Characterization of Lipoprotein Secreted by Cultured Eel Hepatocytes and its Comparison with Serum Lipoproteins

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Key words: apolipoprotein/eel/hepatocytes/lipoproteins/VLDL

ABSTRACT. The lipoprotein secreted by cultured eel hepatocytes was fractionated by density gradient ultracentrifugation and compared with eel serum lipoproteins. Eel hepatocytes were cultured for 7 to 10 days as a monolayer in Williams' medium E containing 5% fetal bovine serum and 0.16 μM insulin on a dish precoated with fibronectin of horse serum. The only lipoprotein secreted by eel hepatocytes was a very-low-density lipoprotein like one which consisted of 69% triglyceride, 15% phospholipid, 4% cholesterol, and 12% protein. On the other hand, very-low-density lipoprotein and high density lipoprotein were found in eel serum, in which high density lipoprotein was a main lipoprotein. The secreted lipoprotein contained apo B and apo A as the main protein components. Furthermore, the lipoprotein contained preapo A-I in addition to apo A-I, which was proved by comparing the amino acid composition of both proteins. In our discussion, we noted that the lipoprotein secreted by eel hepatocytes was a good material for the study of high-density lipoprotein formation.

Several studies on fish serum lipoproteins have been carried out (1-3, 19, 26, 30). Very-low-density lipoprotein (VLDL), low-density lipoprotein (LDL), and high-density lipoprotein (HDL) are found in serum of several fishes of teleostei; the main lipoprotein among these fishes is HDL. It is not known why HDL is the main lipoprotein in fish lipoproteins or whether HDL is synthesized and secreted by fish liver. Few data are currently available on lipoprotein synthesis and secretion in fishes. Lipoproteins are primarily synthesized and secreted by the liver and small intestine. Recently, we developed a method for the primary culture of eel hepatocytes as reported previously (16, 17). We could observe the secretion of apolipoproteins of eel serum HDL by cultured eel hepatocytes (27). These results revealed that cultured eel hepatocytes were as useful for studies of lipoproteins metabolism as mammalian cultured hepatocytes (7, 11, 14). Therefore, we investigated in this report what kind of lipoproteins were synthesized and secreted by cultured eel hepatocytes, and tried to reveal the characteristics of the lipoprotein secreted by the hepatocytes and compare it with eel serum lipoproteins. It was found that the lipoprotein secreted by cultured eel hepatocytes was VLDL-like lipoprotein, and that other lipoproteins such as HDL and LDL were absent. The VLDL-like lipoprotein contained apo A and apo B as the main protein components.

MATERIALS AND METHODS

Materials. Bovine serum albumin was obtained from Armour Pharmaceutical Co. Fetal bovine serum was purchased from Gibco BRL and insulin of hog or bovine pancreas was from Sigma Chemical Co. Collagenase was obtained from Wako Pure Chemical Ind. Sephadex G-25 and Bio gel P-10 were obtained from Pharmacia and Bio-Rad, respectively. Bicinchoninic acid for protein assay according to the method of Smith et al. (31) and 4N methansulfonic acid containing 0.2% 3-(2-aminoethyl)indole were purchased from Pierce. L-(U-14C)-Leucine (11.4 GBq/mmol) was obtained from Radiochemical Center, Amersham. Williams' medium E was from Flow Laboratories Inc. Other chemicals were obtained from Wako Pure Chemical Ind. or Nakarai Tesque.

Eels. Cultured eels (Anguilla japonica) weighing 200 to 250 g were purchased from Sueyoshi Co. in Kagoshima. They were starved for several days before experiments.

Primary culture of eel hepatocytes. The liver (about 2.5 g wet weight) was isolated from eel anesthetized by 0.2% 2-phenoxyethanol and perfused with 50 ml of Ringer solution containing 35 mg collagenase and 50 mg bovine serum albumin at 25°C for 30 min, as described previously (15). After 30 min digestion by collagenase, the liver was further perfused with 80 ml of Ca2+- and Mg2+-free Ringer solution containing 20 mM EDTA, removed from the perfusion apparatus and rinsed with Ringer solution on a clean bench. Then isolated
hepatocytes were prepared as described previously (15). Finally, cells were suspended in 10 ml of Ringer solution. The cells were counted by the trypan blue exclusion method.

