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Influence of Additional Lipid on Electrophoretic Behavior of Carp Plasma Lipoprotein

Heisuke NAKAGAWA and Mitsu KAYAMA

Department of Fisheries, Faculty of Fisheries and Animal Husbandry, Hiroshima University, Fukuyama

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(Figs. 1–5; Tables 1–4)

The studies on fish plasma lipoprotein demonstrated some marked differences in the electrophoretic diagrams among individuals. Disc electrophoresis of carp plasma revealed at least 10 lipoprotein components which showed relatively variable proportions and mobility[1]. These data were also found in other fish, such as yellow tail (*Seriola quinqueradiata*) and rainbow trout (*Salmo gairdneri irideus*).

In carp plasma (*Cyprinus carpio*), the presence of a certain albumin, which seemed to have an ability to combine with low molecular substances, was confirmed, although some chemical properties were different from those of human serum albumin[1]. In human blood, it is well known that low molecular substances are transported in the form of albumin complex. It would be interesting to know how lower molecular substances are transported in the blood of fish.

In human serum, GORDON[2] had reported that fatty acid if bound to serum lipoprotein increased the mobility. Furthermore, in recent years an interaction between lipid and electrophoretic mobility of lipoprotein was determined with reference to clinical aspects[3]. TSUKAMOTO *et al.*[4,5] and SUGANO *et al.*[6] extended this finding to experiments of conjugation mechanism of free fatty acid with human serum lipoprotein. The present paper describes the influence of lipids on the electrophoretic mobility of fish plasma lipoprotein.

MATERIALS AND METHODS

Carp plasma and human serum : Pooled plasma was obtained from fancy carp (*Cyprinus carpio*) of 100–300g in body weight, by cardiac puncture. An anticoagulant, Angrot (Nippon Shoji Co., Ltd.), was used. The blood was centrifuged at 3,000 rpm for 10 min. Human control serum, Consera (Nissui Seiyaku Co., Ltd.) was used for the reference to carp plasma.

Lipid specimens : Tripalmitin, triolein, oleyl alcohol, cetyl alcohol, cetyl oleate, squalene, palmitic acid, oleic acid, linoleic acid, linolenic acid and cholesterol were used for the adding tests with plasma protein.

Lipid addition to carp plasma : Lipids were added to the plasma by the following procedure. (1) Various lipids were added to 1 m*l* of plasma and incubated by shaking at

37°C for 1 hr. After incubation 0.5 m*l* of *n*-hexane was added and centrifuged at 3,000 rpm for 5 min. in order to remove the unreacted lipid. Thus obtained reactants were used for the determination of lipid content and cellulose acetate electrophoresis. Quantitative determination of lipid in the reactants was carried out by the sulfo-phosphate-vaniline method. (II) Various amounts of sodium oleate were added to each test tube containing carp plasma. After incubation at 37°C for 1 hr., the mixtures were applied to electrophoretic analyses.

Cellulose acetate electrophoresis : Four μl of plasma was spotted on a cellulose acetate film (Sartorius-Membranfilter) of 7 cm in length and 2 cm in width, and the electrophoresis was carried out in a field strength of 0.5 mA per cm width for 60 min. in veronal buffer of pH 8.6 and ionic strength 0.06. The cellulose acetate film was divided into halves, then one piece was visualized with Ponceau 3R for protein, and the other with Schiff's reagent after preliminary ozonization for the lipid staining.

Agarose gel electrophoresis : One μl of carp plasma was applied in sample well on agarose gel plate of Pol-E-film (Pfizer Co., Inc., USA). Protein fractions were detected by Nigrosin staining. For lipoprotein detection, 3 μl of plasma was applied and staining was made with Sudan black B.

Disc electrophoresis : Disc electrophoresis was carried out by using 7.5 % polyacrylamide gel. Three or 5 μl of plasma was applied on the sample gel with tris-glycine buffer-containing 2 *M* sucrose. After migration, protein was visualized by staining with Coomasie brilliant blue, and lipid by Sudan black B.

Measurement of relative mobility : Mobility change of individual lipoprotein was presented as a relative mobility. The relative mobility of carp and human lipoprotein was determined in relation to the migration of fraction IV of carp plasma (Fig. 1) and human serum albumin, respectively.

