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Author(s)	MURATA, Mie; SUGIMOTO, Chihiro; KODAMA, Hiroshi et al.
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N-TERMINAL AMINO ACID SEQUENCE OF
A 28 kDa MAJOR SERUM HIGH DENSITY
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ONCORHYNCHUS MYKISS

Mie MURATA¹, Chihiro SUGIMOTO², Hiroshi KODAMA³,
and Misao ONUMA²

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ABSTRACT

As there are few data on primary structures of fish apolipoproteins (apo), we determined the N-terminal sequence of a 28 kDa major serum protein of rainbow trout, a possible homologue of apoAI protein in higher vertebrates. Rainbow trout serum was separated by two-dimensional electrophoresis. A spot of the 28 kDa protein (pI 5.45) was cut out and directly applied to a protein sequencer. We succeeded in sequencing 30 amino acids from the N-terminal. The sequence of the 28 kDa protein shared a high similarity with that of apoAI of Atlantic salmon (*Salmo salar*). These results showed that the 28 kDa protein is apoAI of rainbow trout.

Key words: High-density serum lipoprotein; rainbow trout (*Oncorhynchus mykiss*); salmonid

It has been established that serum lipoproteins function to transport cholesterol and fatty acids in a number of fish species, including salmonids^{2,8-10,22}. In salmonids, the concentration of high density lipoproteins (HDL) in the sera reaches 15-20 mg/ml². Several classes of fish lipoproteins were found to be analogous to those of mammals in immunological cross-reactivity, electrophoretic behavior and structural appearance by electron microscopy^{6,17,21}. The major HDL 25 and 13 kilodalton (kDa) apolipoproteins in trout appear to be homologous to human apoAI and apoAII in electrophoretic analyses^{3,6,21}. The amino acid composition of salmonid apoAI is similar to that of human apoAI²¹. These results suggest that lipoproteins were

¹) Department of Veterinary Science, National Institute of Health, Toyama 1-23-1, Shinjuku Tokyo, 162, Japan.

²) Department of Epizootiology, Faculty of Veterinary Medicine, Hokkaido University, Sapporo, 060, Japan.

³) Laboratory of Veterinary Immunology, Department of Veterinary Science, College of Agriculture, University of Osaka Prefecture, Gakuen 1-1, Sakai, Osaka, 593, Japan.

established early in evolutionary history and that they have been structurally and functionally preserved throughout the development of species. Nevertheless, few data on the primary structures of apolipoproteins are available in lower vertebrates^{18,19}. Therefore, the present study was carried out to characterize serum apolipoprotein (apoAI homologue) of the rainbow trout (*Oncorhynchus mykiss*).

Young rainbow trout weighing 50–70g were purchased from a commercial farm. They were acclimatized to laboratory conditions for several weeks in 100 l plastic aquaria filled with aerated ground water at 14–15°C (flow rate of 30 l/h). The fish were fed with commercial pellets. Blood collected from the dorsal arteries and veins of ten trout was centrifuged at 1,000rpm at 4°C for 10 min, and the supernatant was recentrifuged at 10,000rpm for 15 min at 4°C. The sera from 10 trout were pooled and used to prepare lipoproteins.

Lipoproteins were prepared by floatation centrifugation using different concentrations of KBr solutions as described by Skinner and Rogie²¹. After removal of chylomicrons, very low density (VLD) and low density (LD) lipoproteins ($d < 1.085$ g/ml) were separated by centrifugation at 80,000 rpm for 5h (Hitachi CS-100, RP-80AT rotor). After removal of VLD and LD, HDL ($d > 1.085$) were isolated by centrifuging further at 80,000 rpm for 10h. Serum proteins and isolated HDL were analyzed by one- and two-dimensional sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE). One-dimensional SDS-PAGE was carried out using 12% acrylamide slab gel¹³. For two-dimensional (2D) SDS-PAGE, samples were treated with the SDS mixture (2% w/v SDS, 1% w/v DTT, 10% v/v glycerol, 0.05M cyclohexylaminoethane sulfonic acid [CHES], pH 9.5) at 100°C for 2 min¹. Carrier ampholytes used in the first dimensional isoelectric focusing tube gels were a 2 : 1 mixture of pH 3.5–9.5 and pH 4–6 ampholines (Pharmacia LKB Biotechnology). The second dimensional SDS-PAGE was carried out as described above. Proteins separated by SDS-PAGE were stained by the silver staining method. For analysis of the HDL fraction, SDS-glycerol-PAGE was performed⁷.