Isolated hepatocytes of 1.5 x 10^7 cells were inoculated into 7 ml of culture medium in a 10-cm plastic dish (Falcon) which was precoated with fibronectin isolated from horse serum by affinity chromatography using gelatin-Sepharose (12, 25). The culture medium consisted of Williams’ medium E (WE medium) containing 23 mM NaHCO₃, 5% fetal bovine serum (FBS) and 0.16 μM insulin of hog or bovine pancreas. Hepatocytes were cultured at 28°C under saturated humidity in 5% CO₂ in an air atmosphere.

Incorporation of ¹⁴C-leucine into lipoprotein secreted by cultured eel hepatocytes. After hepatocytes were cultured in WE medium containing 5% FBS and 0.16 μM insulin for 5 to 7 days, hepatocytes were washed three times with serum- and insulin-free WE medium and 7 ml of the serum- and insulin-free medium and ¹⁴C-leucine was added to the cells. Then hepatocytes were incubated at 28°C. After incubation, the medium was recovered and the cells were washed with 3 ml of phosphate-buffered saline (PBS). The medium and PBS used to wash the cells were combined. Three ml of 0.1 N NaOH was added to the cells on a dish, and dissolved cells were transferred into a test tube with a Pasteur pipet. The dish was washed with 2 ml of 0.1 N NaOH, and the washing solution was transferred to the same test tube. Then 1.3 ml of 50% trichloroacetic acid (TCA) was added to the test tube, the solution was centrifuged at 3,000 rpm for 5 min, and the precipitate was washed with 2 ml of 5% TCA. This washing procedure was repeated three times. The resulting precipitate was dissolved in 2 ml of 0.1 N NaOH, and the solution was used for radioactivity measurement and protein assay.

The medium pooled from 6 to 10 dishes was centrifuged at 3,000 rpm for 5 min to remove cell and cell debris, and the supernatant was applied to a Sephadex G-25 column (5 x 18.6 cm) equilibrated with PBS. The protein fraction was pooled and concentrated by membrane of YM10 (Amicon) for lipoprotein fractionation.

Lipoprotein fractionation. Lipoprotein was fractionated by density gradient ultracentrifugation (10). An equal amount of 0.75% NaCl was gently laid over 19 ml of 0.15% NaCl was gently laid over 19 ml of 10% sucrose in a 10-cm tube by a peristaltic pump and a fraction collector (Phar-macia). Five hundred μl taken from each fraction was used for counting radioactivity. Two hundred μl taken from each fraction was dialyzed against water and used for SDS-polyacrylamide gel electrophoresis (SDS-PAGE).

SDS-PAGE and autoradiography. Samples for electrophoresis were treated for 5 min at 90°C in sample buffer at a final concentration of 1.7% (W/V) SDS, 0.56 M 2-mercaptoethanol, 52 mM Tris-HCl (pH 6.8), 6% (V/V) glycerol, and 0.004% (W/V) bromophenol blue. Before electrophoresis, lipoprotein with a density of less than 1.063 g/ml was dialyzed against water, lyophilized and treated with 2 ml of diethyl ether twice to remove lipids. Proteins were resolved by electro- phoresis on 5-17.5% acrylamide gradient slab gels according to the method of Laemmli (23). Proteins were stained in 0.25% (W/V) Coomassie Brilliant Blue R-250 in 45% ethanol-10% glacial acetic acid and destained with 25.5% ethanol-8% glacial acetic acid. Calibration proteins for molecular weight in the range of 20,000 to 340,000 (Boehringer Mannheim) and 14,000 to 94,000 (Pharmacia) were used. The former contained α1-macroglobulin (340,000), phosphorylase b (97,000), glutamate dehydrogenase (55,400), lactate dehydrogenase (36,500), and trypsin inhibitor (20,100), and the latter contained phosphorylase b (94,000), albumin (67,000), ovalbumin (43,000), carbonic anhydrase (30,000), trypsin inhibitor (20,100), and α-lactalbumin (14,400).

For autoradiography, after electrophoresis, the gels were soaked in ENHANCE autoradiography enhancer fluor (NEN Research Products) for 1 h and then in water for 30 min. After fluor was precipitated with water, the gels were dried and exposed to Fuji X-ray film at -70°C. ¹⁴C-Methylated proteins (Radiochemical Center, Amersham) were used as molecular weight markers. They were myosin (200,000), phosphorylase b (97,400), bovine serum albumin (69,000), ovalbumin (46,000), carbonic anhydrase (30,000), and lysozyme (14,300).

