Mem. Fac. Fish. Kagoshima Univ.,
Vol. 44, pp. 23~30 (1995)

Affinity of Fish Plasma Lipoproteins for Dextran Sulfate Cellulose

Seiichi Ando*

Keywords : Lipoprotein, dextran sulfate cellulose, vitellogenin, rainbow trout, Japanese eel.

Abstract

Characteristics of the plasma lipoproteins from rainbow trout and Japanese eel were investigated using dextran sulfate cellulose (DSC) gel. Binding profiles of very low density lipoprotein (VLDL), low density lipoprotein (LDL), high density lipoprotein (HDL), and vitellogenin (VTG) to the DSC gel were consistent with a simple Langmuir's adsorption isotherm. The dissociation constants of VTG and VLDL were lower than those of LDL and HDL, suggesting high affinities of VTG and VLDL for the DSC gel. VTG and VLDL revealed to have specific capacities for binding to the DSC gel from values of the maximum binding level. The structural similarity was suggested between apolipoproteins in VTG and VLDL from rainbow trout and Japanese eel.

In the circulatory fluid of animals including fish, most lipids are complexed with specific apolipoproteins known as lipoproteins[1]. The plasma lipoproteins in human are divided into five major classes according to the densities at which they are isolated: chylomicrons, density (d)< 0.93 g/mℓ; very low density lipoprotein (VLDL), 0.93 < d < 1.006 g/mℓ; intermediate density lipoprotein (IDL), 1.006 < d < 1.019 g/mℓ; low density lipoprotein (LDL), 1.019 < d < 1.063 g/mℓ; high density lipoprotein (HDL), 1.063 < d < 1.21 g/mℓ[2]. The basal structure of lipoprotein particles is a microemulsion composed of a core of non-polar lipids such as triacylglycerol (TG) and cholesterol ester, and the surface monolayer membrane, mainly containing phospholipids and free cholesterol. Apolipoproteins are present on the surface of the lipoprotein particles to stabilize the surface itself and to give biological activities to the particles as regards metabolism[2]. Chylomicrons possess apolipoprotein (apo) B-48 and apo A-Ⅳ as main components. Apo B-100 is the predominant protein in LDL and VLDL. Both apo A-

* Laboratory of Food Chemistry, Faculty of Fisheries, Kagoshima University, 50-20 Shimoarata 4, Kagoshima, 890 Japan.

I and apo A-II are the main components of HDL. VLDL and IDL contain apo E. Apo Cs composed of apo C-I, C-II, and C-III are present in TG-rich lipoproteins such as chylomicrons and VLDL. Apo A-I and apo A-II are activator and inhibitor for lecithin: cholesterol acyltransferase, respectively. Apo C-II activates lipoprotein lipase. Apo B and apo E are known to recognize and bind to LDL receptor.

Fish lipoproteins consist of VLDL, LDL, and HDL as well as other vertebrates, although the density of each lipoprotein class in fish is slightly different from that of mammals[1,3]. In addition to these lipoproteins, vitellogenin (VTG), precursor of egg yolk protein, appears in the blood of spawning fish[4-10]. High levels of lipoprotein have been found in fish plasma, reflecting high utilization of lipids[11]. The plasma profiles of most teleosts are dominated by high levels of HDL[1,3], whose concentrations may exceed 15 mg/mℓ. Fish lipoproteins have also been reported to possess several apolipoproteins such as apo B-, A-I, A-II-, and C-like proteins[12,13]. Although fish lipoproteins have been considerably investigated in detail, most of reports are limited to the chemical analysis of lipoprotein components[8,10,12-23]. Studies on the relationship between structure and function of apolipoproteins are needed to understand lipoprotein metabolism in fish.

Dextran sulfate cellulose (DSC) has been developed for LDL apheresis treatment of the patients with familial hypercholesterolemia[24,25]. The DSC has a specific affinity for apo B-100 protein but not for apo A-I and apo A-II by which LDL is selectively removed from the human plasma[26]. The DSC also seems to be a useful tool for the characterization of fish lipoproteins with different apolipoproteins. The present paper deals with the behavior of plasma lipoproteins from rainbow trout and Japanese eel against the DSC.

