METABOLIC PATHWAYS OF CAROTENE TO VITAMIN A IN ANIMALS

III. DEGRADATION OF β -CAROTENE AND VITAMIN A IN RAT INTESTINE

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In the previous paper (1), it was reported that two metabolic intermediates were detected on paper chromatograms in the experiment using the intestine homogenate of rats. A compound with an absorption maximum at 296 m μ in ethanol was identified as β -ionone, and another compound having an absorption maximum at 365 m μ in petroleum ether was assumed to be a vitamin A aldehyde-protein-complex. Further, the conversion of β -carotene to vitamin A in the homogenate of the intestine from thiamine-deficient rats was found to be extremely low. The addition of one of the coenzymes such as NAD and FAD showed scarcely any effect on the conversion of β -carotene to vitamin A alcohol, but the addition of both NAD and FAD resulted always in positive results (2).

In the present experiment, the degradations of β -carotene, vitamin A and its derivatives by rat-intestine enzymes under aerobic condition were further investigated using the synthetic preparations and the physicochemical properties of the products were studied by paper chromatography and by determining the absorption spectra. Oxygen uptake of β -carotene, vitamin A and related compounds by the homogenate was further determined manometrically using Warburg's apparatus. It was always found to be rather low when β -carotene, vitamin A alcohol and vitamin A aldehyde were used as substrates. On the other hand, additive effects of succinate, malonate, fumarate or α -ketoglutarate on the degradation of the vitamins were observed, and vitamin A acid was always detected in the final degradation products. Further, the degradation products were also tested by paper chromatography and spectrophotometry after addition of NAD, FAD or cytochrome c (3).

The findings suggested that the "central cleavage" of β -carotene might unexpectedly take place *in vivo*, but β -carotene also seems to be metabolized to vitamin A by terminal oxidation because of the appearance of β -ionone besides protein-bound vitamin A aldehyde, which seems to be further converted to vitamin A alcohol and vitamin A acid (4—6).

It seems therefore to be plausible that the metabolic pathway of β -carotene to

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vitamin A in rat intestine can be as follows (Fig. 1).

FIG. 1 Possible Metabolic Pathways of β -Carotene and Vitamin A

EXPERIMENTAL AND RESULTS

1. Standard Preparations

Standard preparations used in the experiments were commercial products, and the purity was ascertained from the physicochemical properties. Free vitamin A alcohol was prepared from synthetic vitamin A palmitate or acetate. Standard vitamin

FIG. 2 Preparation of Vitamin A Aldehyde

TABLE I
Ultraviolet Absorption Maxima of Standard Preparations

Preparation	$E \frac{1}{1\text{ cm}} \%$ at λ_{max} in			
	Petroleum ether	Ethanol	Chloroform	References
Vitamin A alcohol	1655 (327)	1510 (327)	1510 (335)	—
Vitamin A acetate	—	—	1480 (328)	(325-8)
Vitamin A palmitate	—	—	1500 (328)	(325-8)
Vitamin A aldehyde	1688 (368)	1051 (370)	1326 (388)	1685 (369.5) ^a 1050 (368) ^b 1303 (389) ^c
Vitamin A acid	—	1400 (350)	1480 (370)	1415 (350) ^b
β -Carotene	1742 (450)	(470)	1352 (465)	(450) ^a
Vitamin A alcohol-vitamin A acid-ester	(342-347)	(347)	(355)	(466) ^c
β -ionone	(280)	(296)	(295)	(295) ^c
Vitamin A alcohol plus the acid	—	—	(355)	—

^a Petroleum ether, ^b Ethanol, ^c Chloroform

FIG. 3 Paper Chromatograms of Vitamin A Alcohol, Vitamin A Aldehyde and Vitamin A Acid

$\text{Ca}(\text{H}_2\text{PO}_4)_2 \cdot \text{NH}_4\text{OH}$ paper developed with petroleum ether.

a, vitamin A alcohol; b, vitamin A aldehyde; c, all-*trans*-vitamin A acid.

A aldehyde was synthesized from vitamin A alcohol by treating with freshly prepared MnO_2 at 20° for 10 days in petroleum ether (Fig. 2). Vitamin A acid and vitamin A alcohol-vitamin A acid-ester were synthetic preparations.

Natural vitamin A acid was produced from natural vitamin A alcohol by treating with dilute alkaline solution by Cannizzaro's reaction. The ultraviolet absorption spectra and paper chromatograms of the standard preparations are given in Table I and Fig. 3.