Preparation of eel serum lipoprotein (d<1.063 g/ml) and HDL. After the eel was anethetized by 0.2% 2-phenoxyethanol, the abdomen was opened and blood was collected from the posterior vena by perfusing liver with PBS containing 1 mM Ca²⁺ and 0.5 mM Mg²⁺. Blood was pooled usually from 12 eels and left at room temperature for several hours. The clotted blood was centrifuged at 3,000 rpm for 20 min to obtain serum. Chloramphenicol, NaN₃, and phenylmethane- sulfonyl fluoride were added to the serum at a final concentration of 80 mg/ml, 0.01%, and 1 mM, respectively. Serum lipoprotein (d<1.063 g/ml) and HDL were isolated by density gradient ultracentrifugation as described above. Lipoprotein (d<1.063 g/ml) and HDL were pooled from fractions 37 to 40 and 15 to 27 (1.08<d<1.17 g/ml), respectively.

Purification of apo A-I and apo A-I-like proteins secreted by cultured eel hepatocytes. Lipoprotein (d<1.063 g/ml) secreted by cultured hepatocytes was isolated by density gradient ultracentrifugation, and apo A-I and apo A-I like proteins were separated by SDS-PAGE. The molecular weight of apo A-I-like protein was about 1,000 daltons higher than that of apo A-I. Apo A-I and apo A-I like protein stained with Coomassie Brilliant Blue on a gel were sliced away from the gel and the proteins in the sliced gel were eluted by electrophoretic elution, as described by Hunkapiller et al. (18). The eluate containing the protein, salt, SDS, and dye was lyophilized. The lyophilized sample dissolved in 50 μl of 70% formic acid (Merck) was applied to a Bio-Gel P-10 column (1 ml of bed volume) equilibrated with 70% formic acid. The protein was recovered from 9 to 16 drops and the recovered protein was evaporated by a rotary evaporator, dissolved in

Fu-gong Yu, S. Ando, and S. Hayashi
200 μl of water and lyophilized. The lyophilized protein was used for protein assay and analysis of amino acid composition.

**Analysis of amino acid composition.** Lyophilized sample (about 1 nmol) was dissolved in 20 μl of 4N methansulfonic acid containing 0.2% 3-(2-aminoethyl)indole, sealed under reduced pressure and hydrolyzed at 115°C for 24 h. After this hydrolysis, cystine reduced by dithiothreitol and cysteine was converted to sulfocysteine by Na-tetrathionate according to the method of Simpson et al. (29). Amino acids containing tryptophan and sulfocysteine were analyzed by the Shimazu LC-6A system using o-phthalaldehyde.

**Analysis of lipids.** Triglyceride, phospholipid, total and free cholesterol contents of lipoproteins were determined by means of enzymatic kits (Kyowa Medex) known as Determiner TG-S555, Determiner PL, Determiner TC555, and Determiner FC555, respectively.

Protein content was assayed by the method of Bradford (8) in the range of 0.1 to 1.5 mg/ml and by the method of Smith et al. (31) in the range of 1 to 20 μg/ml.

**RESULTS**

*Cultured eel hepatocytes.* Eel hepatocytes were obtained at a yield of (2.66 ± 1.10) × 10⁸ cells per g liver from 200 to 250 g eels (n = 5). The viability of the prepared hepatocytes examined by the trypan blue exclusion method was over 90%. Figure 1-A shows eel hepatocytes immediately after their inoculation to the dish. Cells attached to a dish precoated with fibronectin after 2 to 3 days' culture. Matrices such as collagen (type I) or p-N-p-vinylbenzyl-d-lactonamide (Nagase Chemicals Ltd.) were not effective for attachment of eel hepatocytes. Furthermore, if the entire medium was changed within 2 to 3 days, cells were detached from the dish. Figure 1-B shows eel hepatocytes before the change of medium, which were still round-shaped. After the entire medium was changed, the cells lost their round shape and spread as a monolayer within 24 h, as shown in Fig. 1-C. The morphology was maintained for at least 10 days, but after 14 days’ culture, nonparenchymal cells appeared (Fig. 1-D). It was assumed that the very small number of nonparenchymal cells contami-
nated during preparation of hepatocytes increased as the parenchymal cells died.