RESULTS

Lipid combination test : The results of combination test in carp plasma and human serum are summerized in Table 1. The amount of lipid combined with plasma protein differed remarkably by the different kinds of lipid. Unsaturated fatty acids were preferentially incorporated into both carp plasma and human serum. The combination ability with triglyceride and cholesterol was somewhat lower in carp plasma than in human serum.

Mobility change of lipoprotein : As shown in Fig. 1, two lipoprotein components were observed in the pooled carp plasma. The most prominent lipid was localized on fraction II in carp plasma protein pattern. The lipoprotein band with higher mobility in carp plasma was designated as lipoprotein A, and the lower as lipoprotein B. Addition of triolein, cetyl alcohol, squalene or cholesterol to carp plasma revealed slight decrease in the mobility of lipoprotein. It was noted that the lipoprotein A greatly increased its mobility by addition of the fatty acid. When the unsaturated fatty acids were added, partial resolution of lipoprotein B into two indistinct bands was seen. The mobility of

Table 1. Combination of carp plasma and human serum with several lipids

Lipid	Additional amount (μmole)	Lipid amount (mg/100ml) Carp plasma	Human serum
Initial		588	644
Tripalmitin	3	708	755
	9	748	755
Triolein	3	708	772
	9	1131	1050
Palmitic acid	3	689	835
	9	735	1040
Oleic acid	3	1026	1428
	9	1255	2039
Linoleic acid	3	1018	1692
	9	1113	2200
Linolenic acid	3	1346	1431
	9	1447	1679
Cholesterol	3	638	1253
	9	638	1253
Oleyl alcohol	3	776	886
	9	790	1075
Cetyl alcohol	3	748	814
	9	776	1010
Cetyl oleate	3	741	754
	9	864	850
Squalene	3	*	964
	9	694	1327

* not analyzed.

Table 2. Changes of relative electrophoretic mobility* of carp plasma lipoprotein with the addition of lipids

Lipid	Additional amount (μmole)	Lipoprotein region A		B
Initial		2.18		1.56
Tripalmitin	3	2.48		1.65
	9	2.81		1.85
Triolein	3	2.02		1.48
	9	2.10		1.54
Oleyl alcohol	3	2.48		1.65
	9	2.81		1.85
Cetyl alcohol	3	2.06		1.45
	9	2.09		1.45
Cetyl oleate	3	2.50		1.63
	9	2.57		1.68
Squalene	3	2.02		1.38
	9	2.13		1.57
Palmitic acid	3	2.59		1.76
	9	2.59		1.80
Oleic acid	3	2.94	2.00	1.73
	9	3.16	2.00	1.86
Linoleic acid	3	2.74	1.83	1.65
	9	2.74	1.86	1.71
Linolenic acid	3	2.37	1.66	1.37
	9	2.37		1.55
Cholesterol	3	2.01		1.38
	9	2.02		1.44

* Calculated from the mobility of Fraction IV as 1.00.

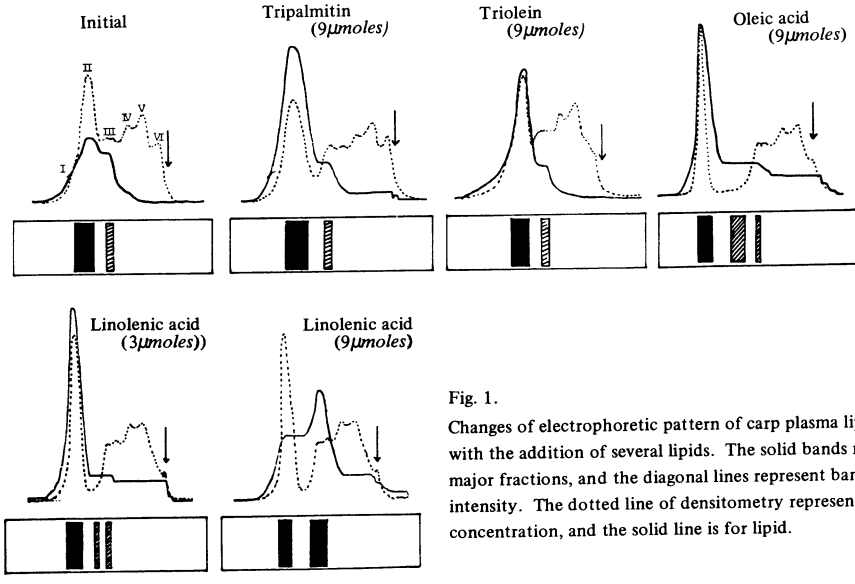

Fig. 1.
Changes of electrophoretic pattern of carp plasma lipoprotein with the addition of several lipids. The solid bands represent major fractions, and the diagonal lines represent bands of medium intensity. The dotted line of densitometry represents protein concentration, and the solid line is for lipid.

