

Lipid transfer protein-catalyzed exchange of cholesteryl ester between high density lipoproteins and apoB-containing lipoproteins

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Abstract Low density lipoproteins (LDL), lipoprotein (a)(Lp(a)), and lipoprotein(a) after removal of the a-protein (Lp(a-)) were compared with respect to their ability to accept cholesteryl ester from high density lipoproteins (HDL). The incubations were performed at constant concentrations of HDL and various concentrations of either LDL, Lp(a), or Lp(a-). Lp(a) exchanged cholesteryl ester with HDL, but at a rate that was only $48.5 \pm 3.8\%$ of the exchange rate found in the presence of autologous LDL. Cleavage of the apo(a) from Lp(a) resulted in Lp(a-), an LDL-like particle, with characteristics of cholesteryl ester exchange very similar to LDL.—Groener, J. E. M., and G. M. Kostner. Lipid transfer protein-catalyzed exchange of cholesteryl ester between high density lipoproteins and apoB-containing lipoproteins. *J. Lipid Res.* 1987. 28: 1053–1056.

Supplementary key words cholesteryl ester transfer/exchange • low density lipoproteins • lipoprotein(a) • lipoprotein(a-)

Cholesteryl esters are components of all the major lipoprotein classes and are localized predominantly within the hydrophobic core of these particles. In human plasma, cholesteryl esters are synthesized mainly in HDL₃ by the action of lecithin:cholesterol acyltransferase (LCAT) (1) and subsequently transferred to or exchanged between very low density lipoproteins (VLDL), low density lipoproteins (LDL), and high density lipoproteins (HDL). This process is catalyzed by lipid transfer proteins (LTPs) (2–5).

Lp(a), first described by Berg (6), is a lipoprotein present in most human plasmas in concentrations ranging between less than 1 and more than 100 mg/dl. Lp(a) is of particular interest since it seems to be an independent risk factor in atherogenesis. Lp(a) is similar to LDL with respect to many of its physical and chemical properties. The lipid composition is almost identical with that of LDL. The main apoprotein in both lipoproteins is apoB. In Lp(a), however, an additional specific protein, apo(a), is present, which is bound to apoB by disulfide linkage (7–9). At present, the sites(s) of Lp(a) synthesis and catabolism are not completely clear. Lp(a) does not seem to

be a metabolic product of other apoB-containing lipoproteins and it seems to be synthesized as a separate lipoprotein (10, 11). Contradictory results have been reported as to whether Lp(a) may be cleared by the LDL receptor pathway (12–14).

No information is available on the characteristics of Lp(a) with respect to the capability of Lp(a) to transfer/exchange cholesteryl ester (CE) with HDL.

In this study, we have compared the characteristics of the cholesteryl ester exchange between HDL and LDL, Lp(a), and Lp(a-).

METHODS

Isolation of lipoproteins: LDL, Lp(a), Lp(a-), and HDL

Fresh human plasma was screened for Lp(a) immunoreactivity by immunodiffusion (15). Strongly Lp(a)-positive plasmas were collected in 1 mM EDTA containing 0.01% sodium azide, and immediately subjected to ultracentrifugation. Lp(a) was isolated by ultracentrifugation of the plasmas between d 1.060 and 1.125 g/ml, followed by size exclusion chromatography (10, 11). The fractions containing apo(a) were combined and concentrated by pressure dialysis.

Part of the Lp(a) was reduced with dithiothreitol (DTT) as described by Armstrong, Walli, and Seidel (16). DTT was freshly added, 10 μ mol of DTT/mg of protein, to the

Abbreviations: VLDL, very low density lipoproteins; LDL, low density lipoproteins; HDL, high density lipoproteins; Lp(a), lipoprotein(a); Lp(a-), lipoprotein(a) after removal of the a-protein; LTP, lipid transfer protein; CETP, cholesteryl ester transfer protein; LCAT, lecithin:cholesterol acyltransferase; CE, cholesteryl ester; DTT, dithiothreitol.

