Biochemical Studies on Carp Plasma Protein—IV

Lipid Analysis of Lipoproteins

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The lipid class and fatty acid composition of carp serum lipoproteins of a globulin fraction, as well as carp serum albumin (CSA), were analyzed. The main lipid classes of the lipoproteins were sterol esters and triglycerides; their sum amounted to 40–50% of the total. These lipoproteins differed from each other only slightly in lipid class composition, whereas they differed markedly in fatty acid composition.

The fatty acid compositions of nonpolar lipids (sterol esters and wax esters), triglycerides, non-esterified fatty acids, partial glycerides, and phospholipids separated from the lipid of CSA were examined. Saturated fatty acids were high in nonpolar lipids, and polyunsaturated fatty acids were characteristically distributed in partial glycerides and phospholipids.

Significant diversities in lipid class and fatty acid composition were observed between the carp and the other animals, including fish. CSA has some similarity in chemical composition to both albumin and high density lipoprotein of mammalian serum.

One of the lipoproteins was observed to disappear in the cold season, but the others did not show any significant seasonal variations.

The lipid composition of fish serum lipoprotein classes separated by ultracentrifugation has been presented by many workers¹⁻⁶. In a previous paper⁷, it was described that approximately 70% of serum lipid is conjugated with an albumin, and the remainder lipid with several lipoproteins of globulin fraction. It is necessary to consider the presence of unusual lipid components in fish tissues for elucidation of lipid transport mechanism, since explanation of lipid metabolism in mammalian serum is probably inapplicable to that of fish.

The presence of a lipid-containing albumin and 5 lipoproteins was previously confirmed in carp serum^{7,8}. This time were analyzed the lipid composition and fatty acid distribution in the lipoproteins and the albumin, to obtain basic information on the physiological role of the lipoprotein.

Materials and Methods

Lipid Extraction from Lipoproteins

Five lipoproteins having globulin character were fractionated from non-fasting carp serum by gel filtration and DEAE-cellulose column chromatography, as described previously⁷. Lipid was extracted once with ether-ethanol (3:2, v/v) and

twice with ether-ethanol (3:1, v/v). The extracts were combined and evaporated under nitrogen stream and the remainder lipid was submitted to thin-layer and gas chromatography.

Lipid of Carp Serum Albumin (CSA)

The albumin fraction salted-out with 50% saturated ammonium sulfate solution⁸ was passed through a Sephadex G-200 column. The second fraction, of which lipid solely comprises that of CSA⁸, was collected. The lipid extracted from the fraction by the above mentioned procedure.

Thin-layer Chromatography

For quantitative analysis, lipid classes were separated by thin-layer chromatography on a plate coated with Silicagel G (E. Merck, Darmstadt). The solvent system used was petroleum ether-ether-acetic acid (85:15:1, v/v/v). For separation of nonpolar lipids, petroleum ether-ether-acetic acid (95:5:1, v/v/v) was used. Lipid components located by charring with the sulfuric acid-dichromate reagent were quantitatively measured by an Ozumori densitometer 82 (Asuka Kogyo Co., Ltd.). In order to determine the fatty acid composition in lipid classes of CSA, the lipid classes separated on a thin-layer plate were determined by spraying a dichlorofluorescein ethanol solution.

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The lipid components extracted with a methanol-chloroform mixture (1:1, v/v) from the silicagel were saponified, and fatty acids were converted into methyl esters by treating with diazomethane in ether.

Gas Chromatography

The methyl esters were analyzed by a Shimadzu GC-2C gas chromatograph at 190°C. A stainless column of 3 mm in diameter and 280 mm in length was packed with 10% diethylene glycol succinate (DEGS) on Diasolid. A hydrogen ionization detector was employed. Identification of fatty acids was carried out by the relative retention time and the value of equivalent chain length of HOFSTETTER *et al.*⁹⁾

Seasonal Variation of Lipoproteins and Albumin

The blood samples were collected at the end of every season of a year from the carp cultured in an out-door pond of Kumano Freshwater Biological Station of Hiroshima University, as described previously¹⁰⁾. The first blood collection was started in June. Subsequent collections were performed in September, November, and then January of the next year. Blood (13–19 fish in each group) was collected by cardiac puncture. In order to facilitate the examination of other blood values, which were reported in a previous paper¹⁰⁾, a heparinized syringe was used. The blood was centrifuged and the plasma obtained was stored at –20°C. The lipoproteins were separated by combination of both gel-filtration and DEAE-cellulose chromatography. The percentage composition was calculated from the proportions of lipid in lipoproteins and albumin as estimated by the sulfo-phospho-vanilline method¹¹⁾.