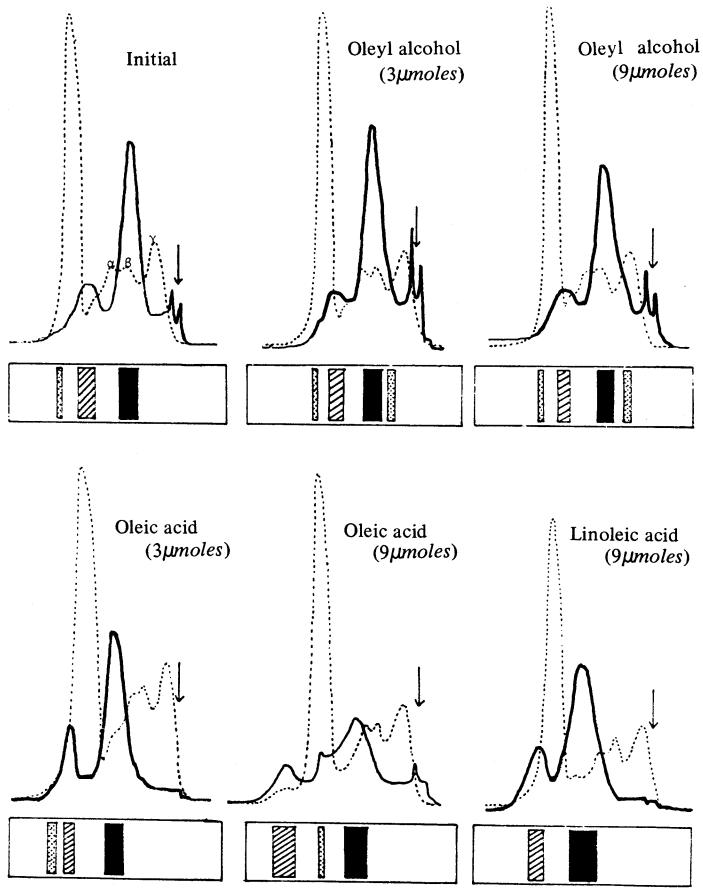

Fig. 2. Changes of electrophoretic pattern of human serum lipoprotein with the addition of several lipids. The explanations of the figure are same as indicated in Fig. 1.

lipoprotein A markedly increased not only by the addition of fatty acid, but also of tripalmitin, oleyl alcohol, and cetyl oleate.

In human serum, three distinct lipid-positive bands were illustrated. However, under the conditions used here the resolution of β- and pre-β-lipoproteins was incapable. Fatty acid and fatty alcohol were effective for the mobility change of human serum lipoprotein. The addition of these lipids to the serum complicated the lipoprotein pattern on the cellulose acetate film, as shown in Fig. 2 and Table 3. In the other lipids, no marked difference appeared in the lipoprotein pattern. The addition of fatty alcohol led to the occurrence of subfraction in β-lipoprotein region. As a result of the addition of linoleic acid the β-lipoprotein overaped the α-lipoprotein.

Table 4 shows the result of the examination on recovery of mobility change. The mobility increased by addition of oleic acid was partially recovered by dilution of fatty acid concentration.

Effect of sodium oleate on mobility : It was shown in the above experiment that oleic acid addition to carp plasma altered markedly the lipoprotein pattern. Therefore,

Table 3. Changes of relative electrophoretic mobility* of human serum lipoprotein with the addition of lipids

Lipid	Additional amount (μmole)	Lipoprotein region			
		α	β	A1	
Initial		0.44	0.88	1.07	
Tripalmitin	3	0.48	0.96	1.08	
	9	0.49	0.96	1.08	
Triolein	3	0.53	0.92	1.05	
	9	0.53	0.92	1.05	
Oleyl alcohol	3	0.53	0.90	1.09	
	9	0.53	0.92	1.09	
Cetyl alcohol	3	0.47	0.93	1.10	
	9	0.51	0.93	1.10	
Cetyl oleate	3	0.56	0.98	1.09	
	9	0.58		1.15	
Squalene	3	0.45	0.90	1.05	
	9	0.49	0.91	1.07	
Palmitic acid	3	0.53		1.09	
	9	0.57		1.11	
Oleic acid	3	0.69		1.11	
	9	0.72		1.11	1.30
Linoleic acid	3	0.66		1.09	
	9	0.71		1.12	
Linolenic acid	3	0.64		1.08	
	9	0.69		1.09	
Cholesterol	3	0.44	0.89	1.09	
	9	0.44	0.89	1.09	

* Calculated from the mobility of albumin as 1.00.

