Conversion of Intravenously Administered β-Cryptoxanthin to a β-Carotene-Like Compound in Mouse Lung

Yoshifumi Tomita 1,*, Gamarallage Vijitha Kumara Senanayake 1, Tomoko Yoshioka 1, Hoyoku Nishino 2 and Masamichi Yano 3

1Laboratory of Animal Nutrition and Biochemistry, Miyazaki University, 1-1, Gakuen Kibana Dai-Nishi, Miyazaki 889-2192, Japan
2Department of Biochemistry, Kyoto Prefectural University of Medicine, Kajii-cho 465, Kamigyo-ku, Kyoto 602-0841, Japan
3Fruit Tree Research Station, Ministry of Agriculture, Forest and Fishery, Okitsu, Shimizu, Shizuoka 424-0292, Japan

(Received March 18, 2002)

Summary The intravenous administration of β-cryptoxanthin (320 μg/mouse) into 4-wk-old ddY mice caused a new peak in addition to the peak of β-cryptoxanthin during high-performance liquid chromatography (HPLC) of the lipid fraction of lung homogenate. Different HPLC conditions revealed that the new peak might be attributed to a β-carotene-like compound. The average retention times for the new peak and authentic all-trans-β-carotene were 14.97 and 15.00 min, respectively, in a HPLC system using a YMC-Pack ODS-A column and methanol-based mobile phase, and 27.05 and 26.93 min, respectively, in a HPLC system using a Waters Nova Pack C18 column and methanol-based mobile phase. In a HPLC system using a Waters Nova Pack C18 column and acetonitrile-based mobile phase, the retention times were 10.73, 10.48 and 10.70 min for the new peak, authentic all-trans-β-carotene, and 9-cis-β-carotene, respectively. Spectrophotometry with a photodiode array detector showed maximum absorption of 447 and 475 nm for the new peak, and 450 and 475 nm for authentic all-trans-β-carotene. This new peak was not observed in the lung tissue of control mice. These findings indicate the possible conversion of β-cryptoxanthin to a β-carotene-like compound in ddY mice.

Key Words β-cryptoxanthin metabolism, β-carotene formation, reductive metabolism of carotenoids, mouse lung

Previous researchers have shown that many animals can oxidize and reduce carotenoids (1–3), while mammals cannot metabolize them except into retinol (4). However, the available evidence that mammals are unable to metabolize carotenoids is not sufficient to conclude the assumption as being fact. Therefore, we investigated the metabolism of carotenoids in mammals by employing different experimental approaches. This paper describes the reductive conversion of β-cryptoxanthin into a β-carotene-like compound in ddY mice, which is nutritionally and evolutionarily important as it suggests the possibility that provitamin A carotenoids are formed, in part, from non-provitamin A carotenoids by reductive pathways.

MATERIALS AND METHODS

Chemicals. β-Carotene was purchased from Nacalai Tesque Co. (Kyoto, Japan). β-Cryptoxanthin was prepared from Citrus unshiu oranges and purified by one of the authors using open-column chromatography. Organic solvents were of HPLC grade and other reagents were special pure grades from Wako Pure Chemical Industries, Ltd. (Osaka, Japan) unless otherwise stated.

Animals and protocol. β-Cryptoxanthin (1.6 mg) was dissolved into 100 μL of tetrahydrofuran (Katayama Chemicals, Osaka, Japan) and mixed with 1,900 μL of 0.9% NaCl to make a final concentration of 320 μg/0.2 mL. The solution (0.2 mL) was intravenously administered to six ddY male mice at the age of 4 wk and weighing 18–19 g (Kyudo Co., Kumamoto, Japan). Four other mice (i.e., control group) were injected with the same solution without β-cryptoxanthin. Four-week-old ICR male mice (Kyudo Co.) were also used to compare strains. Organs (liver, lung, testes, kidney, adrenal, spleen and brain) were collected from animals anesthetized with ether and sacrificed by bleeding 15, 30, 60 and 120 min after injection.

Animal handling and care were performed according to the accepted ethical standards of Miyazaki University.

Carotenoid extraction. The pooled organs were minced and homogenized with 0.1% butylated hydroxytoluene (BHT); 99.9% ethanol solution, and then
The lipid fractions were extracted with n-hexane after saponification with 10% NaOH at 60°C for 30 min. The hexane layer was evaporated, the residue was dissolved in a 0.1% BHT: 99.9% ethanol solution and then analyzed by conducting high-performance liquid chromatography (HPLC). A preparation of β-cryptoxanthin was subjected to the same assay procedure as the tissue samples and analyzed by HPLC to examine whether or not any new peaks are formed as artifacts.

**High-performance liquid chromatography.** Two different systems of HPLC (systems I and II) were used for the analysis of β-cryptoxanthin and other carotenoids. NIST SRM 968b sera (National Institute of Standards and Technology, Gaithersburg, USA) and authentic carotenoids were used for the calibration and standardization of the HPLC systems.