Materials and Methods

Cultured female rainbow trout *Oncorhynchus mykiss* (average body weight 1,580 g) in vitellogenesis were obtained from Salmon Farm Station, Okuchi Branch, Kagoshima. Cultured Japanese eel *Anguilla japonica* (average body weight 220 g) were purchased from a local fish market. Blood was removed over ethylenediaminetetraacetic acid (EDTA, 5 mg/mℓ blood) dissolved in 0.15 M NaCl using a syringe inserted in the caudal vasculature of live fish and kept at 4 ℃ throughout the procedure. Plasma was obtained by centrifugation (3,000× g, 15 min). Japanese eel were also injected intraperitoneally with estradiol-17β (E_2, 2 mg/kg body weight) on days 0, 4, and 8 to induce VTG. The injection volume was 0.8 mℓ/kg body weight. E_2 was dissolved in anhydrous ethanol (2.5 mg/mℓ ethanol). At day 10 blood was collected from the E_2-treated eel in a similar manner as other specimens. Plasmas were used for the isolation of lipoproteins.

Plasma lipoproteins were separated by sequential ultracentrifugation[27] in a KBr

solution at densities less than 1.006 g/mℓ for VLDL, from 1.006 to 1.085 g/mℓ for LDL, from 1.085 to 1.210 g/mℓ for HDL, from 1.085 to 1.100 g/mℓ for HDL_2, from 1.100 to 1.210 g/mℓ for HDL_3, and 1.210 to 1.280 g/mℓ for VTG. Each lipoprotein fraction was dialyzed after separation against 10 mM sodium phosphate buffer (pH 7.4) containing 0.15 M NaCl and 1.3 mM EDTA (PBS-saline solution).

The procedures for binding of plasma lipoproteins to DSC gel were as follows. Varying amounts of each lipoprotein were incubated with a constant amount of DSC gel (LA01), which was kindly donated by Kanegafuchi Chemical Industrial Company, Ltd., Osaka, at room temperature for 60 min with gentle stirring. The incubation mixtures were then centrifuged at 3,000 rpm for 10 min. The protein concentration in the supernatant was determined, and its decrease due to the presence of the gel was considered as the protein bound to the gel. For typical experiments with VLDL, LDL, and VTG, the DSC gel was first equilibrated with PBS-saline solution. Two mℓ wet-packed volume of DSC gel was suspended in 6 mℓ of the same buffer. The incubation mixtures contained 200 μℓ of the gel in suspension and lipoprotein in the same buffer, giving a final volume of 600 μℓ. In control incubation mixtures, 200 μℓ of buffer substituted for the DSC gel suspension. For HDL, HDL_2, and HDL_3, 500 μℓ of the gel in suspension, prepared by suspending 3 mℓ wet-packed gel in 6 mℓ buffer, was added to the lipoprotein solution, giving a final incubation volume of 1 mℓ. Bindings of lipoproteins reached maxima within 30 min. Therefore, the incubation time of 60 min was chosen for all binding experiments.

Results and Discussion

The typical profiles of different lipoproteins from rainbow trout and Japanese eel bound to the DSC gel are shown in Fig. 1. The amount of lipoprotein bound to a con-

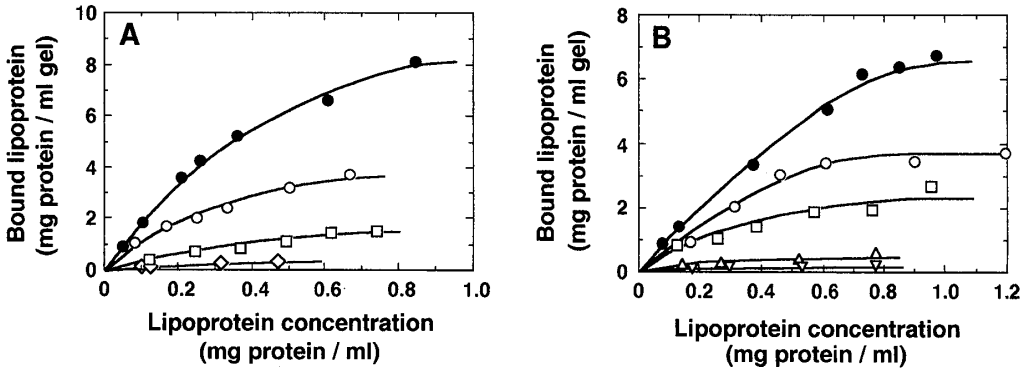

Fig. 1 Typical binding profiles of plasma lipoproteins from rainbow trout (A) and Japanese eel (B) to dextran sulfate cellulose.