Table 4. Recovery of mobility of lipoprotein

	Carp plasma			Human serum			
	A		B	β	α	A1	
No. 1. Initial	2.18		1.56	0.44	0.88	1.07	
No. 2. Addition of 9μmoles of oleic acid	3.16	2.00	1.86	0.72		1.08	1.30
No. 3. No.1 + No.2 (1 : 1)	2.82		1.72	0.64		1.07	

various amounts of sodium oleate were added to carp plasma and the mobility changes were measured by electrophoresis. The agarose gel and the polyacrylamide gel disc electrophoretic diagrams are illustrated in Fig. 3 and 4. Correlation between oleate concentration and the relative mobility was obtained by calculating the electrophoretic diagram on cellulose acetate film. Lipoprotein A was splitted by addition of 0.2–1 μmole oleate, and lipoprotein B 10–50 μmoles, as seen in Fig. 5. In agarose and cellulose acetate electrophoreses, there appeared marked increase of the mobility in lipoprotein A by addition of the oleate, however, disc electrophoretic pattern showed slight increase.

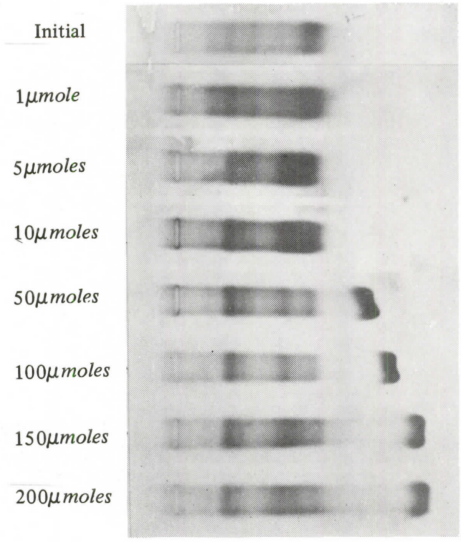

Fig. 3. Changes of electrophoretic patterns of carp plasma protein on agarose film with the addition of varing amount of sodium oleate. Photographs were taken after staining with Nigrosine.

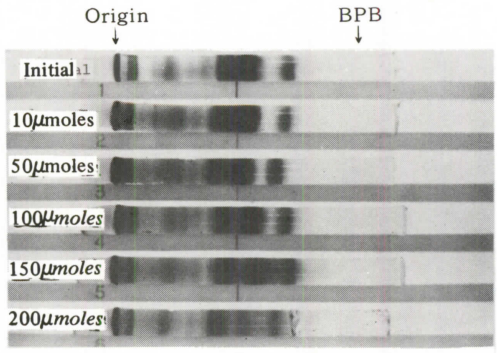

Fig. 4. Disc electrophoretic patterns of carp plasma protein with the addition of varing amount of sodium oleate. The bands were visualized with Coomasie brilliant blue staining.

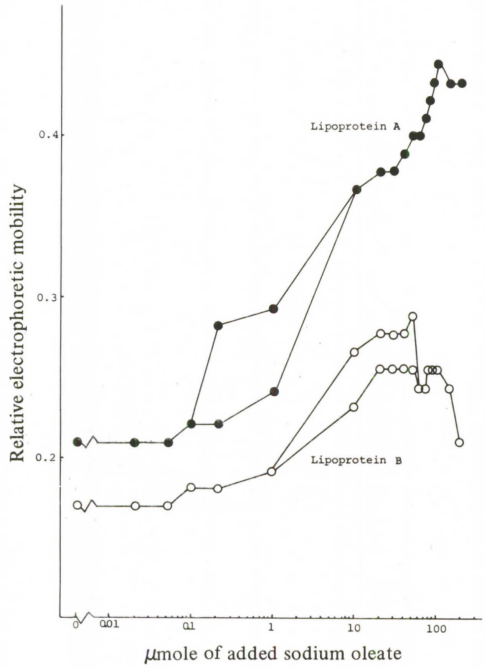

Fig. 5. Correlation between added sodium oleate and relative mobility of carp plasma lipoprotein.