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Lp(a) solution and the solution was incubated at 37°C for 3 hr. After the incubation the mixture was applied to a heparin-Sepharose column (20 × 1.5 cm), equilibrated with 10 mM Tris-HCl, 50 mM NaCl, 1 mM EDTA, and 0.02% NaN₃ (pH 7.6). Apo(a) eluted first, followed by DTT. After increasing the salt concentration to 0.5 mM NaCl, Lp(a-) eluted from the column. The Lp(a-) was further dialyzed and concentrated by pressure dialysis.

LDL and HDL were isolated by ultracentrifugation of plasma at densities between 1.020 and 1.060 g/ml and 1.070 and 1.210 g/ml, respectively. Both lipoproteins were washed once at the appropriate densities. All lipoproteins were dialyzed against 10 mM Tris-HCl, 150 mM NaCl, 1 mM EDTA, 0.01% NaN₃ (pH 7.4).

Purification of cholesteryl ester transfer protein (CETP)

CETP was partially purified from the $d > 1.18$ g/ml supernate of human plasma as described by Pattnaik et al. (17). The supernate was chromatographed on a Phenyl-sepharose CL 4B column (Pharmacia, Fine Chemicals), followed by cation exchange chromatography on a CM cellulose column (Whatman CM-52). The specific activity of the partially purified CETP fraction was increased 800-fold in relation to plasma. The partially purified CETP preparation was free of LCAT. LCAT activity was assayed as described by Glomset and Wright (18). CETP activity in the purification procedure was measured between [¹⁴C]-labeled CE]LDL and HDL as described before (19).

Cholesteryl ester exchange between HDL and LDL, Lp(a), and Lp(a-)

HDL was labeled with [¹⁴C]cholesteryl oleate (Amersham Int.) by a modification (19) of the lipid dispersion technique of Morton and Zilversmit (20). ¹⁴C-Labeled HDL (150 nmol of cholesteryl ester, 10,000 cpm) was incubated with various amounts of either LDL, Lp(a), or Lp(a-) in 50 mM phosphate buffer and 2% BSA in the presence of partially purified CETP for 2 hr at 37°C. The amounts of LDL, Lp(a), and Lp(a-) were adjusted to equivalent concentrations of CE. Total volume was 0.7 ml. Since LCAT was not present in the CETP preparation, as checked by the method of Glomset and Wright (18), no inhibitor for this enzyme was added. After incubation the tubes were placed on ice and LDL, Lp(a), and Lp(a-) were precipitated, after the addition of 2 μmol of LDL-total cholesterol, with 0.1 vol of 2% dextran sulfate-2 M MgCl₂ (1:1). The precipitate was washed once by resuspension in 150 mM NaCl and precipitated again. Finally the precipitate was solubilized in 1 ml of 10% NaCl and the radioactivity was measured by liquid scintillation counting (LKB 1219 Rackbeta). In all experiments control incubations in the absence of CETP were performed, and the measured radioactivity in these incubations was subtracted as a blank.

Mass transfer between HDL and LDL, Lp(a), or Lp(a-) was estimated by chemical analysis of all fractions after incubation. CETP activity was calculated according to Barter and Jones (21) as previously outlined (19).

Lipid and lipoprotein analysis

Total cholesterol, free cholesterol, triglycerides, and phospholipids were measured by enzymatic methods. The following kits were used: total cholesterol, kit number 237 574 (Boehringer); free cholesterol, kit number 14106 (Merck); triglycerides, kit number 612318 (BioMerieux); and phospholipids, kit number 61410 (BioMerieux). Cholesteryl ester was calculated as the difference between total cholesterol and free cholesterol. Protein was determined according to the method of Lowry et al. (22). The amount of apoB in Lp(a) and Lp(a-) was estimated by rocket electrophoresis (23).

RESULTS

LDL, Lp(a), and Lp(a-) were isolated from the same batch of plasma. The fractions were assayed for protein, cholesteryl ester, free cholesterol, phospholipids, triglycerides, and apoB. The lipid values are expressed as weight percentage of the total lipoprotein mass. In two experiments, LDL and Lp(a) were investigated; in two other experiments, LDL, Lp(a), and Lp(a-) were investigated. The results are presented in Table 1. Lp(a) differed from LDL in its higher protein content and its slightly lower content of cholesteryl ester. Lp(a-) and LDL had similar lipid and protein compositions. Measurements of apoB, by rocket electrophoresis, in LDL, Lp(a), and Lp(a-) showed that in LDL and in Lp(a-) 95% of the total protein was apoB, whereas in Lp(a) this value was 65%.