System I consisted of a Shimadzu HPLC system (Shimadzu Corp., Kyoto, Japan) with a LC-10AD pump, SIL-10ADvp auto-injector, SPD-10A UV-VIS detector (436 nm) and fitted with a Waters C18 column (Nova pack cartridge C18, 4 μm, 0.8×10 cm, Waters Corp., MA, USA). Methanol (100%) was used as the mobile phase at a flow rate of 2 mL/min and the column temperature was maintained at 25°C. The experiment was repeated with an acetonitrile-based mobile phase (acetonitrile: methanol: dichloromethane, 2:2:1 by vol., with 2% H2O, flow rate 2.3 mL/min) under the same system conditions for further confirmation.

A spectral analysis was made with system II, a Shimadzu HPLC system (Shimadzu Corp.) consisting of a DGU-14A degasser, SLC-10ADvp pump, SILL-10ADvp auto-injector, CTO-10ACvp column oven, SPD-M10Avp photodiode array detector and CBM-10A communications bus module fitted with a YMC-Pack ODS-A column (5.5 μm, 0.46×15 cm, YMC, Inc., c/o Waters, Milford, USA). The mobile phase used was 100% methanol at a flow rate of 2 mL/min and the column temperature was maintained at 40°C.

**RESULTS**

When the ddY mice were administered β-cryptoxanthin (320 μg/mouse) intravenously and organs removed and extracted 15, 30, 60 and 120 min after administration, transient induction of a new peak, in addition to the peak of β-cryptoxanthin, was seen in the lungs of the β-cryptoxanthin-treated mice by HPLC analysis (Fig. 1C). This new peak was observed under different HPLC conditions (systems I and II) in a similar manner. The peak was clearly found in the lung and plasma samples. The concentration of β-cryptoxanthin in other organs (testes, kidney, brain and adrenal) peaked within 120 min post-injection.

In HPLC system I using a Waters C18 column, the retention times with HPLC system II using a YMC-Pack ODS-A column were 14.97 and 15.00 min for the new peak and authentic β-carotene, respectively. The retention time of all-trans-β-carotene was 10.70 min in the acetonitrile-based mobile phase.

Retention times with HPLC system II using a YMC-Pack ODS-A column were 14.97 and 15.00 min for the new peak and authentic β-carotene, respectively. The chromatogram of authentic all-trans-β-carotene was given in Fig. 1D for the comparison of retention time. Three absorption peaks were discernible in the spectrum of the new chromatographic peak when the spectrum was obtained in the HPLC system II using a photodiode array detector (Fig. 2). Therefore, this new peak was attributed to a carotenoid. The maximum absorption wavelengths of the new peak were 447 and 475 nm, and those of the all-trans-β-carotene were 450 and 471 nm. The maximum absorption wavelength of the new peak was characteristic of the β-carotene, but the wavelength was slightly lower than that of authentic all-trans-β-carotene. Maximum absorption for authentic 9-cis-β-carotene was 445 and 471 nm. A small hypsochromic shift (usually 2–6 nm) is possible for the maximum absorption of a mono-cis carotenoid, in relation to its all-trans compound (5). Therefore, it is possible that the new peak could include a cis isomer(s) of β-carotene.

In the case of the liver, a β-carotene-like compound was present in mice treated with and without β-cryptoxanthin, and the retention time and UV spectra agreed closely with those of the all-trans-β-carotene-like compound found in the lungs from β-cryptoxanthin-treated mice. The concentration of β-carotene-like compound in the mice administered β-cryptoxanthin was higher in the lungs than that in the liver (Table 1). A peak corresponding to the β-carotene-like compound was not observed in other organs.

The concentration of β-carotene in the lungs and other sinusoidal organs (liver and spleen) peaked within 30 min post-injection and gradually diminished thereafter. Another experiment showed that plasma β-cryptoxanthin disappeared rapidly within 15 min after administration and no new peak was detected in plasma samples. The concentration of β-cryptoxanthin in other organs (testes, kidney, brain and adrenal) peaked within 120 min post-injection.

The β-cryptoxanthin preparation did not contain β-carotene-like compounds as seen in Fig. 1A. Furthermore, when a concentrated solution of the β-cryptoxanthin preparation was analyzed by HPLC, possible contaminants eluting close to β-carotene were not detected (Fig. 3A). The assay procedure used did not produce β-carotene-like artifacts as shown in Fig. 3B and C. Therefore, the new peak could not be a compound contaminated in the injected β-cryptoxanthin preparation or produced during the extraction process.

In ICR mice injected with β-cryptoxanthin, the peak for the carotenoid was found in the lungs and liver 15 and 60 min after injection. The β-carotene-like compound was detected in the liver, but not in the lungs, regardless of administration, which was in contrast to ddY strain mice.
Mouse Lung Converts β-Cryptoxanthin to β-Carotene

**DISCUSSION**

In animals other than mammals, astaxanthin is converted to zeaxanthin (2) and lutein is converted to dehydroretinol and 3-hydroxyretinol (3). The presence of echinenone in the liver of rats fed a diet containing canthaxanthin was reported, and a reductive pathway from canthaxanthin to β-carotene via echinenone was proposed (6). However, further studies on the pathways of carotenoids in mammals have not been published.

