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Review

Molecular and dietary regulation of β,β-carotene 15,15′-monooxygenase 1 (BCMO1)

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ABSTRACT

β,β-Carotene 15,15′-monooxygenase-1 (BCMO1) is a key enzyme in vitamin A metabolism in mammals. Various dietary components such as non-pro-vitamin A carotenoids, fat, and polyphenols have been shown to influence the intestinal absorption and conversion of pro-vitamin A carotenoids. Furthermore, vitamin A deficiency has been shown to induce BCMO1 expression, whereas supplementation with vitamin A or its activ\e metabolites, all-*trans* and 9-*cis* retinoic acid, dose-dependently reverse these effects. A diet-responsive regulatory network involving the intestine specific homeodomain transcription factor ISX has been shown to regulate the intestinal vitamin A uptake and production via a negative feedback control. Furthermore, non-synonymous single nucleotide polymorphisms in the human *BCMO1* gene have been discovered causing observably reduced BCMO1 activity. Detailed knowledge about BCMO1 regulation as well as genetic variations causing variable cleavage activities may provide a background, on which individual and/or population based dietary recommendations for β-carotene and vitamin A intake could be established.

Introduction

Vitamin A (retinal and retinol) and its derivative retinoic acid (RA)[1] are involved in numerous physiological processes. The isomer 11-*cis* retinal is crucial for vision, whereas the transcriptional regulator RA exerts most vitamin A functions, e.g., in embryonic development, reproduction, cell growth and differentiation, and immunity [1–3]. RA is supposed to be involved in regulation of more than 500 genes [4]. Because animals and humans are unable to synthesise vitamin A *de novo*, they must consume diets with preformed vitamin A, predominantly as retinyl esters, or pro-vitamin A carotenoids, such as β-carotene and carotenoids containing an unsubstituted β-ionone ring. For absorption hydrophobic β-carotene is incorporated into mixed micelles together with lipids and bile components [5]. These enter the enterocyte either via passive diffusion or through active transport via the cholesterol transporter scavenger receptor class B, type 1 (SR-B1) [6–8]. In humans, between 35% and 90% of absorbed all-*trans* β-carotene is oxidatively cleaved by the β,β-carotene 15,15′-monooxygenase 1 (BCMO1; EC 1.14.99.36) into two molecules of all-*trans* retinal, which subsequently can be oxidised irreversibly to retinoic acid by retinal dehydrogenase or reduced reversibly to retinol by a retinal reductase [5,9]. Developmental inductions of BCMO1, retinal dehydrogenase and retinal reductase in the chick duodenum suggests that a co-ordinated induction mechanism should be operative for these three enzymes [10,11].

Carotenoid bioavailability and bioconversion are heavily influenced by multitudinous extrinsic and intrinsic factors [12–15]. This review focuses on recent advances in the elucidation of activity and transcriptional regulation of BCMO1 by macronutrients, phytochemicals, carotenoids, vitamin A and its derivatives. Furthermore, it summarizes the impact of genetic variations in the *BCMO1* gene that impact on the observed high inter-individual differences for β-carotene cleavage and absorption [16,17].

Biochemical properties

BCMO1 has been biochemically characterised in various species such as human, mouse, rat, chicken, ferret, rabbit, pig, and guinea-pig. The Michaelis–Menten constant (K_m) for the enzyme, with β-carotene as the substrate, is between 0.52 and 31 μmol/l. The maximum reaction velocity (V_{max}) ranges from 0.01 to 420 nmol retinal formed/mg protein ∗ h for different species and experimental conditions (Table 1) [17–27].

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[1] *Abbreviations used:* RA, retinoic acid; SR-B1, scavenger receptor class B, type 1; BCMO1, β,β-carotene 15,15′-monooxygenase 1; Fe^{2+}, ferrous iron; EDTA, ethylenediaminetetraacetic acid; BHT, 2,6-di-*tert*-butyl-4-methylphenol; BHA, butylated hydroxyanisol; TBHQ, *tert*-butylhydroquinone; NDGA, nor-dihydrogaiaretic acid; RAR, retinoic acid receptors; RARE, retinoic acid response element; PPRE, peroxisome proliferator response element; CRE, cAMP response element; MEF2, myocyte enhancer factor 2; LCFAs, long-chain fatty acids; SNPs, single nucleotide polymorphisms.

