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Biosynthesis of Vitamin A from β-Carotene[1,2]

DEWITT S. GOODMAN[3]

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THE BIOSYNTHESIS of vitamin A from β-carotene takes place mainly in the intestinal mucosa, during the absorption of dietary β-carotene. The normal in vivo reaction sequence involves two steps: first, the cleavage of β-carotene to form two molecules of retinal; second, the reduction of retinal to retinol. The newly formed retinol is then mainly esterified with long-chain fatty acids, and the retinyl esters are incorporated into lymph chylomicrons and transported from the intestine via the intestinal lymphatics.

Studies with lymph-fistula rats have demonstrated that virtually no β-carotene is absorbed unchanged beyond the intestinal mucosa (1-3). Instead, the β-carotene is apparently absorbed into the mucosal cells and there converted into retinal. Although a small portion of the retinal may be oxidized to retinoic acid, under physiological conditions, retinal is mainly reduced to retinol (4). The retinol is next esterified with fatty acids, and transported via the lymphatics as described above. In the rat, 90% of the radioactivity absorbed into the lymph, after the feeding of either labeled retinol or labeled β-carotene, was found as labeled retinyl esters (3). Man differs from the rat in that the human intestine is able to absorb a small amount of unchanged dietary β-carotene into the lymph (5). The quantitative difference in the ability of these two species to absorb intact β-carotene is, however, not very great, since most of the radioactivity absorbed into human lymph after feeding labeled β-carotene was also found in retinyl esters, and not in unchanged β-carotene (5). In both man (5) and the rat (3) the composition of the lymph retinyl esters was found to be remarkably constant, regardless of the fatty acid composition of the diet and regardless of whether the retinyl esters were derived from preformed vitamin A or from β-carotene. Retinyl palmitate predominated in all samples, and saturated esters consistently comprised approximately three-fourths of the labeled retinyl esters. After entering the vascular compartment, the newly formed retinyl esters are mainly taken up by the liver (6), where they undergo turnover, and where vitamin A is mainly stored in ester form in the body.

We have extensively studied the in vitro cleavage of β-carotene into retinal with soluble enzyme preparations from rat intestinal mucosa (7-9). The reaction appears to be a dioxygenase reaction (8, 10), in which molecular oxygen reacts with the central two carbon atoms of β-carotene, followed by the cleavage of the central double bond of carotene to yield two molecules of retinal. The evidence for this reaction mechanism includes the following: *1*) the reaction shows an absolute requirement for molecular oxygen (7); *2*) the stoichiometry of the results indicates the conversion of one molecule of β-carotene

[1] From the Department of Medicine, Columbia University College of Physicians and Surgeons, New York, New York 10032.

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[3] Career Scientist of the Health Research Council of the City of New York under Contract I-399.

into two molecules of retinal (7); *3*) studies with doubly labeled β-carotene demonstrated that the hydrogen atoms attached to the two central carbon atoms of β-carotene are completely retained during the conversion of β-carotene into retinal (8); and *4*) the reaction is inhibited by the chelating agents α,α′-dipyridyl and *o*-phenanthroline (9). The enzyme has been referred to as β-carotene-15,15′ dioxygenase (10), or, as the carotene cleavage enzyme. In addition to molecular oxygen, the in vitro reaction requires the addition of an appropriate detergent or detergent–lipid mixture. A great deal of empirical information has been obtained about the detergent–lipid requirement (9), but the role which the detergent–lipid mixture plays in the in vitro reaction is still not clear.

Studies have been conducted recently on the conversion of β-carotene to retinal with enzyme preparations from homogenates of hog intestinal mucosa (11). The hog mucosal enzyme was purified approximately 27-fold by precipitation with ammonium sulfate, chromatography on DEAE-Sephadex, and gel filtration on Sephadex G-200. The reaction displayed a narrow optimal pH range (ca. 7.8–8.2). The enzyme was stimulated strongly by the addition of thiols and was inhibited by thiol inhibitors and by the chelating agents α,α′-dipyridyl and *o*-phenanthroline. The reaction required the addition of an appropriate detergent (or bile salt); maximal activity was obtained by addition of an appropriate combination of detergents and lipid. The reaction displayed Michaelis kinetics with an apparent K_m of 1.3×10^{-6} M and a V_{max} of 1.1×10^{-9} moles of retinal formed per hour (for 0.7 mg enzyme protein). The properties of the hog cleavage enzyme are generally similar to those previously observed with the less purified rat enzyme preparation.

The reduction of retinal to retinol was studied with an approximately 13-fold purified enzyme preparation from rat intestinal mucosa (12). The enzyme was relatively heat stable and had a molecular weight approximately in the range of 60,000–80,000. The partly purified reductase was unable to oxidize ethanol in the presence of NAD^+. Retinal reduction required NADH or NADPH as cofactor. Both reduced nucleotides were effective; at low nucleotide concentration NADH was more effective, whereas at high concentrations the reaction rate was slightly greater with NADPH. The reaction was stimulated by the addition of glutathione and was inhibited by —SH inhibitors. No other cofactors were required. There was a sharp pH optimum near 6.3. Retinal reduction displayed Michaelis kinetics, with a V_{max} of 2×10^{-6} moles of retinol formed per hour per mg of protein and with an apparent K_m of 2×10^{-5} M. The enzyme appears to be a relatively nonspecific aldehyde reductase. Short- and medium-chain aliphatic aldehydes, of length C_2 to C_{14}, were actively reduced, with greatest activity being seen with aldehydes of length C_4 to C_8. Unsaturated C_{18} fatty aldehydes were reduced at a lesser rate, but saturated aldehydes of length C_{16} or greater were not reduced. The enzyme was stereospecific for 4*R*-NADH-4-$^3H_1$, and did not incorporate tritium from 4*S*-NADH-4-$^3H_1$ into the product retinol.

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