Immunological evidence for common antigenic sites in high-density lipoproteins from rainbow trout and man

Marise AYRAULT-JARRIER,*† Jacqueline BURDIN,* Lucie FREMONT† and Marie-Thérèse GOZZELINO†

*U.A. 524 CNRS, Laboratoire de Biochimie, Faculté de Médecine Saint-Antoine, 27 rue Chaligny, 75571-Paris Cédex 12, France, and †Station de Recherches de Nutrition, Centre de Recherches INRA, 78350-Jouy-en-Josas, France

The plasma high-density lipoproteins (HDL, ρ 1.085–1.21 g/ml) of rainbow trout (*Salmo gairdneri*) have been shown to contain large amounts of apolipoprotein (apo) AI (M_r 28000) and several other apolipoproteins of M_r < 14000 (apo C-like) and of M_r 37000–38000, 44000–45000 and 53000–54000. Comparison of apo AI from trout and human HDL shows them to be similar in M_r and to have some cross-immunoreactivity, whereas apo AII differs in M_r but also possesses common antigenic sites. It is suggested that the major apolipoproteins of fish and human HDL may fulfil similar roles in lipid transport.

INTRODUCTION

Atherosclerosis is at present a major factor in morbidity, and extensive evidence has accumulated for a principal role of the serum high-density lipoprotein (HDL) as an anti-atherogenic factor, at least in man [1–3].

Studies on the structure of HDL in a wide variety of animal species [4,5] have revealed that these lipoproteins share a M_r -28000 apoprotein akin to human apolipoprotein AI (apo AI). Several immunological studies of the serum low-density lipoprotein (LDL) from both mammalian and non-mammalian species have indicated that the antigenic structure(s) of LDL has been relatively highly conserved during evolution [6,7]; however, only a few comparative immunological studies of serum HDL have been described. Thus dog [8], chicken [9], and marmoset [10] apo AI gave a pattern of partial identity with the human protein.

In the present studies, we compare the cross-reactivity of the major apolipoproteins of human and rainbow-trout HDL with a view to understanding their importance in the molecular structure and function of these complex particles.

MATERIALS AND METHODS

Blood samples

Adult male or female rainbow trout (*Salmo gairdneri*) (1.0–1.5 kg), maintained in fresh water at 17–18 °C, were bled from the caudal artery 36 h after feeding. After blood clotting, serum was obtained by centrifugation (3000 g for 20 min at 20 °C). Three or four samples were pooled, and EDTA (0.1 mg/ml) and anti-bacterial agents (0.001 % sodium merthiolate and 0.01 % NaN_3) were added.

Isolation of HDL

Lipoproteins were separated by sequential preparative ultracentrifugation [11] at 10 °C, with a Beckman L8 55

ultracentrifuge and type 50 Ti rotor. HDL (ρ 1.085–1.21 g/ml) were recovered after centrifugation for 24 h at 165000 g (r_{av} 5.9 cm) from trout serum cleared of apo B-containing particles, which have been shown to be distributed up to densities of 1.015–1.085 g/ml [12,13]. Lipoprotein fractions were exhaustively dialysed at 4 °C against 0.15 M-NaCl/2.6 mM-EDTA/3 mM- NaN_3 , pH 7.4.

Isolation of apolipoproteins

HDL were delipidated with organic solvents at –20 °C [14]. The apolipoproteins were solubilized in 0.1 M-Tris/HCl buffer containing 6 M-urea at pH 7.6 and stored at –20 °C until use.

The apo HDL were fractionated by gel filtration on Sephadex G-150 with 0.05 M-Tris/HCl buffer containing 6 M-urea (freshly prepared to avoid artefactual carbamylation) at pH 7.6 [15]. Appropriate fractions were pooled and were exhaustively dialysed against 0.05 M- $(\text{NH}_4)_2\text{CO}_3$ at pH 9, then freeze-dried and re-solubilized in 0.01 M-Tris/HCl or 6 M-urea at pH 8.2. Protein content was determined as described by Lowry *et al.* [16], with bovine serum albumin as the standard.

Electrophoretic procedures

Electrophoresis in SDS/polyacrylamide gel (SDS/PAGE) with an acrylamide concentration gradient of 3–20 % was performed on slab gels in 0.375 M-Tris/HCl at pH 8.8 in accordance with Weber *et al.* [17]. Electrophoresis was carried out in a buffer containing 0.025 M-Tris, 0.192 M-glycine and 1 % SDS at pH 8.3.

