Halobacterium* [9]. Additionally, asymmetric oxidative cleavage of carotenoids has been described in animals leading to the production of apocarotenoids different from retinoids [10]. Thus, carotenoids and their apocarotenoid derivatives exert critical physiological functions throughout many living organisms in the kingdoms of nature.

1. Out of the wild green yonder: carotenoid cleaving enzymes

Vitamin A was recognized as an essential factor in food a century ago. Early research suggested that certain yellow plant pigments had the same activity as vitamin A. This phenomenon was explained in 1930 by Moore [11], who described a conversion of β,β-carotene into vitamin A in the small intestine of the rat, thus providing the first evidence that a plant-derived carotenoid is the direct precursor of retinoids. Karrer [12] elucidated the structure of β,β-carotene and proposed a central cleavage of the C (15,15′) double bond of β,β-carotene to form two molecules of retinaldehyde. In cell free-extracts, cleavage of β,β-carotene by a 15,15′-oxygenase to produce two molecules of RAL has been reported [13,14]. Enzymatic oxidative cleavage of carotenoids at a specific position of the polyene chain also has been proposed in bacteria and plants as a method for the synthesis of apocarotenoids. By analyzing the molecular basis of the ABA-deficient maize mutant, *vp14* (*viviparous* 14), the first carotenoid-cleaving enzyme was molecularly characterized [15]. These researchers proposed that enzymes related to VP14 catalyze oxidative cleavage of carotenoids in other organisms as well. Indeed, this breakthrough was followed by the molecular cloning and biochemical characterization of related carotenoid cleavage enzymes (CCEs) not only in plants but also in animals [16,17], fungi [18] , and bacteria [19]. CCEs depend on ferrous iron as a cofactor but do not contain a heme ring. Thus, CCEs belong to the family of non-heme iron oxygenases, but it is still controversial whether they act in a monooxygenase or dioxygenase fashion [20,21]. Initially, evidence for a monooxygenase mechanism was provided for a vertebrate enzyme. However, exchange of the oxygen label of retinaldeyde with bulk water may have hampered this analysis. Studies with an eccentrically cleaving plant enzyme producing a ketone cleavage product (no exchange of the oxygen label) indicated a dioxygenase mechanism and thus incorporation of both oxygen atoms into the substrate. Studies with mammalian β,β-carotene-15,15′-monooxygenase 1 (BCMO1) suggest that carotenoid cleavage involves the formation of a carbocation intermediate and cation-π stabilization by aromatic residues in the carotenoid-binding cleft [22]. Studies with animal CCEs revealed an additional enzymatic property of these enzymes; they can isomerize the C10,C11 double bonds of their substrates [23]. This reaction is critical for the formation of 11-*cis*-retinal or derivatives thereof such as 11-*cis*-3-OH-retinal. This geometric state of the chromophore can bind to the protein moiety (opsin) to form functional visual pigments. In insects, the oxidative cleavage of carotenoids and the all-*trans* to 11-*cis* isomerization of carotenoids is catalyzed by a single protein named NinaB [24]. In mammals, carotenoid cleavage and all-*trans* to 11-*cis* isomerization is catalyzed by two distinct family members, respectively named BCMO1 and retinal pigment epithelium 65 kDa protein (RPE65). RPE65 is not an actual carotenoid-oxygenase, but the long-sought retinoid isomerase in the vertebrate visual cycle and catalyst of the conversion of retinyl esters (RE) to 11-*cis*-retinol [25–28]. In contrast to carotenoid-oxygenases, RPE65 does not incorporate oxygen into its substrate, but the reaction also depends on ferrous iron [29]. Mutations in NinaB and RPE65 cause chromophore deficiency and blindness [30–32], thus emphasizing the importance of this class of proteins for animal vision.

The crystal structure has been resolved for three family members of carotenoid-oxygenase; a bacterial ACO (apocarotenoid-oxygenase) [33], plant VP14 [34], and vertebrate RPE65 protein [35]. The analyses of these structures revealed that the structural fold of CCEs is well conserved between family members of different kingdoms; the basic structural motif being a 7-bladed β-propeller. The iron cofactor is coordinated by four conserved histidine residues and three second shell glutamate residues. The iron is accessible through a long non-polar tunnel juxtaposing the substrate to the active center. This ground-breaking structural work will allow comparisons between CCEs for identification of functional site residues that participate in the isomerization and/or oxidative cleavage reaction at specific sites of the polyene carbon backbone of the substrates.

2. Mammalian genomes encode three different CCE family members

In mammals, three different members of the CCE family have been molecularly identified and biochemically characterized. RPE65 is expressed in the retinal pigment epithelium of the eyes and localizes to the endoplasmatic reticulum. The critical role of RPE65 in visual chromophore production and regeneration is well established and has been extensively reviewed (e.g., [23]). The other two family members, BCMO1 and β,β-carotene-9,10-dioxygenase 2 (BCDO2), are true carotenoid-oxygenases and catalyze the oxidative cleavage of