Since no mass transfer was found between HDL and LDL, Lp(a), or Lp(a-) during the 2-hr incubation, the data are presented as exchange rates. Exchange of CE between HDL and LDL, Lp(a), and Lp(a-) was estimated in incubations in which the amount of HDL (150 nmol of cholesteryl ester) was constant and the amount of LDL, Lp(a), and Lp(a-) was between 50 and 650 nmol of cholesteryl ester. Incubations were performed for 2 hr. In previous experiments we ascertained that the exchange process was linear for at least 4 hr. Fig. 1 shows the results of such an experiment. Over the whole concentration range CETP activity was the lowest in incubations in which Lp(a) served as acceptor for CE from HDL. However, in the presence of Lp(a-) as acceptor for CE, the CETP activity was quite similar to that of LDL.

We compared the CETP activity of the incubations in the presence of LDL with the incubations in the presence of Lp(a) or Lp(a-) at four different concentrations. The CETP activity in the presence of LDL was taken as 100%. The CETP activity in the presence of Lp(a) and Lp(a-) is expressed as % of the transfer activity in the presence

TABLE 1. Comparison of the composition of LDL, Lp(a), and Lp(a-)

Sample	Percent of Total Lipoprotein Mass				
	Free Cholesterol	Cholesteryl Ester	Triglycerides	Phospholipids	Protein
LDL (n = 4)	9.2 ± 0.4 ^a	40.3 ± 1.7	4.5 ± 1.6	20.4 ± 0.6	25.6 ± 2.0
Lp(a) (n = 4)	8.8 ± 0.3	37.8 ± 2.7	4.0 ± 0.6	20.2 ± 0.8	29.2 ± 2.5
Lp(a-) (n = 2)	9.0 ± 0.2	40.6 ± 1.5	5.6 ± 0.6	20.5 ± 0.5	24.4 ± 1.5

^aValues represent means ± SD of n observations.

of LDL. There were no significant differences among the four concentrations. For all concentrations the CETP activity in the presence of Lp(a) was $48.5 \pm 3.8\%$, and in the presence of Lp(a-) $85.8 \pm 5.7\%$ of the CETP activity found in the presence of LDL.

DISCUSSION

Krempler et al. (10) suggested that Lp(a) is synthesized by the liver with no apparent precursor. Since, on the other hand, the fatty acid composition of CE in Lp(a) and LDL is almost identical, and LCAT is not active on Lp(a) (F.

Krempler and G. M. Kostner, unpublished results), CE transfer/exchange is the only process in the plasma that can yield such a particle.

The present study shows that Lp(a) is an acceptor of CE from HDL. This process can explain the similar fatty acid composition of cholesteryl ester in Lp(a) and in other lipoproteins. No mass transfer was found under the conditions chosen for our study. This might be due to the fact that in fasted plasma an equilibrium exists between the cholesteryl ester in HDL and Lp(a) as has been found for HDL and LDL (24). Noticable is the lower exchange activity of Lp(a) as compared to autologous LDL. However, reduction of Lp(a), yielding an LDL-like particle, results in an improvement of the particle as acceptor for CE, reaching up to 86% of the activity measured for LDL.

In a recent study, Eisenberg (25) noticed a difference in the rate of cholesteryl ester exchange and transfer between HDL and VLDL and LDL of different sizes. Based on this, Eisenberg suggested that lipid transfer is a surface reaction and that a large surface area positively influences the amount of transferred molecules. Whether mass transfer occurs is more dependent on the composition of the lipoprotein core. Lp(a) is a larger particle than LDL (7). Recent studies show that there is only a slight difference in the moles of CE per particle (26). LDL, Lp(a), and Lp(a-) contained almost equal amounts of CE per particle (1600–1900 nmol). Therefore, in our experiments in which the incubations were adjusted to equivalent concentrations of CE, an approximately equal number of particles was present in the incubations. Since one would expect a greater probability of exchange when the surface is larger (25), the approximately 50% lower exchange activity was surprising. Two possible explanations can be envisaged. First, the apo(a) protein is located on the surface of the particle in such a way that the actual surface that is available for the CE exchange is much smaller than for the comparable LDL particle. Second, Lp(a) binds CETP to a much lower extent than LDL does. Steric hindrance with respect to the exchange process may occur. This assumption is in line with the findings in the present work, where exchange activity increases after removal of apo(a). The finding in this study supports a previous view that LDL and Lp(a-) behave similarly. Armstrong et al. (16) reported the high similarity of LDL and Lp(a-) with respect to their cellular uptake by the LDL receptor in

