Note

Tocopherol Levels in the Plasma Lipoproteins from Japanese Eel Anguilla japonica

Seiichi ANDO

Department of Fisheries Science, Kagoshima University, 4-50-20 Shimoarata, Kagoshima 890, Japan
Received June 5, 1995

The \( \alpha \)- and \( \gamma \)-tocopherol levels in the plasma of Japanese eel (Anguilla japonica) were 53.9 \( \mu \)g and 1.3 \( \mu \)g/ml of plasma, respectively. The \( \alpha \)-tocopherol in the plasma was mainly distributed as very-low-density lipoprotein and low-density lipoprotein (LDL), while LDL and high-density lipoprotein constituted most of the \( \gamma \)-tocopherol. A highly positive coefficient of correlation was observed between the \( \gamma \)-tocopherol and lipoprotein contents in Japanese eel plasma.

The circulatory fluid of animals, including fish, shows most lipids complexed with proteins in the form of lipoproteins. Lipoproteins of all animals function as major carriers of lipid and other hydrophobic compounds. Lipoproteins are labile to oxidation, and high levels of low-density lipoprotein (LDL) are closely associated with an increased risk of atherosclerosis.\(^{1,2}\) Tocopherols dissolved in the lipid moiety of lipoproteins are generally regarded as the most important lipid-soluble antioxidant in human blood plasma.\(^{23}\) The lipoprotein levels in fish plasma are known to be several times higher than those in mammals,\(^{3,4}\) suggesting that fish lipoproteins are susceptible to oxidation. The tocopherol levels in fish plasma are of interest in connection with lipoprotein oxidation, although few data on the distribution of tocopherols in fish plasma lipoproteins are available.\(^{5}\) The purpose of this study is to investigate the tocopherol levels in the plasma lipoproteins from Japanese eel which contain large amount of lipid in the body.\(^{6}\)

Cultured Japanese eel (Anguilla japonica) with an average body weight of 220 g were purchased from a local fish market and used as the materials. Blood was removed in ethylenediaminetetraacetic acid (5 mg/ml of blood) dissolved in 0.15 M NaCl by using a syringe inserted into the caudal vasculature of the live fish and maintained at a temperature of 4°C throughout the procedure. Plasma was obtained by centrifugation (3000 \( \times \) g for 15 min), the plasma volume being corrected for the dilution introduced by the anticoagulant. Very-low-density lipoprotein (VLDL), LDL, and high-density lipoprotein (HDL-\( \gamma \) and HDL-\( \delta \)) in the plasma were obtained by sequential ultracentrifugal flotation, by using a Hitachi CS100 ultracentrifuge equipped with a RPS80AT rotor (267,000 \( \times \) g at 15°C).\(^{7}\) The density (\( d \)) intervals of the lipoproteins were as follows: VLDL, \( d < 1.006 \) g/ml; LDL, 1.006 < \( d < 1.085 \) g/ml; HDL-\( \gamma \), 1.085 < \( d < 1.100 \) g/ml; HDL-\( \delta \), 1.100 < \( d < 1.210 \) g/ml. Lipid was extracted from each lipoprotein fraction by the method of Bligh and Dyer.\(^{8}\)

The protein content of each lipoprotein fraction was determined by using a Bio-Rad protein assay kit with bovine serum albumin as a standard. The lipoprotein concentration for each fraction was calculated from the combined contents of lipid and protein. An analysis of the tocopherol content in the lipid extracted from each lipoprotein fraction was carried out by high-performance liquid chromatography (HPLC).\(^{9}\) The HPLC system was composed of the following components: an LC-9A solvent delivery system (Shimadzu), an SPD-6AD variable-wavelength detector (Shimadzu), a 7161 injector with a 20-\( \mu \)l loop (Rheodyne), and a D-2500 Chromato-Integrator (Hitachi). Chromatographic separation of the \( \alpha \)-, \( \beta \)-, \( \gamma \)-, and \( \delta \)-tocopherols was carried out in a normal-phase silica column (Zorbax Sil, 4.6 mm i.d. x 25 cm, 5 \( \mu \)m) with a Guard-Pak precolumn module (Waters) and was monitored at 295 nm. The mobile phase used was \( n \)-hexane/dioxane/isopropylalcohol (985: 10:5, by volume), its flow rate being maintained at 1.0 ml/min. The tocopherols were identified by their retention times and were quantified by using calibration curves for the corresponding tocopherols. The tocopherol standards were kindly donated by Eisai Co., Tokyo, the purity of \( d \)-\( \alpha \)-, \( d \)-\( \beta \)-, \( d \)-\( \gamma \)-, and \( d \)-\( \delta \)-tocopherols being greater than 98.5%. Data were analyzed by a one-way analysis of variance, which was followed by Student's \( t \) test.