Analytical isoelectric focusing was accomplished in the pH range 3.5–10 in cylindrical gels containing ampholine and in the presence of 6 M-urea [18].

Immunological methods

Antiserum to trout apo HDL was prepared in rabbits. Trout apo HDL (5 mg) in 0.5 ml of 0.15 M-NaCl was mixed with an equal volume of Freund's complete

Abbreviations used: LDL, low-density lipoproteins; HDL, high-density lipoproteins (density 1.085–1.21 g/ml); apo HDL, delipidated HDL; apo AI, apolipoprotein AI; apo AII, apolipoprotein AII; PAGE, polyacrylamide-gel electrophoresis.

†To whom all correspondence should be addressed, at: Laboratoire de Biochimie B, Hôpital Saint-Antoine, 184 rue du Faubourg Saint-Antoine, 75571-Paris Cédex 12, France.

adjuvant and injected intradermally. At 3 and 5 weeks after the first injection, the animals were re-injected with apo HDL (5 mg) in Freund's complete adjuvant. Blood of immunized animals was drawn by carotid puncture 1 week after the last injection. Antisera to human apo HDL, to human apo AI and to human apo AII were prepared by similar procedures with apo HDL (4 mg), apo AI (200 μ g) and apo AII (300 μ g) respectively as previously described [19].

Electroimmunodiffusion in 1% agarose gel containing specific antibodies was performed under conditions described previously [20].

Apolipoproteins were separated by SDS/PAGE (12%-acrylamide gel) [21] and transferred to nitrocellulose sheets by electrophoresis at 20 V overnight by using a buffer containing 0.025 M-Tris and 0.192 M-glycine at pH 8.3 [22]. Blots were incubated with antibodies for 2 h in phosphate-buffered saline containing 3% (w/v) bovine serum albumin [22]. The bound antibodies were detected by incubation with peroxidase-labelled sheep antibodies to rabbit IgG. The peroxidase enzyme was revealed by incubation with 4-chloro-1-naphthol containing H_2O_2 .

RESULTS

Gel filtration of lipid-free apolipoproteins on a Sephadex G-150 column, in accordance with Skinner & Rogie [13], gave a reproducible elution profile (Fig. 1a). A single band was only found in the pooled fractions II and IV, shown by SDS/PAGE, with M_r in the range 27000–28000 and < 14000 respectively (Fig. 1b).

Electrofocusing in the pH range 3.5–10 showed that trout apo AI (fraction II) gave multiple bands in the pH 5–5.8 region (Fig. 1c), as observed previously for human apo AI. However, the focusing pattern was similar to, but not identical with, that for human apo AI (three or four isoforms), since nine isoforms indicate a marked degree of polymorphism for trout apo AI. The apolipoproteins of trout fraction IV were resolved as nine bands situated in the pH range 4–7 by electrofocusing (Fig. 1). This focusing pattern was distinct from that previously observed for human C apolipoproteins by Utermann [18].

In spite of their different focusing patterns, the similarity in M_r of trout and human apo AI and apo C may indicate a partial or total analogy of their structure. To confirm this hypothesis, trout HDL and apo HDL were studied by electroimmunodiffusion with antisera to trout apo HDL, to human apo HDL, to human apo AI and to human apo AII. Both trout HDL and human HDL reacted with antiserum to human apo HDL to give a single precipitin line (with respect to the amount of antigen used). These reactions necessitated use of 50-fold larger amounts of trout HDL (Fig. 2a); 30-fold larger amounts of trout apo HDL and 10-fold larger amounts of human apo HDL were necessary with heterologous antisera than with homologous antisera, indicating that only certain specific common antigenic sites may exist in human and trout apo HDL (Figs. 2b and 2c). As observed in Fig. 2(b), two precipitin lines appeared when trout apo HDL and human apo HDL were tested with antiserum to human apo HDL, suggesting a partial or total identity. To explore further the possibility of a structural identity among the apolipoproteins of trout and human apo HDL, electroimmunodiffusion was

Fig. 1. Electrophoretic patterns of the apolipoproteins isolated by Sephadex G-150 chromatography from trout HDL (1.085–1.21 g/ml).