Results

The lipid compositions of various lipoproteins from non-fasting carp serum are listed in Table 1. The high proportions of triglycerides (TG) and sterol esters (SE) were common to the lipoproteins. These lipoproteins were insufficiently differentiated by the lipid class composition. TG and fatty alcohols were only lipid classes the proportions of which showed some distinct differences between Lp-I and the others. A high level of SE, as noted in mammalian sera, was also observed in carp serum. In addition, total sterol was present at an almost constant level through the lipoproteins. The nonesterified fatty acid (NEFA) levels in the lipoproteins were quite low, compared to that of CSA. In contrast to sardine serum lipoproteins lacking in long-chain alcohols and wax esters (WE)²⁾, a fairly amount of WE was found in carp serum lipoproteins.

The fatty acid composition of the lipoproteins and CSA was analyzed by gas chromatography (Table 2). A DEGS column did not separate 18:3 and 20:1. The fatty acid composition of carp lipoproteins significantly differed from that of mammals. The results showed that each lipoprotein is characterized by a specific fatty acid composition. In Lp-I the predominant fatty acids were found to be 16:1, 22:2, and 20:5. However, there was relatively little 16:0 which was dominant in the other lipoproteins. Lp-I was characterized by a low content of saturated fatty acids (*ca.* 20%). Lp-II, of which major fatty acids were 18:1, 16:0, 16:1, and 18:2, apparently contained a high percentage of monounsaturated fatty acids (>50%). A high content of polyunsaturated fatty acids (>50%) was a feature of Lp-III, in

Table 1. Lipid class composition of carp serum lipoproteins

Lipid content*	Lp-I	Lp-II	Lp-III	Lp-IV	Lp-V
		63.3%	29.9%	63.5%	39.1%
Sterol esters	26.3%	23.0%	22.1%	25.2%	24.2%
Wax esters	16.1	16.1	15.0	15.7	14.5
Diacylglyceryl ethers	0.8	1.5	1.7	1.5	0.5
Triglycerides	31.3	22.5	19.5	23.2	25.4
Fatty acids	4.5	6.0	6.0	2.9	2.0
Fatty alcohols	3.2	7.0	8.1	6.8	10.2
Unknown	1.2	—	2.0	1.5	1.0
Cholesterol	8.0	11.5	9.4	8.7	9.1
Partial glycerides	2.1	3.5	3.4	3.4	3.3
Phospholipids	6.4	9.0	12.7	11.1	9.7

* Cited from the previous data.⁷⁾

Table 2. Percentage composition of fatty acid in carp serum lipoproteins

Fatty acid	Lp-I	Lp-II	Lp-III	Lp-IV	Lp-V	CPA
12:0	3.3	trace	trace	trace	trace	1.1
13:0	3.7	trace	—	—	—	—
14:0	5.1	1.9	3.9	4.1	4.4	2.9
14:1	6.7	trace	1.7	2.1	1.6	1.0
16:0	6.4	22.7	15.7	25.3	27.8	29.7
16:1	18.2	14.0	14.3	13.9	17.6	14.1
16:2	5.0	—	—	1.2	trace	—
18:0	1.4	3.5	1.1	1.7	1.5	4.0
18:1	7.6	31.8	8.5	19.1	24.4	17.9
18:2	1.6	10.8	3.1	9.7	9.2	9.6
18:3) 20:1)	3.8	5.4	2.2	2.2	2.3	2.0
20:2	—	trace	2.0	trace	trace	trace
20:3	4.0	—	5.7	1.1	1.1	1.3
20:4	3.7	1.1	—	trace	trace	2.0
22:2	15.3	—	31.5	12.9	10.1	—
20:5	9.2	2.3	—	—	—	6.7
22:4	trace	trace	6.6	—	—	—
22:5	5.0	6.4	—	6.8	—	7.7
22:6	trace	trace	3.6	trace	—	trace

Table 3. Fatty acid composition of CPA

Fatty acid	SE WE	TG	NEFA	PG	PL
12:0	2.5	trace	trace	trace	trace
14:0	4.0	3.5	2.0	3.0	trace
14:1	4.4	1.1	trace	trace	trace
16:0	18.0	20.4	33.0	13.6	22.5
16:1	7.4	18.3	11.6	10.0	8.7
18:0	23.7	trace	4.5	1.7	3.3
18:1	4.5	31.4	23.1	10.8	13.4
18:2	3.1	13.3	7.7	6.6	6.6
18:3) 20:1)	1.8	4.2	4.2	1.5	1.6
20:2	3.3	—	trace	—	trace
20:3	1.3	—	—	—	1.8
20:4	3.8	trace	trace	1.2	4.2
20:5	14.1	4.3	3.5	9.2	6.0
22:4	2.9	trace	—	—	—
22:5	trace	3.6	10.5	5.3	22.4
22:6	5.2	—	—	37.1	9.5

Abbreviations: SE, sterol esters; WE, wax esters; TG, triglycerides; NEFA, nonesterified fatty acids; PG, partial glycerides; PL, phospholipids.

which over 30% of fatty acids were 22:2. In Lp-IV and Lp-V, there appeared some resemblance in fatty acid pattern, with the exception of 22:5. The fatty acid composition of CPA differed from those of lipoproteins in having more saturated fatty acids. The major fatty acids of CPA were 16:0, 18:1, and 16:1. The CPA had a slightly similar fatty acid pattern to Lp-IV. However, the differences may be characterized by markedly differ-

Table 4. Seasonal variation of lipoprotein composition

Lipo-protein	Date of sampling			
	1975 June 9* ¹	1975 Sept. 4* ²	1975 Nov. 6* ³	1976 Jan. 20* ⁴
Albumin	68.8%	76.1%	71.9%	77.4%
Lp-I	trace	3.0	4.4	trace
Lp-II	11.9	8.5	12.3	11.9
Lp-III	2.8	2.3	1.4	1.3
L/-IV	8.0	5.4	5.7	5.2
L/-V	8.5	4.7	4.4	4.2

*¹ Pooled sample from 19 specimens.