In our previous papers on fish lipoproteins,\(^{10,11}\) a close relationship was found between the plasma lipoprotein and muscle lipid levels. Fish with high levels of muscle lipid tended to secrete lipoproteins actively in their plasma, Japanese eel containing much more lipid in their muscle tissue (13.1 ± 0.6 g/100 g of muscle (mean ± S.D.)), and the muscle lipid levels determined in this study were comparable to those reported to date.\(^6\) Japanese eel were presumed to possess high levels of lipoprotein in their plasma, the plasma lipoproteins being made up of VLDL (24.5 ± 11.0 mg/ml of plasma), LDL (16.1 ± 1.13 mg/ml of plasma), HDL-\( \gamma \) (3.59 ± 1.02 mg/ml of plasma), and HDL-\( \delta \) (9.97 ± 2.00 mg/ml of plasma). VLDL was the main lipoprotein in the plasma of Japanese eel, although most teleosts possessed HDL as the main component in their plasma.\(^{12-15}\) The plasma lipoprotein levels of Japanese eel were in excess of 50 mg/ml of plasma, suggesting that Japanese eel were in the plasma hyperlipidemia and hyperlipoproteinemia category. The tocopherol levels of the plasma lipoproteins from Japanese eel are of interest, since tocopherols are the most important lipid-soluble antioxidants. The basal diet for Japanese eel is supplemented with tocopherols,\(^{16}\) the tocopherols found in the plasma lipoproteins seeming to arise from the diet.

The \( \alpha \)- and \( \gamma \)-tocopherol levels per mg of lipoprotein are shown in the Table. The highest and lowest levels of \( \alpha \)-tocopherol were found in VLDL and HDL-\( \gamma \), respectively. Although the \( \gamma \)-tocopherol levels of the plasma lipoproteins were much lower than those of \( \alpha \)-tocopherol, the \( \gamma \)-tocopherol was significantly distributed throughout LDL, HDL-\( \gamma \), and HDL-\( \delta \). The \( \gamma \)-tocopherol levels seemed to be independent of the lipid levels in the plasma lipoproteins. The \( \gamma \)-tocopherol was hardly apparent in VLDL, regardless of the high lipid level in VLDL, and no \( \beta \)- and \( \delta \)-tocopherol were detected in the plasma of Japanese eel.

The \( \alpha \)-tocopherol level per mg of lipoprotein was closely associated with the lipid content in the lipoproteins, a direct correlation being observed between the \( \alpha \)-tocopherol and lipid levels in the plasma lipoproteins (Fig. 1). The regression line shown reflects a highly significant positive correlation (\( r = 0.963, p < 0.0001 \)) between the two parameters. No correlation was observed between the \( \gamma \)-tocopherol and lipid levels in the plasma lipoproteins. The \( \alpha \)-tocopherol, but not \( \gamma \)-tocopherol, tended to be distributed in the plasma lipoproteins with high levels of lipid such as VLDL and LDL. The proportions of \( \alpha \)- and \( \gamma \)-tocopherols

---

**Abbreviations**: HPLC, high-performance liquid chromatography; VLDL, very-low-density lipoprotein; LDL, low-density lipoprotein; HDL-\( \gamma \), high-density lipoprotein 2; HDL-\( \delta \), high-density lipoprotein 3.
Table 2- and γ-Tocopherol Levels in the Plasma Lipoproteins from Japanese Eel