The effect of temperature on the morphology of cultured eel hepatocytes was as follows. If the temperature was over 31°C, the cells peeled from the dish and died. When the cells were cultured at below 25°C, they barely attached and spread. Therefore, eel hepatocytes should be cultured at between 25 to 30°C; we usually cultured them at 28°C.

**Time course of protein synthesis by cultured hepatocytes.** Syntheses of intra- and extracellular protein were investigated by incorporation of ¹⁴C-leucine during 2- to 32 h-incubation. Both of the protein syntheses were increased during the 32 h, and the synthesis of intracellular protein was always higher than that of extracellular protein (Fig. 2-A). A fluorogram of extracellular protein showed that after a 16 h incubation, almost all proteins corresponding to eel serum proteins were observed (Fig. 2-B). In subsequent experiments, we used a 24 h incubation period.

**Lipoprotein secreted by eel hepatocytes and its comparison with eel serum lipoproteins.** Proteins secreted by cultured hepatocytes were fractionated by density gradient ultracentrifugation as described in "Materials and Methods" (Fig. 3A-1). Radioactivity of proteins labelled by ¹⁴C-leucine and proteins determined by the colorimetric method were observed at densities above 1.21 g/ml and below 1.603 g/ml. Fractions with densities below 1.063 g/ml had triglyceride as the main lipid component, as shown in Fig. 3A-2.

On the other hand, eel serum proteins were also fractionated in the same manner as secreted proteins, and proteins were observed at densities above 1.21 g/ml, between 1.08 and 1.17 g/ml, and below 1.063 g/ml (Fig. 3B-1). Fractions with densities between 1.08 and 1.17 g/ml had phospholipid and cholesterol as the main lipid component (Fig. 3B-2). The fraction (1.08<d<1.17 g/ml) had a protein content and lipid composition typical of human or rat serum HDL (9), as shown in Table I. Furthermore, the fraction consisted of two main proteins corresponding to apo A-I and A-II of mammalian serum HDL. We defined this fraction as HDL. However, secreted proteins contained no lipoprotein corresponding to serum HDL, as shown in Fig. 4A and 4B. As eel serum lipoprotein with densities below 1.063 g/ml had a protein content and lipid composition simi-
Fig. 3. Fractionation of secreted and serum lipoproteins by density gradient ultracentrifugation. After hepatocytes cultured on a 10-cm dish were incubated with 14C-leucine (37 KBq/dish) for 24 h, the medium pooled from 10 dishes was centrifuged and applied to a Sephadex G-25 column (5 × 18.6 cm). Then protein fraction was fractionated by density gradient ultracentrifugation. Eel serum was prepared as described in "Materials and Methods" and fractionated in the same way.

A, Fractionation of secreted lipoprotein. 1, Distribution of protein (●●●); 2, Distribution of radioactivity (■■■); B, Fractionation of serum lipoproteins. 1, Distribution of protein (●●●); 2, Distribution of lipid. Lipid (●●●, triglyceride; ○○○, phospholipid; △△△, cholesteryl ester; ×××, free cholesterol).

Table I:

<table>
<thead>
<tr>
<th>Protein (mg/ml)</th>
<th>Density (g/ml)</th>
<th>Triglyceride (mg/ml)</th>
<th>Phospholipid (mg/ml)</th>
<th>Cholesterol (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Secreted VLDL</td>
<td>1.063</td>
<td>0.500</td>
<td>0.150</td>
<td>0.040</td>
</tr>
<tr>
<td>Serum VLDL</td>
<td>1.030</td>
<td>0.350</td>
<td>0.100</td>
<td>0.030</td>
</tr>
</tbody>
</table>