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BCMO1 enzyme activity can be significantly inhibited by various ferrous iron (Fe^{2+}) chelators, such as *o*-phenanthroline and α,α′-dipyridyl and possibly by ethylenediaminetetraacetic acid (EDTA). Furthermore, BCMO1 is sensitive to the sulfhydryl alkylating agents *N*-ethylmaleimide, *p*-chloromercuribenzoate, iodoacetamid, sodium arsenite, and *p*-hydroxymercuribenzoate (Table 2) [21,24,27,28]. *N*-Ethylmaleimide or *p*-chloromercuribenzoate might inhibit BCMO1 by preventing the binding of the substrate by the alkylation of one or several free thiols within the enzyme [24], though the inhibition caused by *p*-(OH or Cl)-mercuribenzoate was almost completely reversed by the addition of glutathione [27,29]. Addition of sulfhydryl reducing agents, such as mercaptoethanol and glutathione, can even maximise BCMO1 activity (Table 2) [21,27,29,30]. It was recently shown that tyrosine Y235 and Y326 in mouse BCMO1 fix the position of the substrate on the two sides of the 15,15′-double bond most likely due to a mechanism implicating cation π-stabilization [31]. Diphenylamine inhibits BCMO1 at physiological pH either due to its ability to protonate (non-competitive inhibition) or to its similarity to transition state carbocation intermediates (competitive inhibition) which are formed during the cleavage reaction by BCMO1 (Table 2) [31]. BCMO1 has high specificity towards the double bonds at the 15,15′-position of carotenoids but quite broad specificity towards carotenoids containing at least one unsubstituted β-ionone ring (Table 3) [22,24,25,29,32]. However, enzyme activities measured with carotenoids different from β-carotene are significantly lower [22].

Tissue specific expression and activity

BCMO1 activity and expression was found in several tissues of various vertebrate species, including humans. The enzyme activity was detected in the intestinal mucosa from human, guinea-pig, mouse, rat, ferret, monkey, and rabbit [24,33–38]. β-Carotene conversion is not exclusively restricted to the digestive tract, and BCMO1 activity has also been demonstrated in liver, kidney, lung, brain, testis, and adipose tissue of animals [37,39–41]. In human tissues, BCMO1 expression is present at high levels in liver and kidney and lower levels in prostate, testis, ovary, colon, and skeletal muscle [24]. BCMO1 was predominantly found in epithelial cells of several tissues including stomach, intestine, colon, prostate glands, and endometrium. In the kidney the enzyme was localised in the distal and proximal tubular structures. Moreover, immunohistochemical analysis revealed BCMO1 expression in the exocrine portion of the pancreas, epidermis of the skin, and ciliary body pigment epithelia and RPE of the eye. Moderate expression of BCMO1 was found in androgen-producing Leydig and Sertoli cells of the testes. *BCMO1* mRNA was also found in the cortex of the adrenal gland, the subset of skeletal muscles as well as the oestrogen-producing granulosa cells and steroid-producing theca interna of the ovary [34].

During development, BCMO1 activity in the chicken is already detected at the embryonic state and significantly increases around hatching and up to seven days thereafter. Furthermore, the induction patterns of other enzymes in the vitamin A pathway such as retinol reductase, CRBP 2 and retinol dehydrogenase, were very similar to that of BCMO1 [10]. *In situ* hybridisation of tissues of five-day old chicken displayed the presence of BCMO1 in the central nervous system, lungs, limbs, heart, kidney, and cardiovascular system [42].

Similar to the distribution of BCMO1 in peripheral tissues, it was shown that highest β-carotene levels in humans were found in liver and the adrenal gland. Furthermore, β-carotene accumulation was also detected in testis, kidney, ovary, and adipose tissue [43,44]. Thus, it is likely that BCMO1 plays a significant physiological role in the local regulation of vitamin A and RA in reproduction and development.

Table 1
Biochemical properties of BCMO1.

	Rat [22,27,28,30]	Pig [19–21]	Rabbit [29]	Guinea-pig [20]	Mouse 2001 [23] (recombinant)	Human [17,18,24] (recombinant)	Chicken [25] (recombinant)
Molecular weight (kDa)	100–200	100–200			63	64	240
pH-Optimum (at 37 °C)	7.7	7.8–8.2	7.8			7.5–8.0	8.0
K_m (μmol/l)	0.52–5.91	1.3; 5.6			6	6–31	26
V_{max} (nmol retinal/mg protein ∗ h)	0.099; 1.19	1.6; 0.01		0.06	2.16	66–420	

Table 2
Inhibitors and stimulators of BCMO1 activity.

	Rat [27,28,30]	Pig [21,58]	Rabbit [29,84]	Mouse [31]	Human [24]*
Ferrous-ion chelators					
α,α′-Dipyridyl	I	I/-	I		I
o-Phenanthroline	I	I	I		I
EDTA	I/-	-			
Cyanide	S/I		-		
8-Hydroxyquinoline			I		
Sulfhydryl alkylating agents					
N-Ethylmaleimide	I	I	I/-		I
Iodoacetamide	I		I/-		
p-(OH or Cl) Mercurizoate	I		I		I
Na-Arsenite	I				
o-Iodosobenzoate			I		
Sulfhydryl reducing agents					
Reduced glutathione	S reversed inhibition	Omission reduced enzyme activity	Reversed inhibition		S
Mercaptoethanol	S	S			S
Diphenylamine				I	

S, Stimulation of the enzyme activity; I, inhibition of the enzyme activity; –, no effect; *, recombinant enzyme was used in the experiments.