<table>
<thead>
<tr>
<th>Lipoprotein</th>
<th>2-Tocopherol (μg/mg of lipoprotein)</th>
<th>γ-Tocopherol (μg/mg of lipoprotein)</th>
<th>2-Tocopherol (μg/ml of plasma)</th>
<th>γ-Tocopherol (μg/ml of plasma)</th>
</tr>
</thead>
<tbody>
<tr>
<td>VLDL</td>
<td>1.383 ± 0.149* (99.8)</td>
<td>0.003 ± 0.005* (0.2)</td>
<td>33.31 ± 14.20* (60.2)</td>
<td>0.063 ± 0.109* (7.5)</td>
</tr>
<tr>
<td>LDL</td>
<td>0.935 ± 0.126 (95.3)</td>
<td>0.045 ± 0.024 (4.7)</td>
<td>14.96 ± 1.41 (29.2)</td>
<td>0.720 ± 0.399 (52.6)</td>
</tr>
<tr>
<td>HDL2</td>
<td>0.416 ± 0.080 (89.2)</td>
<td>0.051 ± 0.016 (10.8)</td>
<td>1.46 ± 0.35 (2.7)</td>
<td>0.182 ± 0.082 (14.0)</td>
</tr>
<tr>
<td>HDL3</td>
<td>0.449 ± 0.005 (92.8)</td>
<td>0.035 ± 0.001 (7.2)</td>
<td>4.14 ± 0.71 (7.9)</td>
<td>0.319 ± 0.050 (25.9)</td>
</tr>
<tr>
<td>Total</td>
<td>53.86 ± 14.13 (100)</td>
<td>1.284 ± 0.406 (100)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Mean ± S.D. (n = 3).

Fig. 1. Correlation between the Tocopherol and Lipid Contents in the Plasma Lipoproteins from Japanese Eel.

The regression line presents a highly significant positive correlation between the 2-tocopherol and lipid contents in the plasma lipoproteins (r = 0.963, p < 0.0001). Symbols are the same as those shown in Fig. 1.

thus differed among the plasma lipoproteins (Table). Plasma lipoproteins other than VLDL possessed certain proportions of γ-tocopherol (4.7–10.8% of the tocopherol level), while the 2-tocopherol was the main component of VLDL. A different function was considered to be acting between VLDL and the other plasma lipoproteins regarding the transport of tocopherols.

The Table also shows the 2- and γ-tocopherol levels in the plasma are 53.9 μg and 1.3 μg/ml of plasma, respectively. The 2-tocopherol in the plasma was mainly distributed in VLDL (60.2%) and LDL (29.2%), while plasma lipoproteins other than VLDL made up most of the γ-tocopherol. As shown in Fig. 2, a highly positive coefficient of correlation was observed between the 2-tocopherol and lipoprotein contents in the plasma (r = 0.960, p < 0.0001), suggesting that the 2-tocopherol tended to be distributed in the main plasma lipoprotein (VLDL) of Japanese eel. The tocopherol levels in Japanese eel plasma are comparable to those in Atlantic salmon (Salmo salar), although the 2-tocopherol in the plasma from Japanese eel and Atlantic salmon was mainly distributed in VLDL and HDL, respectively. HDL is the main plasma lipoprotein of Atlantic salmon, and the 2-tocopherol is considered to circulate in the plasma along with the main lipoprotein. The tocopherol levels in Japanese eel plasma were higher than those in humans (6.5–17 μg/ml). High levels of plasma tocopherols in fish may be attributed to the high plasma lipoprotein levels. Taking into account the high levels of tocopherols and n-3 polyunsaturated fatty acids in fish plasma lipoproteins, the oxidative stability of fish plasma lipoproteins is particularly interesting.

Acknowledgment. Thanks are given to Messrs. M. Matsuaki and O. Suena for their outstanding technical assistance.

References