When secreted lipoprotein with densities below 1.063 g/ml was compared with eel serum VLDL, protein (12%) and triglyceride (69%) contents of secreted lipoprotein were found to be higher than those of eel serum VLDL (Table I). On the other hand, the cholesterol (4%) and phospholipid (15%) contents of the secreted lipoprotein were found to be lower. Apolipoproteins of the secreted lipoprotein consisted of apo A and B as the main components (Fig. 4A). Molecular weights of secreted apolipoproteins were as follows: proteins corresponding to apo B were 290,000, 265,000, 245,000 and 220,000, and proteins corresponding to apo A-I and A-II were 26,000 and 13,000, respectively. These apolipoproteins were found in eel serum VLDL. Therefore, we concluded that the secreted lipoprotein was a VLDL-like lipoprotein. VLDL-like lipoprotein was secreted to the medium at a rate of 10.93 ± 4.34 µg protein per mg cell protein per 24 h (n = 6). In rat hepatocytes, it was reported that VLDL was secreted at a rate of 0.20 µg protein per mg cell protein per 17 h (7). This
value was much lower than that of eel hepatocytes. However, LDL and HDL were increased during the 17 h incubation in the medium of cultured rat hepatocytes (7).

Apo A-I and apo A-I-like protein secreted by cultured hepatocytes. As shown in Fig. 5, secreted lipoprotein contained apolipoprotein with a molecular weight about 1,000 daltons higher than that of apo A-I of serum HDL. This protein on a gel was eluted electrophoretically according to the method of Hunkapiller et al. (18). The amino acid composition of the eluted protein (referred to as apo A-I-like protein) was analyzed and compared with that of apo A-I of eel serum HDL (Table II). The amino acid compositions of both proteins were very similar, especially in alanine, half cystine, glutamate, leucine, methionine, serine and valine contents. Apo A-I-like protein contained more arginine, aspartate, glycine, isoleucine, phenylalanine, and
Lipoprotein Secreted by Cultured Eel Hepatocytes

Table I. Composition of VLDL-like lipoprotein secreted by eel hepatocytes, serum VLDL, and HDL.

<table>
<thead>
<tr>
<th></th>
<th>VLDL-like lipoprotein (%)</th>
<th>Eel serum (VLDL %)</th>
<th>HDL (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>FC</td>
<td>3.8±0.3</td>
<td>9.6±0.8</td>
<td>3.8±0.3</td>
</tr>
<tr>
<td>CE</td>
<td>0.36±0.50</td>
<td>4.5±1.1</td>
<td>14.0±2.2</td>
</tr>
<tr>
<td>TG</td>
<td>69.0±5.1</td>
<td>53.3±7.0</td>
<td>4.2±1.7</td>
</tr>
<tr>
<td>PL</td>
<td>14.7±2.9</td>
<td>26.4±4.7</td>
<td>32.0±2.8</td>
</tr>
<tr>
<td>Protein</td>
<td>12.0±4.8</td>
<td>6.2±1.1</td>
<td>46.1±1.0</td>
</tr>
</tbody>
</table>

VLDL-like lipoprotein was pooled from fractions 37 to 40 shown in Fig. 3A. The values of VLDL-like lipoprotein, VLDL and HDL are Mean±S.D. for 5, 4, and 4 experiments, respectively. FC, free cholesterol; CE, cholesteryl ester; TG, triglyceride; PL, phospholipid.

Table II. Amino acid composition of apo A-I-like protein of VLDL-like lipoprotein and apo A-I of serum HDL.

<table>
<thead>
<tr>
<th>Amino acid residues</th>
<th>apo A-I-like protein</th>
<th>apo A-I</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ala</td>
<td>19</td>
<td>19</td>
</tr>
<tr>
<td>Arg</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td>Asp</td>
<td>30</td>
<td>19</td>
</tr>
<tr>
<td>Cys</td>
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<td>1</td>
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<tr>
<td>Glu</td>
<td>42</td>
<td>44</td>
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<tr>
<td>Gly</td>
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<td>5</td>
</tr>
<tr>
<td>His</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>Ile</td>
<td>10</td>
<td>6</td>
</tr>
<tr>
<td>Leu</td>
<td>29</td>
<td>30</td>
</tr>
<tr>
<td>Lys</td>
<td>17</td>
<td>25</td>
</tr>
<tr>
<td>Met</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Phe</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Pro</td>
<td>6</td>
<td>11</td>
</tr>
<tr>
<td>Ser</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Thr</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Trp</td>
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<td>0</td>
</tr>
<tr>
<td>Tyr</td>
<td>5</td>
<td>9</td>
</tr>
<tr>
<td>Val</td>
<td>13</td>
<td>15</td>
</tr>
</tbody>
</table>

The values are number of amino acid residues of one molecule of apo A-I-like protein (M.W., 27,000) and apo A-I (M.W., 26,000). threonine than did apo A-I of serum HDL. These results suggest that apo A-I-like protein is proapo A-I of serum HDL.