○, very low density lipoprotein (VLDL); □, low density lipoprotein (LDL); ◇, high density lipoprotein (HDL); △, high density lipoprotein 2 (HDL_2); ▽, high density lipoprotein 3 (HDL_3); ●, vitellogenin (VTG).

stant volume of gel was displayed as a function of the concentration of added protein. The amount of lipoprotein bound to the DSC gel increased as the lipoprotein concentration increased until saturation was reached. When tested for different lipoproteins from rainbow trout and Japanese eel, the saturating levels for HDL, HDL_2, and HDL_3 were much lower than for VTG and VLDL. LDL displayed a moderate saturating level. The binding data were analyzed according to the Langmuir's adsorption isotherm[28] using the equation: $(S-LP_b)\,LP_f/LP_b=K_d$, where S is the saturating level of lipoprotein binding, LP_b and LP_f are concentrations of bound and free lipoproteins, and K_d is the dissociation constant. The plot of LP_f against $LP_f/(LP_b/V)$ gives the K_d value as the ordinate intercept in terms of lipoprotein concentration and the S/V value as the slope in terms of lipoprotein concentration per volume of the DSC gel (V). The linear plot of the data according to Langmuir's adsorption isotherm is shown in Fig. 2. The data for respective lipoprotein fractions from rainbow trout and Japanese eel fitted to a straight line, suggesting that binding of lipoprotein to the DSC gel was a simple Langmuir-type adsorptive binding. The values of K_d and S are summarized in Table 1. The K_d values (inverse of affinity) of VTG and VLDL from rainbow trout and Japanese eel were lower than those of LDL and HDL, suggesting that both VTG and VLDL had a high affinity to the DSC gel. The S values also indicated that the DSC gel had a specific capacity for binding VTG and VLDL from rainbow trout and Japanese eel, as well as human LDL. Such adsorption seemed to occur because VTG and VLDL from rainbow trout and Japanese eel have a strongly positive charge and because the binding of dextran sulfate covalently linked to porous cellulose beads to these lipoproteins is based on its negative charge[25]. This suggests strongly the structural similarity between VTG and VLDL from rainbow trout and Japanese eel.

VLDL and VTG from laying hens have recently been reported to be endocytosed by the growing oocyte *via* binding to the same receptor, termed the VLDL/VTG receptor, localized on the oocyte plasma membrane[29-31]. VLDL from the plasma of laying hens possesses apo B-like protein and apo VLDL-Ⅱ of molecular weight (Mr) 500 K and 9.5 K, respectively, as the main components[29]. The binding of VLDL to the receptor has been mediated by apo B-like protein but not by apo VLDL-Ⅱ[31]. VTG isolated from the plasma of laying hens consists of apolipoprotein with Mr 240 K[30]. Although the difference in molecular weight is observed between apo B-like protein in VLDL and apolipoprotein in VTG, these two apolipoproteins may have homologous domains in terms of binding to the VLDL/VTG receptor. VLDL and VTG from the plasma of laying hens are the main egg yolk precursors synthesized under the transcriptional control of estrogen in the liver, from where they are secreted into the blood stream. VLDL and VTG appear to deliver TG and protein components into the oocyte of laying hens by the process of receptor-mediated endocytosis, respectively. In addition, VTG has been capable of transporting inorganic phosphate, calcium, and riboflavin to the oocyte[32].

Table 1 Binding parameters of plasma lipoproteins from rainbow trout and Japanese eel to dextran sulfate cellulose.

