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Review

Towards a better understanding of carotenoid metabolism in animals

Johannes von Lintig*, Susanne Hessel, Andrea Isken, Cornelia Kiefer, Johanna M. Lampert, Olaf Voolstra, Klaus Vogt

Institute of Biology I, Dept. of Animal Physiology and Neurobiology, University of Freiburg, Hauptstrasse 1, D-79104 Freiburg, Germany

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Abstract

Vitamin A derivatives (retinoids) are essential components in vision; they contribute to pattern formation during development and exert multiple effects on cell differentiation with important clinical implications. All naturally occurring vitamin A derives by enzymatic oxidative cleavage from carotenoids with provitamin A activity. To become biologically active, these plant-derived compounds must first be absorbed, then delivered to the site of action in the body, and metabolically converted to the real vitamin. Recently, molecular players of this pathway were identified by the analysis of blind *Drosophila* mutants. Similar genome sequences were found in vertebrates. Subsequently, these homologous genes were cloned and their gene products were functionally characterized. This review will summarize the advanced state of knowledge about the vitamin A biosynthetic pathway and will discuss biochemical, physiological, developmental and medical aspects of carotenoids and their numerous derivatives.

Keywords: Vitamin A; Carotenoid metabolism in animal; bco; bco2; rpe65; Retinoic acid signaling

We are all familiar with carotenoids as the yellow to red coloring of fruits, flowers and vegetables. These colored compounds, C40 isoprenoids, are synthesized in plants, certain fungi and bacteria. Their characteristic chemical and physical properties are responsible for their light absorption as well as for the inactivation of free radicals (for a recent review, see Ref. [1]). Among the various classes of pigments found in nature, the diverse family of carotenoids is the most widespread, with important functions not only in carotenoid-producing organisms. Some animals use dietary carotenoids for coloration, well-known examples are the feathers of flamingos and the red color of salmon. However, carotenoids not only color the world around us, but are being intensively investigated currently regarding their potential to prevent chronic disease and vitamin A deficiency (VAD). Thanks to their anti-oxidative properties, beneficial effects have been reported for carotenoids in reducing the risk of coronary heart diseases, certain kinds of cancer and age-related macular degeneration (AMD) (reviewed in Ref. [2]). Most importantly, certain carotenoids are the precursors (provitamins) for the formation of vitamin A in animals.

In humans, VAD leads to night blindness in milder forms, while more severe progression results in corneal malformations, e.g., xerophthalmia. Besides visual defects, this deficiency affects the immune system, leads to infertility or causes malformations during embryogenesis. The molecular basis for these diverse effects is found in the dual role exerted by vitamin A derivatives in animal physiology.

In the entire animal kingdom, 11-*cis*-retinal or closely related compounds such as 11-*cis*-3-hydroxyretinal serve as the chromophores of the visual pigments (rhodopsin) [3,4]. Light activation of these G protein-coupled receptors is the first step in phototransduction, the process by which light is converted into a photoreceptor's electrical response. Besides being essential for vision, in vertebrates the vitamin A derivative retinoic acid (RA) is a major signal controlling a wide range of biological processes. RA is the ligand of two

* Corresponding author. Tel.: +49 761 203 2539; fax: +49 761 203 2921.
E-mail address: johannes.von.lintig@biologie.uni-freiburg.de (J. von Lintig).

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classes of nuclear receptors, the retinoic acid receptors (RARs) and the retinoid X receptors (RXRs) [5,6] (reviewed in Refs. [7,8]). The active receptor complex is a RAR/RXR heterodimer that binds DNA regulatory sequences and regulates gene transcription in response to RA binding. RXR is not only the heterodimer partner of the RAR receptor but also an obligate partner for other nuclear receptors (for recent review, see Ref. [9]). The pleiotropic effects of vitamin A are explained by the discovery that the RA-responsive target genes are involved in a panoply of biological processes as diverse as pattern formation during embryonic development, cell differentiation and control of certain metabolic activities.

VAD is still a major problem particularly in developing countries. Vitamin A demand can be met either by preformed vitamin A or by carotenoids with provitamin A activity. Today we know that all naturally occurring vitamin A in the food chain derives from provitamin A conversion and that the world's population mainly relies on carotenoids from staple food sources to satisfy their vitamin A need [10]. Despite the importance of provitamin A metabolism, its molecular details have remained elusive for long time. This review will focus on recent advances in this field of research. The use of genetically well-defined model organisms led to the identification of respective genes and loss-of-function analyses provided new insights into basic principles of this metabolism, e.g., of the tissue specificity of provitamin A conversion and the regulation of vitamin A homeostasis, all with substantial impact on animal physiology and human health.