2. Aerobic Degradation of β -Carotene, Vitamin A and Related Compounds by Rat-Intestine Homogenate

The metabolisms of β -carotene, vitamin A and related compounds were studied using the intestine homogenate under aerobic condition. At first, O_2 uptake of β -carotene, vitamin A and related compounds by rat-intestine homogenate was determined manometrically. The reaction systems are shown in Table II.

TABLE II
Reaction System for Oxidation of β -Carotene, Vitamin A Alcohol and Vitamin A Aldehyde by Rat-Intestine Homogenate

Rat-intestine homogenate solution	5 ml
0.25 M sucrose buffer solution	1 ml
β -Carotene	5 mg
Vitamin A aldehyde	5 mg
Vitamin A alcohol	5 mg
0.01 M sodium succinate	1 ml
0.01 M sodium malonate	1 ml
0.01 M sodium fumarate	1 ml
0.01 M sodium α -ketoglutarate	1 ml

FIG. 4 O_2 Uptake of β -Carotene, Vitamin A Aldehyde and Vitamin A Alcohol by Rat Intestine Homogenate

Δ , β -carotene; $\times$, vitamin A aldehyde; $\circ$, vitamin A alcohol.

It was always found to be rather low in β -carotene, vitamin A alcohol and vitamin A aldehyde (Fig. 4). In parallel experiments, additive effects of succinate, fumarate, malonate and α -ketoglutarate respectively, were tested on the degradation of β -carotene, vitamin A alcohol and vitamin A aldehyde. A marked increase was seen in succinate (Fig. 5). The effects of NAD, FAD and cytochrome c, respectively, on the degradation of the vitamin were tested by paper chromatography and spectrophotometry with the results shown in Fig. 6.

FIG. 5 Additive Effect of Succinate on the O₂ Uptake of Vitamin A Aldehyde by Rat Intestine Homogenate

○, vitamin A aldehyde as substrate; Δ, succinate as substrate; ×, additive effect of succinate on the O₂ uptake of vitamin A aldehyde.

FIG. 6 Ultraviolet Absorption Spectra of the Degradation Products of β -Carotene after Incubation with Rat Intestine Homogenate under Aerobic Condition

a, β -carotene as substrate; b, β -carotene as substrate with NAD; c, β -carotene as substrate with NAD plus FAD; d, β -carotene as substrate with cytochrome c.

3. Isolation and Identification of Vitamin A Acid in the Degradation Products

The degradation mixture of β -carotene after incubation with intestine homogenate was tested by paper chromatography with $\text{Ca}(\text{H}_2\text{PO}_4)_2\text{-NH}_4\text{OH}$ paper, using petroleum benzine as a mobile phase (7). The picture were quite similar as shown in Fig. 4. The spot, R_F 0.0, was cut out of the paper, 2 cm in length, and the products remaining at the start were eluted with petroleum ether or chloroform. As reported in the foregoing paper, the petroleum ether-soluble compound was identified as β -ionone and the chloroform-soluble one as vitamin A aldehyde-protein complex which gave purplish-red color by Carr-Price reagent. The residual portion was treated with dilute acetic acid at 20–25° for 60 minutes and extracted with chloroform. The absorption spectrum of the chloroform-soluble compound was determined by Beckman spectrophotometer, and it was found to be vitamin A acid (Fig. 7).

In the parallel experiments, natural vitamin A aldehyde was treated with a dilute alkaline solution according to Cannizzaro's reaction, and the equimolar mixture of vitamin A acid and vitamin A alcohol was obtained, having λ_{max} at 349 $m\mu$ (Fig. 8).

FIG. 7 Ultraviolet Absorption Spectrum of the Mixture of Vitamin A Aldehyde and Succinate with Rat Intestine Homogenate after Incubation at 37° for One Hour

FIG. 8 Detection of Vitamin A Acid in the Reaction Mixture A, vitamin A alcohol; B, vitamin A acid; C, equimolar mixture of vitamin A alcohol and vitamin A acid.

SUMMARY

1. Using pure standard preparations it was found that vitamin A acid could be produced from natural vitamin A aldehyde by treating with a dilute alkaline solution according to Cannizzaro's reaction, judging from the findings of spectrophotometry and paper chromatography.

2. Metabolic pathways of β -carotene, vitamin A aldehyde and vitamin A alcohol under anaerobic condition were studied using the intestine of rats, and it was demonstrated that the terminal fission of β -carotene might take place, yielding protein-bound vitamin A aldehyde and β -ionone.

3. Further, the same experiments were performed under aerobic condition, and the

fates of β -carotene and vitamin A were studied. The results suggest that at the final step of vitamin A metabolism in the intestine, protein-bound vitamin A aldehyde might be converted to vitamin A alcohol and vitamin A acid.

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