Lipoprotein	K_d (mg protein / mℓ)		S (mg protein / mℓ gel)	
	Rainbow trout	Japanese eel	Rainbow trout	Japanese eel
VLDL	0.1473	0.1468	4.478	4.254
LDL	0.4554	0.2059	2.358	2.750
HDL	0.4902	–	0.7031	–
HDL_2	–	0.2804	–	0.7404
HDL_3	–	0.1634	–	0.2308
VTG	0.1311	0.08306	9.676	7.568

K_d is the dissociation constant, expressed as concentration of protein.
S is the saturating level of lipoprotein binding in terms of lipoprotein concentration, expressed as milligrams of protein per milliliter of wet-packed gel.

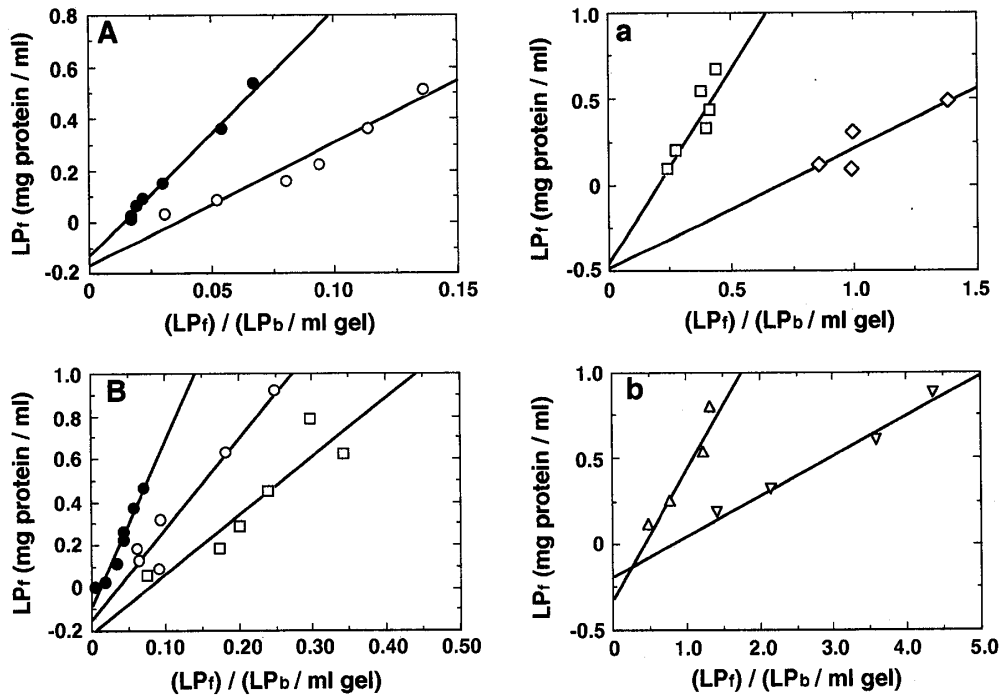

Fig. 2 Linearized plot of binding profiles of plasma lipoproteins from rainbow trout (A, a) and Japanese eel (B, b) to dextran sulfate cellulose.

A and a, Binding data for vitellogenin (VTG, ●), very low density lipoprotein (VLDL, ○), low density lipoprotein (LDL, □), and high density lipoprotein (HDL, ◇) from rainbow trout. B and b, Binding data for vitellogenin (VTG, ●), very low density lipoprotein (VLDL, ○), low density lipoprotein (LDL, □), high density lipoprotein 2 (HDL_2, △), and high density lipoprotein 3 (HDL_3, ▽) from Japanese eel. The Langmuir equation, $(S-LP_b)\,LP_f/LP_b = K_d$, was converted to $LP_f = S \cdot LP_f/LP_b - K_d$. Both S and LP_b in the equation were divided by the volume of the DSC gel present in the incubation mixture (V), giving the equation $LP_f = (S/V)\,LP_f/(LP_b/V) - K_d$. S is the saturating level of lipoprotein binding in terms of lipoprotein concentration; K_d is the dissociation constant; LP_f and LP_b are free and bound lipoprotein concentrations, respectively. The plot of LP_f against $LP_f/(LP_b/V)$ gives the K_d value as the ordinate intercept in terms of lipoprotein concentration and the S/V value as the slope in terms of lipoprotein concentration per volume of the DSC gel.