Proteins separated by 2D-SDS PAGE were blotted to a polyvinylidene difluoride (PVDF) membrane (Immobilon P^{SQ}, Millipore Co., MA) using a semi-dry transfer apparatus with 30mM Tris-17mM borate-0.055% w/v SDS-20% v/v methanol¹². The sheet was stained with Commassie brilliant blue R250 (Bio-Rad) in 50% methanol, destained with 50% methanol and washed with a 50mM NaCl solution followed by distilled water. Protein spots were cut out and dried before sequence analysis. The N-terminal amino acid sequence was determined by an automated protein sequencer (model 477A, Applied Biosystems). Amino acid sequences were compared with the data stored in SWISS-PROT and NBRF-PIR data bases using the DANASIS program (Hitachi Software Engineering).

Two microliters of the trout serum was analyzed by 2D-SDS PAGE (Fig. 1A). Protein spots of 28 kDa and pI 5.45 were predominant over other serum proteins.

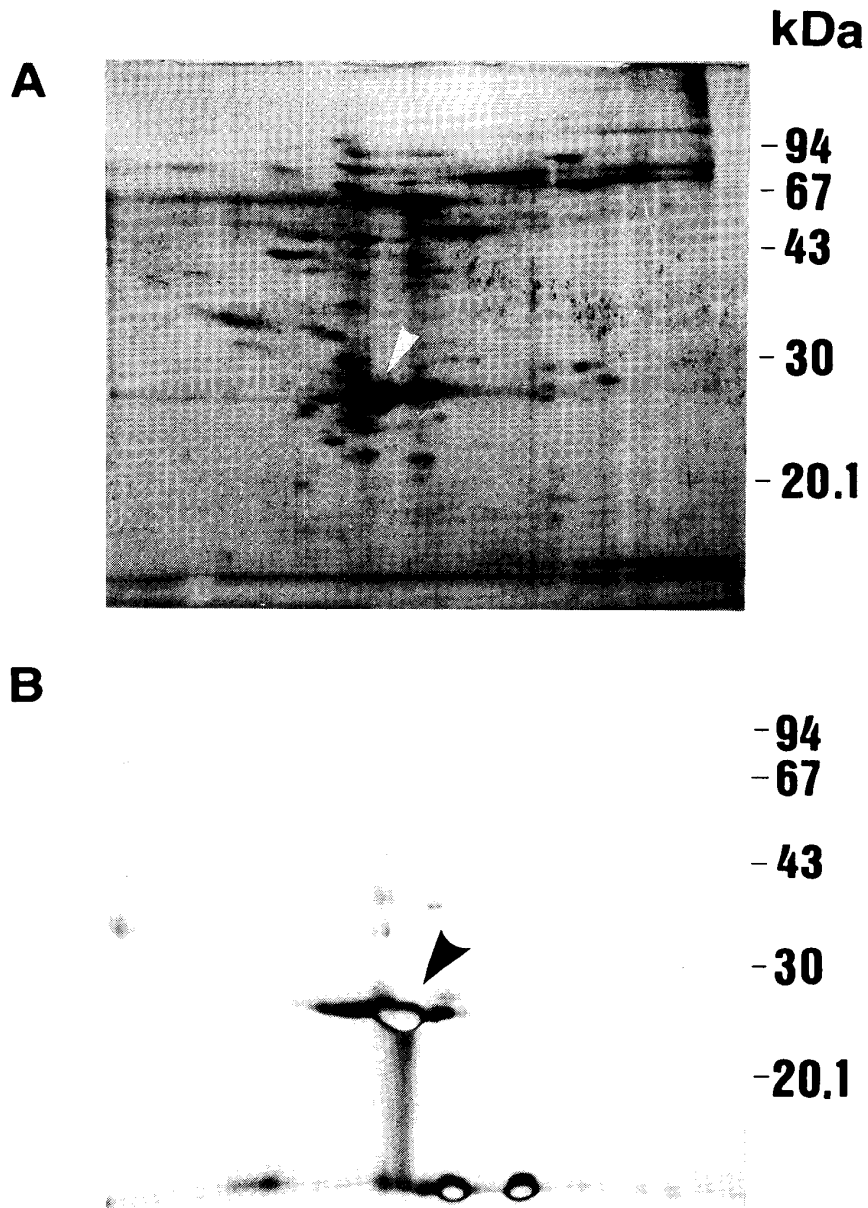


Figure 1. A: Two-dimensional SDS-PAGE analysis of rainbow trout serum. Two μ l of the serum was treated with the SDS mixture for isoelectric focusing SDS-PAGE. The protein spot of 28 kDa used for sequence analysis is indicated by an arrow head. The acidic end is oriented to the left and the basic end to the right.

B: Two-dimensional SDS-glycerol-PAGE of the trout HDL fraction. The HDL fraction separated by KBr flotation was applied to the first-dimensional gel for isoelectric focusing. SDS-glycerol-PAGE was performed with 12% polyacrylamide and 8% glycerol. A protein spot of apoAI is indicated by an arrow head. The acidic end is oriented to the left and the basic end to the right.

The N-terminal amino acid sequence of the major 28 kDa protein was determined after transfer to a PVDF membrane (Fig. 2). The serum proteins were fractionated by KBr flotation centrifugation. As shown in Figure 1B, the 28 kDa protein was detected in the fraction of HDL ($d > 1.085$ g/ml) and showed electrophoretical mobility similar to that previously reported for HDLs from rainbow trout^{2, 3}. The N-terminal amino acid sequence of 28 kDa protein in the HDL fraction was identical to the result shown in Fig. 2. These results showed that the 28 kDa major serum protein is apoAI of rainbow trout. We could determine 30 amino acids of 28 kDa serum protein from the protein spots separated by 2D-SDS PAGE (Fig. 2). Sequence data of apoAI of rainbow trout revealed a high sequence identity with Atlantic salmon apoAI (93%)¹⁹ and a low similarity with chicken apoAI (35%)^{5, 20}. Sequence similarity was scarcely found between apoAI of rainbow trout and apolipoproteins of mammals^{4, 11, 14-16}.