1. The key step in vitamin A formation: the BCO protein and its gene

In 1930, Moore [11] provided the first evidence that a carotenoid is the precursor of vitamin A by describing β-carotene conversion in the small intestine of mammals. For this reaction, a central cleavage mechanism at the C-15,C-15′ double bond for the conversion of β-carotene to vitamin A was proposed soon thereafter by Karrer et al. [12]. Then Goodman and Huang [13] and Olson and Hayaishi [14] characterized the respective enzymatic activity in cell-free homogenates from rat small intestine. The β-carotene-cleavage enzyme depended on molecular oxygen and thus the enzyme was termed β,β-carotene-15,15′-oxygenase (BCO). It was reported to be soluble, to have a slightly alkaline pH-optimum and to be inhibited by ferrous iron chelators and by sulfhydryl-binding compounds, indicating that it contains a ferrous iron co-factor [15]. Subsequently, this enzyme was also characterized in different mammalian species [16] and substrate specificity was determined for different β-carotene stereoisomers [17]. Recent investigation of the mode of action of BCO provided strong evidence that oxidative cleavage at the central (15,15′) double bond is catalyzed in a monooxygenase mechanism via a transient carotene epoxide [18].

In 2000, two research groups independently succeeded in cloning the key enzyme in vitamin A formation [19–21]. The approach by von Lintig and Vogt relied on sequence homology to the plant carotenoid-cleaving enzyme VP14, which catalyzes 9-*cis*-epoxycarotenoid cleavage in the biosynthetic pathway of the plant growth factor abscisic acid [22]. By employing an expression cloning strategy in an *Escherichia coli* strain genetically engineered to produce all the enzymes needed to synthesize β-carotene de novo, they identified a β,β-carotene-15,15′-oxygenase from the fruit fly *Drosophila melanogaster.* The enzymatic properties of the purified recombinant carotene oxygenase revealed that it catalyzed exclusively the centric cleavage of β-carotene (C40) to yield retinal (C20) and that it depended on ferrous iron as cofactor [19]. Direct genetic evidence that this enzyme catalyzes the key step in vitamin A formation was provided by mutant analysis. Among the various available *Drosophila* mutants affected in visual performance, the *ninaB* mutant lacks the visual chromophore, when raised on standard media with carotenoids as the sole source for vitamin A formation. The *ninaB*-mutation has been cytologically mapped in the *Drosophila* genome on chromosome 3 at position 87E–F [23], coinciding with the physical location of the *Drosophila bco* gene. By analyzing the molecular basis of the blindness of *ninaB* mutants, von Lintig et al. [24] showed that this phenotype is caused by mutations in the *bco/ninaB* gene, thus unequivocally demonstrating that Bco/NinaB actually catalyzes vitamin A synthesis in vivo.

Confirmation that this type of enzyme generally in metazoans catalyzes the first step in vitamin A metabolism was provided by Wyss et al. [20,25] by cloning a Bco from chicken. Their approach relied on partial protein purification and determination of peptide sequences, then using this information to synthesize corresponding degenerate oligonucleotideprimers for PCR to generate a partial cDNA and screen a cDNA library derived from chicken small intestine. Amino acid sequence comparison between the *Drosophila* and chicken Bcos showed an overall similarity with several highly conserved regions and a significant similarity to some domains of the plant carotenoid oxygenase VP14 [21].

Subsequently, *BCO* genes from mouse and human were identified and the recombinant proteins biochemically characterized in several laboratories [26–30]. The mammalian BCO catalyzed the cleavage of carotenoid substrates with at least one unsubstituted β-ionone ring, such as β-carotene and β-cryptoxanthin, and there was no cleavage of lycopene or zeaxanthin [29]. The K_m values for β-carotene were estimated to be in the range of 1–10 μM [19,26,27,29]. BCO exhibits a slightly alkaline pH optimum, and enzymatic activity is sensitive to chelating agents such as *o*-phenanthroline and α,α′-bipyridyl, indicating that it depends on ferrous iron [19,29]. Thus, the purified