Taking into account that both VLDL and VTG from rainbow trout and Japanese eel had a high affinity to the DSC gel (Table 1), the structural similarity was suggested between apolipoproteins in VLDL and VTG. VTG has recently been noted to be an ancestor of apo B-100 in human LDL by analyzing nucleotide databases[33, 34]. The major source of nutrients for the developing embryo in fish seems to be delivered into the oocyte by the similar mechanism to laying hens described above. Thus the DSC gel will be a useful tool to characterize fish plasma lipoproteins with different apolipoproteins.

References

1) M. J. Chapman (1980) : Animal lipoproteins. Chemistry, structure, and comparative aspects. *J. Lipid Res.*, **21**, 789–853.
2) A. M. Gotto, Jr., H. J. Pownall, and R. J. Havel (1986) : Introduction to the plasma lipoproteins. "Plasma lipoproteins" (ed. by J. P. Segrest and J. J. Albers), pp. 3–41, Academic Press Inc., Orlando, FL.
3) P. J. Babin and J.-M. Vernier (1989) : Plasma lipoproteins in fish. *J. Lipid Res.*, **30**, 467–489.
4) S. Ando, T. Takeyama, and M. Hatano (1986) : Transport associated with serum vitellogenin of carotenoid in chum salmon (*Oncorhynchus keta*). *Agric. Biol. Chem.*, **50**, 557–563.
5) S. Ando, T. Takeyama, and M. Hatano (1986) : Isolation and characterization of a carotenoid-carrying lipoprotein in the serum of chum salmon (*Oncorhynchus keta*) during spawning migration. *Agric. Biol. Chem.*, **50**, 907–914.
6) P. J. Babin (1987) : Apolipoproteins and the association of egg yolk proteins with plasma high density lipoproteins after ovulation and follicular atresia in the rainbow trout (*Salmo gairdneri*). *J. Biol. Chem.*, **262**, 4290–4296.
7) A. Hara (1987) : Studies on female-specific serum proteins (vitellogenin) and egg yolk proteins in teleosts. Immunochemical, physicochemical and structural studies. *Mem. Fac. Fish. Hokkaido Univ.*, **34**, 1–59.
8) C. Wallaert and P. J. Babin (1992) : Effects of 17β-estradiol and starvation on trout plasma lipoproteins. *Lipids*, **27**, 1032–1041.
9) M. Kishida and J. L. Specker (1993) : Vitellogenin in tilapia (*Oreochromis mossambicus*) : Induction of two forms by estradiol, quantification in plasma and characterization in oocyte extract. *Fish Physiol. Biochem.*, **12**, 171–182.
10) C. Wallaert and P. J. Babin (1994) : Age-related, sex-related, and seasonal changes of plasma lipoprotein concentrations in trout. *J. Lipid Res.*, **35**, 1619–1633.
11) T. Watanabe (1982) : Lipid nutrition in fish. *Comp. Biochem. Physiol.*, **73B**, 3–15.
12) P. J. Babin (1987) : Plasma lipoprotein and apolipoprotein distribution as a function of density in the rainbow trout (*Salmo gairdneri*). *Biochem. J.*, **246**, 425–429.
13) S. Ando and Y. Mori (1993) : Characteristics of serum lipoprotein features associated with lipid levels of muscle and liver from five species of fish. *Nippon Suisan Gakkaishi*, **59**, 1565–1571.
14) G. L. Mills and C. E. Taylaur (1971) : The distribution and composition of serum lipoproteins in eighteen animals. *Comp. Biochem. Physiol.*, **40B**, 489–501.