(a) Elution profile with 0.05 M-Tris/HCl buffer containing 6 M-urea: II, III, IV, pooled fractions. (b) SDS/PAGE (linear gradient of 3–20% acrylamide). (c) Electrofocusing in polyacrylamide gel (7.5% monomer) with pH range 3.5–10 in 6 M-urea. Gels were stained with Coomassie Blue R250: I, total apo HDL (12 mg); II, fraction II of peak 2 (6 μ g in b; 15 μ g in c); III, fraction III of peak 2 (15 μ g in b); IV, fraction IV of peak 3 (15 μ g in b and c).

Fig. 2. Cross-reactivity of trout and human HDL and apo HDL: electroimmunodiffusion on 1%-agarose gel

(a) and (b), Antiserum to human apo HDL: 1, human HDL (2 μ g of protein); 2, trout HDL (100 μ g of protein); 3, trout apo HDL (60 μ g); 4, human apo HDL (2 μ g). (c) Antiserum to trout apo HDL: 3, trout apo HDL (2 μ g); 4, human apo HDL (20 μ g).

Fig. 3. Cross-reactivity of trout and human apo AI and apo AII: electroimmunodiffusion on 1%-agarose gel

(a) and (b), Antiserum to human apo HDL; (c), antiserum to human apo AI. In (a) and (c): 1, human apo AI (5 µg); 2, trout apo HDL (50 µg); 3, human apo HDL (5 µg). In (b): 2, trout apo HDL (20 µg); 4, human apo AII (5 µg).

performed with isolated human apo AI and apo AII. As shown in Fig. 3, reaction with the antiserum to human apo HDL revealed two precipitin lines, one of which may be in continuity with that for human apo AI (Fig. 3a) and the other in partial identity with that for human apo AII (Fig. 3b). These results were confirmed when antiserum to human apo AI was substituted for the antiserum to human apo HDL (Fig. 3c).

The trout AI and C apolipoproteins isolated by gel filtration reacted with antiserum to apo HDL (Fig. 4a), suggesting that trout apo AI may be similar to human apo AI (M_r 28000), showing some common antigenic sites; in addition, trout apo AII, which exists in monomeric form (M_r < 14000), may also possess some antigenic sites in common with human apo AII, as revealed with antiserum to human apo AII (Fig. 4b).

The immunotransfer blots obtained after SDS/PAGE

of human HDL and trout HDL (Fig. 5b) indicate that the antiserum to human apo AI reacted with human apo AI and trout apo AI, although this reaction was weaker against trout apo AI than for human apo AI, even though a larger amount of trout HDL was used. Conversely, when a greater amount of human HDL (10 µg of protein) was used as compared with that of trout HDL (5 µg of protein), the antiserum to trout HDL reacted more strongly with trout apo AI than with human apo AI (Fig. 5a).

Antiserum to trout HDL reacted with trout apolipoproteins of M_r about 37000–38000, 44000–45000 and 53000–54000, confirming the results previously described by Chapman *et al.* (12). This antiserum also reacted with

Fig. 4. Electroimmunodiffusion on 1%-agarose gel of fractions isolated by Sephadex G-150 chromatography

(a), Antiserum to human apo HDL; (b), antiserum to human apo AII; A, fraction I (40 µg); B, C, fraction II (40 and 20 µg respectively); 1, trout apo HDL (50 µg); 2, human apo HDL (5 µg); 3, human apo AI (5 µg).

Fig. 5. Reactivity of antibodies studied by immunotransfer blots

Rabbit antibodies bound to apolipoproteins were detected by peroxidase-labelled sheep antibodies to rabbit IgG and revealed by reaction with 4-chloro-1-naphthol. (a), Antiserum to trout apo HDL; (b), antiserum to human apo AI: 1 and 3, human apo HDL (10 µg in a; 5 µg in b); 2 and 4, trout apo HDL (5 µg in a; 10 µg in b).

low- M_r apolipoproteins of trout HDL, but failed to react with human apo AII and apo C. It appears that our antiserum to trout apo HDL contains little or no antibodies directed against common antigenic sites in trout and human apo AII.

DISCUSSION

In agreement with the data of Chapman *et al.* [12], Skinner & Rogie [13] and Babin [23] on the HDL of rainbow trout, we have noted a high degree of analogy between the corresponding fish and human apolipoproteins. Trout HDL (ρ 1.085–1.21 g/ml) was shown to contain two major apolipoprotein bands by SDS/PAGE, with M_r 27 000–28 000 (apo AI) and M_r 10 000–13 000 (apo C-like), together with minor apolipoproteins of M_r 37 000–38 000, 44 000–45 000 and 53 000–54 000.