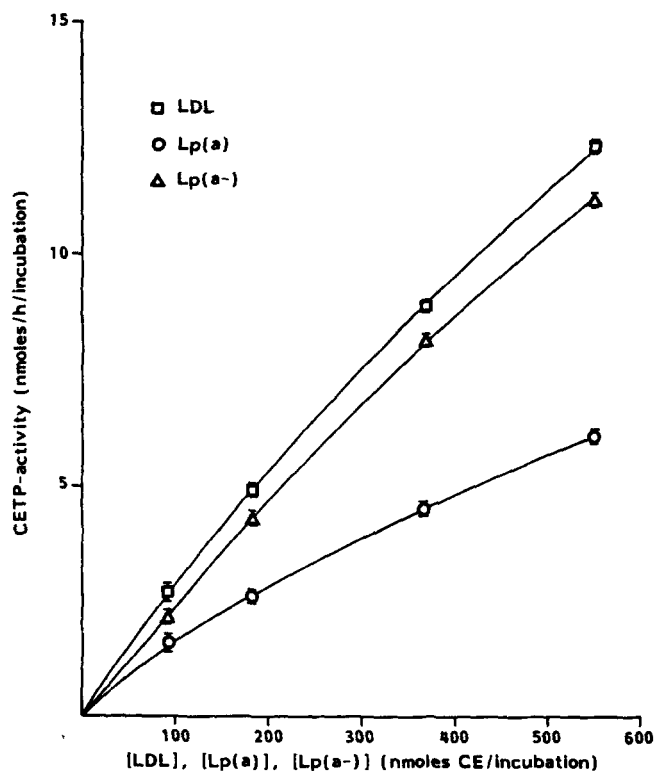


Fig. 1. Cholesteryl ester transfer protein (CETP) activity between HDL and LDL, Lp(a), or Lp(a-) in one characteristic experiment. [¹⁴C]cholesteryl oleate-labeled HDL (150 nmol of CE) was incubated for 2 hr at 37°C with either LDL, Lp(a), or Lp(a-) in the presence of partially purified CETP. The exchange of CE was measured as described in Methods. Results represent means ± SD of three incubations.

cultured human fibroblasts. The present work shows that LDL and Lp(a-) are almost identical with respect to their ability to accept cholesteryl ester from HDL. ■■