15) E. R. Skinner and A. Rogie (1978) : The isolation and partial characterization of the serum lipoproteins and apolipoproteins of the rainbow trout. *Biochem. J.*, **173**, 507–520.
16) A. Rogie and E. R. Skinner (1985) : The roles of the intestine and liver in the biosynthesis of plasma lipoproteins in the rainbow trout, *Salmo gairdnerii* Richardson. *Comp. Biochem. Physiol.*, **81B**, 285–289.
17) D. Black and E. R. Skinner (1986) : Features of the lipid transport system of fish as demonstrated by studies on starvation in the rainbow trout. *J. Comp. Physiol. B*, **156**, 497–502.
18) D. Black and E. R. Skinner (1987) : Changes in plasma lipoproteins and tissue lipoprotein lipase and salt-resistant lipase activities during spawning in the rainbow trout (*Salmo gairdnerii* R.). *Comp. Biochem. Physiol.*, **88B**, 261–267.
19) M. Fainaru, Z. Schafer, D. Gavish, A. Harel, and M. Schwartz (1988) : Interactions between human and carp (*Cyprinus carpio*) low density lipoproteins (LDL) and LDL receptors. *Comp. Biochem. Physiol.*, **91B**, 331–338.
20) R. Amthauer, J. Villanueva, M. I. Vera, M. Concha, and M. Krauskopf (1989) : Characterization of the major plasma apolipoproteins of the high density lipoprotein in the carp (*Cyprinus carpio*). *Comp. Biochem. Physiol.*, **92B**, 787–793.
21) N. Iijima, T. Ueki, S. Yoshitake, and M. Kayama (1990) : Isolation and characterization of carp plasma lipoproteins. *Nippon Suisan Gakkaishi*, **56**, 1113–1122.
22) R. B. MacFarlane, H. R. Harvey, M. J. Bowers, and J. S. Patton (1990) : Serum lipoproteins in striped bass (*Morone saxatilis*). Effects of starvation. *Can. J. Fish. Aquat. Sci.*, **47**, 739–745.
23) N. Iijima, T. Gotou, and M. Kayama (1995) : Isolation and characterization of serum lipoproteins in red sea bream. *Fisheries Sci.*, **61**, 297–303.
24) S. Yokoyama, R. Hayashi, M. Satani, and A. Yamamoto (1985) : Selective removal of low density lipoprotein by plasmapheresis in familial hypercholesterolemia. *Arteriosclerosis*, **5**, 613–622.
25) S. Yokoyama (1988) : Treatment of hypercholesterolemia by chemical adsorption of lipoproteins. *J. Clin. Apheresis*, **4**, 66–71.
26) S. Yokoyama, R. Hayashi, T. Kikkawa, N. Tani, S. Takada, K. Hatanaka, and A. Yamamoto (1984) : Specific sorbent of apolipoprotein B-containing lipoproteins for plasmapheresis. Characterization and experimental use in hypercholesterolemic rabbits. *Arteriosclerosis*, **4**, 276–282.
27) R. J. Havel, H. A. Eder, and J. H. Bragdon (1955) : The distribution and chemical composition of ultracentrifugally separated lipoproteins in human serum. *J. Clin. Invest.*, **34**, 1345–1353.
28) A. W. Adamson (1976) : "Physical Chemistry of Surface", pp. 552–563, John Wiley, New York.
29) R. George, D. L. Barber, and W. J. Schneider (1987) : Characterization of the chicken oocyte receptor for low and very low density lipoproteins. *J. Biol. Chem.*, **262**, 16838–16847.
30) S. Stifani, R. George, and W. J. Schneider (1988) : Solubilization and characterization of the chicken oocyte vitellogenin receptor. *Biochem. J.*, **250**, 467–475.
31) J. Nimpf and W. J. Schneider (1991) : Receptor-mediated lipoprotein transport in laying hens. *J. Nutr.*, **121**, 1471–1474.
32) I. M. Lachlan, J. Nimpf, and W. J. Schneider (1994) : Avian riboflavin binding protein binds to lipoprotein receptors in association with vitellogenin. *J. Biol. Chem.*, **269**, 24127–24132.
33) M. E. Baker (1988) : Is vitellogenin an ancestor of apolipoprotein B-100 of human low-density lipoprotein and human lipoprotein lipase? *Biochem. J.*, **255**, 1057–1060.

34) L. E. Perez, M. J. Fenton, and I. P. Callard (1991) : Vitellogenin-Homologs of mammalian apolipoproteins? *Comp. Biochem. Physiol.*, **100B**, 821-826.