DISCUSSION

Recent studies on human serum lipoprotein indicated that free fatty acid has a significant influence on the electrophoretic mobility of lipoprotein[2,4-6]. Free fatty acids added to human serum fix preferentially on serum albumin, then the excess of fatty acid fixes further on the other lipoproteins[7]. The lipoproteins with added fatty acid showed a higher electrophoretic mobility than the initial lipoproteins. The mechanism of mobility was suggestive by the fact that additional charge contributions would be expected from metal cations, fatty acid anions, and phospholipids[8]. GUSTAFSON *et al.* [9] showed that partial dilipidation of a human lipoprotein resulted electrophoretically in resolving into several components. The dilution of fatty acid concentration in carp plasma allowed a partial recovery of lipoprotein mobility, as shown in Table 4.

In preliminary studies on carp plasma lipoprotein, electrophoretic polymophism was found on disc electrophoresis. The one region was occasionally resolved into two or three components. Carp plasma albumin as confirmed previously is consistent with lipoprotein A as observed on cellulose acetate film. It is a sort of lipoprotein quite different from the human serum albumin in chemical difinition. NAGANO *et al.*[10] reported the presence of a carp serum albumin which had an extremely low concentration of serum. However, the plasma albumin presented by us does not correspond with that of NAGANO *et al.*[10] Interestingly, carp plasma has a predominance of lipid in the lipoprotein A, namely carp plasma albumin, whereas human serum has it preferentially in β-lipoprotein. The lipoprotein A is particularly constructed to serving for transportation of various lipids. Furthermore, it may be thought of as human serum albumin-like protein in a physiological significance. The amount of carp plasma albumin seemed to be subject to the influence of physiological conditions. The high combination ability with lipid in the carp albumin might be leading difference in the lipid transport mechanism with mammals[11].

YAMAGUCHI *et al.*[12] found a blue-green pigmented protein having the lipid-transport function in the eel serum. Its apoprotein was combined with lipid in an energy-free system. The similarity in the mode of lipid binding between fish and human was quite suggestive, although any comparison might be limited by differences in protein composition and its function. It is not easy to tell if there are real compositional differences in protein moiety among the lipoprotein components shown by electrophoretic heterogeneity. In fish, there might be much amounts of unsaturated fatty acid than in mammals. The lipid composition of the lipoproteins is supposed to change with the physiological conditions. Therefore, the veriety of mobility in fish plasma lipoprotein was likely to be dependent on the lipid composition of the prosthetic group or on the transported substance.

SUMMARY

Carp plasma lipoprotein was, *in vitro*, examined for the influences of lipid on electrophoretic behavior. The results obtained are as follows.

1) Plasma lipoproteins were combined with several kinds of lipid of different degrees.

their mobility changed under the influence of additional lipids, especially unsaturated fatty acids.

2) The additional lipids were preferentially accepted by carp plasma albumin which is a sort of lipoprotein, and consequently changed its mobility.

3) The difference of lipid composition in plasma lipoprotein as being the prosthetic group or the transported substance probably revealed the electrophoretic polymorphism of lipoprotein.

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コイ血漿リポ蛋白質の電気泳動的挙動に及ぼす脂質の影響

中川平介・鹿山　光

コイ血漿リポ蛋白質の電気泳動的挙動に及ぼす脂質の影響を *in vitro* で検討し，以下の結果を得た。

1) 血漿リポ蛋白質は添加した脂質を取り込み，易動度が変化する。リポ蛋白質の脂質との結合量および易動度の変化は添加した脂質の種類により異なる。易動度を変化させる脂質として脂肪酸が最も影響が大きく，そのうち特に不飽和脂肪酸が効果的であり，易動度の著しい増加とリポ蛋白質のサブバンドを生じる。

2) コイ血漿アルブミンはリポ蛋白質の一種であり，添加した脂質を優先的に取り込み，その易動度の増加は著しい。

3) コイ血漿リポ蛋白質の電気泳動的多型現象の一因として，補欠分子族又は運搬物としての脂質の組成のちがいが考えられる。