Possible reasons why previous researchers failed to detect reductive metabolic activity may be due to the limitation of carotenoids incorporated into the body and variation of the activities in different species and strains of mammals. Commonly used experimental systems for feeding carotenoids per os do not increase the carotenoid level in the blood or organs sufficiently to allow the detection of such metabolites with available analytical methods.

Rodents are believed to have extremely low β-carotene absorption. However, a high β-carotene diet ranging from 0.2 to 1% caused a limited increase in carotene in the plasma and tissues (4). A small amount of β-carotene and lutein were detected in the livers of ddY and ICR mice fed a basal diet. Subcellular fractionation analysis with an ultra-centrifuge revealed that these carotenoids located mainly in the mitochondrial and lysosomal fractions. A 2-wk oral administration of
Table 1. Concentrations of carotenoids in the lungs and liver.

<table>
<thead>
<tr>
<th></th>
<th>β-Cryptoxanthin (pmol/g)</th>
<th>New peak (pmol/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Lungs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>207.8</td>
<td>101.7</td>
</tr>
<tr>
<td>B. Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>n.d.</td>
<td>69.9</td>
</tr>
<tr>
<td>β-Cryptoxanthin</td>
<td>165.0</td>
<td>71.6</td>
</tr>
</tbody>
</table>

Concentrations of β-cryptoxanthin and new peak (β-carotene-like compound) in the lungs and liver of 4-wk-old ddY male mice 15 min after intravenous administration of β-cryptoxanthin (320 μg) (β-Cryptoxanthin, 3 mice) or 0.9% NaCl (Control, 2 mice). Tissues were pooled in each group before analysis. n.d., not detected.

Fig. 2. Comparison of the spectrum of the new peak shown in Fig. 1C with the spectra of all-trans-β-carotene and 9-cis-β-carotene. The spectra were obtained by a photodiode array detector connected to a HPLC system. The scale of the diagram is arbitrary.

Fig. 3. Effect of assay conditions on modification of the β-cryptoxanthin preparation. The β-cryptoxanthin preparation was subjected to the same assay procedure as that for lung samples and analyzed by HPLC to examine if the carotenoid was modified by the assay conditions to produce the β-carotene-like compound(s) as artifact(s). (A) Concentrated solution of β-cryptoxanthin preparation without being subjected to the assay procedure. (B) The same solution as (A) after being subjected to the assay procedure except saponification. (C) The same solution as above after being subjected to the complete assay procedure including saponification.

β-carotene raised the concentrations (data not shown). Therefore, carotenoids detected in the liver are possibly derived from maternal origin via umbilical cord and dietary origin including colostrum rich in carotenoids. Possible roles of these carotenoids in cells are for conversion into vitamin A and as antioxidants to protect cell membrane and organelle from oxidative stresses. The new peak of β-carotene-like substance was not
Mouse Lung Converts β-Cryptoxanthin to β-Carotene 447

detected in the lungs of ICR mice injected with β-cryptoxanthin 15 or 60 min after injection, suggesting a difference in metabolic activity among strains; that is, detection of the substance is influenced by the activity of the metabolic system and timing of the assay. The peak may appear before 15 min or between 15 and 60 min because the peak was observed in ddY mice earlier than 30 min post-injection. Further studies are necessary to determine strain differences.

The highest accumulation of β-cryptoxanthin in the lungs was found in carotenoid-injected mice, as in the case where β-carotene accumulated in the lungs of rat intravenously injected with β-carotene (7). The reason for highest accumulation occurring in the lungs is not clear, but alveolar macrophages may be involved in the accumulation and conversion of the carotenoid. Another plausible explanation for the increase in the β-carotene-like substance is as follows: Injected β-cryptoxanthin may enhance the absorption of β-carotene, as lycopene has been reported to increase the absorption rate of β-carotene (4), or may accelerate the release of β-carotene stored in the liver. However, these explanations are not convincing in the case of our study because the increase in the lung β-cryptoxanthin level was observed within 15 to 30 min after injection and then diminished.

Our findings strongly suggest a reductive metabolic pathway for carotenoids in mammals. However, further investigations using mass spectrometry are necessary to confirm the structure of the β-carotene-like peak. Further experiments are also needed to determine if reduced carotenoids are metabolized to other substances such as retinol or other carotenoids or transported to other tissues or organs, and to elucidate the differences in reductive metabolic activities in different strains of mice and other animals.

In summary, a metabolic pathway for the conversion of β-cryptoxanthin to a β-carotene-like compound has been described. To our knowledge, this is the first description of a reductive metabolic pathway for β-cryptoxanthin in mammals.

Acknowledgments

The authors are grateful to students in the laboratory for their technical assistance.

REFERENCES