Cross-reactivity between human and trout HDL and apo HDL was revealed by our antisera to human and to trout apo HDL. In addition, we have found cross-reactivity between human and trout apo AII, using our antisera to human apo AII. This result indicates that trout apo AII exists in monomeric form and shares some common antigenic sites with dimeric human apo AII. Trout apo AI, which resembles human apo AI in its M_r , cross-reacts with human apo AI, although a more marked charge polymorphism was observed in trout apo AI. Previously, cross-reactivity has been reported between human apo AI and both dog [8] and chicken [9] apo AI when using antisera raised against human apo AI. In our studies, cross-reactivity was demonstrated by use of antiserum to trout apo HDL, to human apo HDL and to human apo AI, as well by immunoblotting and electroimmunodiffusion, thereby confirming the existence of common antigenic sites in human and trout apo AI.

In conclusion, our data, and those of Skinner & Rogie [13] and Chapman *et al.* [12], suggest that the basic molecular organization of trout HDL does not differ substantially from that characteristic of human HDL. Moreover, if some differences were observed in the amino acid composition of the apolipoproteins [12,13], these appear to be without significant effect on their antigenic structure. It may be assumed that the modified proteins have retained their functional specificity during the evolution of species and that HDL may have an anti-atherogenic role both in fish and in man.

Dr. M. J. Chapman is gratefully acknowledged for careful reading of the manuscript. We thank Mrs. D. Sérébrénik and M. Wertenschlag for their excellent secretarial help, and Mrs. L. Théron for the reprinting of documents.

REFERENCES

1. Miller, G. J. & Miller, M. F. (1975) *Lancet* **i**, 16–20
2. Heiss, G., Johnson, M. J., Reiland, S., Davis, C. E. & Tyroler, H. A. (1980) *Circulation* **62**, 116–136
3. Stein, Y., Glangeaud, M. C., Fainaru, M. & Stein, O. (1975) *Biochim. Biophys. Acta* **380**, 106–118
4. Chapman, M. J. (1980) *J. Lipid Res.* **21**, 789–853
5. Camus, M. C., Chapman, M. J., Forgez, P. & Laplaud, P. M. (1983) *J. Lipid Res.* **24**, 1210–1228
6. Goldstein, S., Chapman, M. J. & Mills, G. L. (1977) *Atherosclerosis* **28**, 93–100
7. Chapman, M. J., Goldstein, S. & Laudat, M. H. (1977) *Biochemistry* **16**, 3006–3015
8. Swaney, J. B. (1980) *Biochim. Biophys. Acta* **617**, 489–502
9. Jackson, R. L., Lin Hu, Y., Chan, L. & Means, A. R. (1976) *Biochim. Biophys. Acta* **420**, 342–348
10. Forgez, P., Chapman, M. J. & Mills, G. L. (1983) *Biochim. Biophys. Acta* **754**, 321–333
11. Havel, R. J., Eder, H. A. & Bragdon, J. H. (1955) *J. Clin. Invest.* **34**, 1345–1353
12. Chapman, M. J., Goldstein, S., Mills, G. L. & Léger, C. (1978) *Biochemistry* **17**, 4455–4464
13. Skinner, E. R. & Rogie, A. (1978) *Biochem. J.* **173**, 507–520
14. Ayrault-Jarrier, M., Lastra, G., Pastier, D., Burdin, J. & Polonovski, J. (1972) *Biochimie* **54**, 973–979
15. Scanu, A. M., Toth, J., Edelstein, C., Koga, S. & Stiller, E. (1969) *Biochemistry* **8**, 3309–3316
16. Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
17. Weber, K., Pringle, J. P., Osborne, M. & Timasheff, S. N. (1972) *Methods Enzymol.* **26**, 3–27
18. Utermann, G. (1977) *Nature (London)* **269**, 604–607
19. Glangeaud, M. C., Pastier, D., Ayrault-Jarrier, M. & Polonovski, J. (1974) *Biochimie* **56**, 245–253
20. Salmon, S., Ayrault-Jarrier, M., Beucler, I. & Polonovski, J. (1975) *Biochimie* **57**, 1155–1162
21. Laemmli, U.K. (1970) *Nature (London)* **227**, 680–685
22. Towbin, H., Staehelin, T. & Gordon, J. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4350–4354
23. Babin, P. J. (1987) *J. Biol. Chem.* **262**, 4290–4296