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REFERENCES

1. Glomset, J. A., and K. R. Norum. 1973. The metabolic role of lecithin:cholesterol acyltransferase. Perspectives from pathology. *Adv. Lipid Res.* **11**: 1-65.
2. Zilversmit, D. B., L. B. Hughes, and J. Balmer. 1975. Stimulation of cholesterol ester exchange by lipoprotein-free plasma. *Biochim. Biophys. Acta.* **409**: 393-398.
3. Fielding, P. E., and C. J. Fielding. 1980. Cholesteryl ester transfer complex in human plasma. *Proc. Natl. Acad. Sci. USA.* **77**: 3327-3330.
4. Ha, Y. C., G. D. Calvert, G. H. McIntosh, and P. J. Barter. 1981. A physiologic role for the esterified cholesterol transfer protein: in vivo studies in rabbits and pigs. *Metabolism.* **30**: 380-383.
5. Tall, A. R. 1986. Plasma lipid transfer proteins. *J. Lipid Res.* **27**: 361-367.
6. Berg, K. 1963. A new serum type system in man—the Lp system. *Acta Pathol. Microbiol. Scand.* **59**: 369-382.
7. Kostner, G. M. 1983. Apolipoproteins and lipoproteins of human serum. *Adv. Lipid Res.* **20**: 1-43.
8. Gaubatz, J. W., C. Heideman, A. M. Gotto, J. D. Morisset, and G. H. Dahlen. 1983. Human plasma lipoprotein(a). Structural properties. *J. Biol. Chem.* **258**: 4582-4589.
9. Utermann, G., and W. Weber. 1983. Protein composition of Lp(a) lipoprotein from human plasma. *FEBS Lett.* **154**: 357-361.
10. Krempler, F., G. M. Kostner, K. Bolzano, and F. Sandhofer. 1979. Lipoprotein(a) is not a metabolic product of other lipoproteins containing apolipoprotein B. *Biochim. Biophys. Acta.* **575**: 63-70.
11. Krempler, F., G. M. Kostner, K. Bolzano, and F. Sandhofer. 1980. Turnover of lipoprotein(a) in man. *J. Clin. Invest.* **65**: 1483-1490.
12. Maartmann-Moe, K., and K. Berg. 1981. Lp(a) lipoprotein enters cultured fibroblasts independently of the plasma membrane low density lipoprotein receptor. *Clin. Genet.* **20**: 352-362.
13. Havekes, L., B. J. Vermeer, T. Brugman, and J. Erneis. 1981. Binding of Lp(a) to the low density lipoprotein receptor of human fibroblasts. *FEBS Lett.* **132**: 169-173.
14. Floren, C. H., J. J. Albers, and E. L. Bierman. 1981. Uptake of Lp(a) lipoprotein by cultured fibroblasts. *Biochem. Biophys. Res. Commun.* **102**: 636-639.
15. Kostner, G. M., J. R. Patsch, S. Sailer, H. Braunsteiner, and A. Holasek. 1974. Polypeptide distribution of the main lipoprotein density classes separated from human plasma by rate zonal ultracentrifugation. *Eur. J. Biochem.* **45**: 61-621.
16. Armstrong, V. W., A. K. Walli, and D. Seidel. 1985. Isolation, characterization, and uptake in human fibroblasts of an apo(a)-free lipoprotein obtained on reduction of lipoprotein(a). *J. Lipid Res.* **26**: 1314-1323.
17. Pattnaik, N. H., A. Montes, L. B. Hughes, and D. B. Zilversmit. 1978. Cholesteryl ester exchange protein in human plasma: isolation and characterization. *Biochim. Biophys. Acta.* **530**: 428-438.
18. Glomset, A. J., and J. L. Wright. 1964. Some properties of a cholesterol esterifying enzyme in human plasma. *Biochim. Biophys. Acta.* **89**: 266-276.
19. Groener, J. E. M., R. W. Pelton, and G. M. Kostner. 1986. Improved estimation of cholesteryl ester transfer/exchange activity in serum or plasma. *Clin. Chem.* **32**: 283-286.
20. Morton, R. E., and D. B. Zilversmit. 1981. A plasma inhibitor of triglyceride and cholesteryl ester transfer activities. *J. Biol. Chem.* **256**: 11992-11995.
21. Barter, P. J., and M. E. Jones. 1979. Rate of exchange of esterified cholesterol between human plasma low and high density lipoproteins. *Atherosclerosis.* **34**: 67-74.
22. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**: 265-275.
23. Laurell, C. B. 1972. Electroimmunoassay. *Scand. J. Clin. Lab. Invest.* **29 Suppl.** **124**: 21-37.
24. Barter, P. J., and J. I. Lally. 1979. In vitro exchanges of esterified cholesterol between serum lipoprotein fractions: studies of humans and rabbits. *Metabolism.* **28**: 230-236.
25. Eisenberg, S. 1985. Preferential enrichment of large-sized very low density lipoprotein populations with transferred cholesteryl esters. *J. Lipid Res.* **26**: 487-494.
26. Fless, G. M., M. E. ZumMallen, and A. M. Scanu. 1986. Physicochemical properties of apolipoprotein(a) and lipoprotein(a-) derived from the dissociation of human plasma lipoprotein(a). *J. Biol. Chem.* **261**: 8712-8718.