DISCUSSION

The culture method for eel hepatocytes was essentially the same as that used for rat hepatocytes (32). However, isolated hepatocytes of fish such as eel or rainbow trout (22, 24) barely attached to the dish if the dish was not precoated with some matrix such as fibronectin. Furthermore, it takes 2 to 3 days for hepatocytes to attach to a dish; this is very different from the case of rat hepatocytes which attached to a dish in a few hours.

After 2 to 3 days' culture, when the entire medium was changed for a fresh medium, a fine monolayer of hepatocytes was observed within 24 h. The reason why fish hepatocytes hardly attach and need a long time to attach is unclear. It may be that the properties of plasma membrane of syntheses of matrix of fish hepatocytes are different from those of rat hepatocytes.

Fig. 5. SDS-polyacrylamide gel electrophoresis (SDS-PAGE) of secreted lipoprotein, serum HDL, and serum proteins. Fifty µg of each sample was loaded into the gel. Gel concentration was 15.5%. a, apo A-I (M.W. 26,000); b, apo A-I-like protein (M.W. 27,000).
in mammals, it was reported that cultured hepatocytes of rat (7, 11), Hep G2 human hepatoma cell line (33), and a perfused liver of rat (28) and rhesus monkey (20) all secreted VLDL and HDL.

The second characteristic is that secreted lipoprotein contained apo A as the main protein component besides apo B. Any VLDL secreted by mammalian cultured hepatocytes (7, 11, 33) or a perfused liver (20, 28) contained no apo A as the main protein component, and the main protein components of the VLDL were apo B and E. Apo A was found in only HDL or HDL-like protein secreted by mammalian cultured hepatocytes (7, 11, 33) or a perfused liver (20, 28). The main apolipoproteins of eel serum HDL were apo A, as shown in Fig. 4-B and Fig. 5, so it is supposed that apo A of the secreted VLDL may be released from the VLDL particle and a nascent HDL will be formed from the released apo A in eel serum, since eel hepatocytes do not secrete HDL. We think that the VLDL secreted by eel hepatocytes is a good material for the study of HDL formation, at least for the study on the transport of apo A of VLDL to HDL. As reported, the intestine secreted VLDL containing apo A (34). The lipoprotein secreted by eel hepatocytes indeed seems to resemble the VLDL secreted by mammalian intestine. Furthermore, the lipoprotein contained only apo B corresponding to apo B-48 (246,000 daltons in human apo B-48). It is known in humans that apo B-48 and B-100 are synthesized in the intestine and liver, respectively (21), although it was reported that rat hepatocytes secreted both apo B-100 and B-48 (35). The lack of apo B-100 was also observed in eel serum VLDL, of which the main proteins were apo B-48, apo A, and 81,000-dalton protein (Fig. 4-B). These characteristics lacking apo B-100 have also been observed in other fish serum lipoproteins (4). It is an interesting but unsolved problem as to whether apo B of secreted VLDL or serum VLDL can in fact bind to a receptor such as LDL or VLDL receptor as ligand.

The third characteristic is that the lipoprotein secreted by eel hepatocytes contained more triglyceride (69%) and protein (12%) than eel serum VLDL, and less cholesterol (4%) and phospholipid (14%) than eel serum VLDL, as shown in Table I. The ratio of free cholesterol to cholesteryl ester of the secreted lipoprotein was 5 times higher than that of serum VLDL. It is supposed that the free cholesterol of the secreted lipoprotein is esterified by lecithin-cholesterol acyl transferase in eel serum.

The secreted lipoprotein contained an apolipoprotein named apo A-I-like protein in addition to apo A-I. The molecular weight of the apo A-I-like protein was about 1,000 daltons heavier than that of apo A-I of eel serum HDL. Furthermore, the amino acid composition of apo A-I-like protein was very similar to that of apo A-I of eel serum HDL. Amino acid compositions of both pro-

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(Received for publication, May 17, 1991 and in revised form, June 29, 1991)