Since evolutionarily lower elasmobranchs have various lipoprotein components present in higher animals¹⁸, the role of apoAI as a lipid transporter seems to be universal among all vertebrates. It is interesting to examine the phylogeny of the lipid transport system by apolipoproteins as well as the range of animal species that conserve functions and structures of the apolipoproteins. The sequence of rainbow trout apoAI is highly conserved but not identical with the sequence deduced from Atlantic salmon apoAI cDNA¹⁹. Although the first amino acid at the N-terminal of the protein of the trout was indefinite due to impurities, the 14th amino acid was serine in rainbow trout and asparagine in Atlantic salmon. Rainbow trout and Atlantic salmon are salmonids and the two species are closely allied. ApoAI sequences of other fish species may be less like those of salmonids. However, sequence data on other fish apoAI are not available. The evolution of fish apoAI will be clarified if such sequence

	1	10	20	30	Homology
Rainbow trout	S	A P S Q L E H V K A A L S	M Y I A Q V K L T A Q R S I	- D L	
Atlantic salmon	D	A P S Q L E H V K A A L N	M Y I A Q V K L T A Q R S I	- D L	93%
Chicken	M	V D V Y L E T V K A S G K D A I	A O F E S S A V G K Q L D L		35%

Figure 2. N-terminal amino acid sequence of a rainbow trout 28 kDa protein, and alignments with Atlantic salmon and chicken apoAI.

A threonine residue with a dot (●) was not definitely identified. —space added for the alignment. Sequences of chicken and Atlantic salmon apoAIs were deduced from their cDNA sequences (refs. 5, 19 and 20). Sequencing of rainbow trout apoAI was carried out twice and sequence data were identical.

data are obtained. In this paper, we presented the first data on the rainbow trout apoAI sequence from the N-terminal and our findings may be useful in evolutionary studies about fish apoAI structures.

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