*² Pooled sample from 13 specimens.

*³ Pooled sample from 15 specimens.

*⁴ Pooled sample from 17 specimens.

ent levels of 22:2 and 20:5.

Table 3 shows fatty acid compositions of non-polar lipids (SE and WE), TG, NEFA, partial glycerides (PG), and phospholipids (PL) which were separated from the lipid of CPA. The differences in fatty acid distribution in these lipid classes were also distinct. The nonpolar lipids were characterized by the lowest level of monounsaturated fatty acids (18%) and the highest level of saturated fatty acids (48%). On the other hand, monounsaturated fatty acids were specifically distributed in TG. Despite of relatively low level of polyunsaturated fatty acids in nonpolar lipids, TG, and NEFA, the fatty acids were abundantly distributed in PG and PL. The PG contained a substantial amount of 22:6. There was a close simi-

larity in fatty acid composition among NEFA, TG, and the whole lipid of CSA.

The seasonal variations of the plasma lipoproteins and albumin are shown in Table 4. More than 70% of the total serum lipid was always conjugated with albumin. The disappearance of Lp-I was found in the plasmas obtained in June and January. Any other significant tendency in seasonal variation was not observed.

Discussion

The content of NEFA was higher in CSA than the lipoproteins having globulin character.⁸⁾ Any marked difference was not observed in lipid compositions among carp serum lipoproteins, while it was found among lipoprotein classes in other fish sera¹⁻⁶⁾. Lp-I having a large molecular weight had a different lipid composition from the mammalian chylomicron. However, the slightly high proportion of TG was only similarity to the chylomicron. In addition, a relatively uniform distribution of fatty acids was characteristic to Lp-I.

While LEE and PUPPONE²⁾ reported the high contents of polyenoic acids in the nonpolar lipid fraction of sardine serum lipoproteins, that of CSA preferentially contained appreciable amounts of saturated acids (48.2%). As a whole, the content of saturated fatty acids was not so low in CSA as in the lipoproteins of globulin fraction. It is noteworthy that the fatty acid composition of NEFA was closely similar to that of unfractionated lipid of CSA. In mammalian sera, free fatty acids are principally transported by albumin. SAIFER and GOLDMAN¹²⁾ described that the fatty acids of human serum are similar to those conjugated with an albumin. Therefore it is supposed that NEFA's of CSA are related to fatty acid transport. Considering the amount of the lipid conjugated with CSA, it seems that the greater part of the serum lipid is transported by CSA.

Assuming that the amount of lipid in carp serum lipoproteins decreases in proportion to density, Lp-II and Lp-IV might correspond to a lipoprotein of lower density in ultracentrifugal classification. The serum of other fish contained a major lipoprotein very similar to human serum HDL in ultracentrifugal and electrophoretic properties and in chemical composition^{5,6,13)}. Transport system of low molecular substance as well as protein function in fish seem not to be differentiated so highly as

that in mammals. A major lipid-containing albumin found in teleost fish sera probably corresponds to the protein designated HDL of those fish^{1,2,4,5)}. There appeared some seeming similarities in both protein- and lipid-moieties among CSA, human serum albumin, and human serum HDL^{7,8)}. With respect to lipid metabolism, therefore, it might exist the possibility that CSA has not only a human serum albumin-like function, but also certain lipoprotein-like function such as HDL.

The disappearance of Lp-I in the cold season seemed to be related with difference of dietary intake. However, the other lipoproteins showed no systematic seasonal variation. It is of interest whether the normally fed carp has constantly such fatty acid lipid composition in the lipoproteins. The lipid composition of fish is strongly influenced by dietary lipid and other physiological factors¹⁴⁾. Both albumin and total lipid content in the plasma were high in the warm season, whereas no clear correlation was observed between both of them¹⁰⁾. Added lipids to the plasma in vitro were preferentially accepted by the albumin¹⁵⁾. YAMAGUCHI *et al.*¹⁶⁾ isolated a biliverdin-lipoprotein complex from eel serum. The protein is capable of conjugating a large amount of lipid, and the proportion of lipid fluctuated considerably. While the lipid moiety of human serum lipoprotein is evidently constant, the proportions of lipid in both lipoproteins and albumin might also fluctuate according to physiological condition.

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Abbreviation: HDL, high density lipoprotein.

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