Interaction of Lecithin:Cholesterol Acyltransferase (LCAT)-\(\alpha_2\)-Macroglobulin Complex with Low Density Lipoprotein Receptor-related Protein (LRP)

EVIDENCE FOR AN \(\alpha_2\)-MACROGLOBULIN/LRP RECEPTOR-MEDIATED SYSTEM PARTICIPATING IN LCAT CLEARANCE*

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The reaction of lecithin:cholesterol acyltransferase (LCAT) with high density lipoproteins (HDL) is of critical importance in reverse cholesterol transport, but the structural and functional pathways involved in the regulation of LCAT have not been established. We present evidence for the direct binding of LCAT to \(\alpha_2\)-macroglobulin (\(\alpha_2\)M) in human plasma to form a complex 18.5 nm in diameter. Forty percent of plasma LCAT-HDL was associated with \(\alpha_2\)M; moreover, most of the LCAT in cerebrospinal fluid and in the medium of cultured human hepatoma cell line was associated with \(\alpha_2\)M. Purified recombinant human LCAT (rLCAT) labeled with \({ }^{125} I\)) bound to native and methylene-activated \(\alpha_2\)M (\(\alpha_2\)M-MA) in vitro in a time- and concentration-dependent manner, and this binding did not depend on the presence of lipid. rLCAT bound to \(\alpha_2\)M-MA with greater affinity than to \(\alpha_2\)M. Furthermore, rLCAT did not activate \(\alpha_2\)M as phosphatidylcholine-specific phospholipase C does. Reconstituted HDL particles (LpA-I) inhibited the binding of rLCAT to \(\alpha_2\)M more efficiently than native HDL did. LCAT associated with \(\alpha_2\)M was enzymatically inactive under both endogenous and exogenous assay conditions. Purified rLCAT alone did not bind to low density lipoprotein receptor-related protein (LRP) as lipoprotein lipase (LPL) does; however, when rLCAT was combined with \(\alpha_2\)M-MA to form a complex, binding, internalization, and degradation of rLCAT took place in LRP-expressing cells (LRP \(+\/+\)) but not in cells deficient in LRP (LRP \(-/-\)). It is concluded that the binding of LCAT to \(\alpha_2\)M inhibits its enzymatic activity. Furthermore, the finding supports the possibility that the LRP receptor can act in vivo to mediate clearance of the LCAT-\(\alpha_2\)M complex and may significantly influence the bioavailability of LCAT.

The reaction of lecithin:cholesterol acyltransferase (LCAT) is a 416-amino acid glycoprotein circulating in plasma associated with lipoproteins containing HDL (HDL) in human plasma. It plays a key role in the esterification of free cholesteryl esters and the reacylation of cholesterol esters in the liver for clearance or recycling. Thus, factors affecting the structure, activity, or concentration of LCAT are likely to affect the homeostasis of plasma cholesterol and the RCT process, one of several proposed mechanisms by which HDL may protect against atherosclerosis.

Recent investigations suggest that LCAT can act as an antioxidant and prevent the accumulation of oxidized lipid in plasma lipoproteins. In human plasma, LCAT is almost entirely associated with lipoproteins containing apoA-I, its principal physiological activator. Francone et al. (6) have shown the presence of LCAT, cholesteryl ester transfer protein, apoD, and a small amount of apoA-I in a complex termed pre-\(\beta\)-LpA-I that is involved in the esterification and transfer of cell-derived cholesterol.

We have recently found (7) an association between apoE and \(\alpha_2\)M in human plasma, when we observed that LCAT and apoE migrate to the same position in two-dimensional electrophoretic gels together with \(\alpha_2\)M, in particles 18.5 nm in diameter. This raises the possibility that LCAT circulates in plasma in association with \(\alpha_2\)M. Human \(\alpha_2\)M, the largest known proteinase inhibitor (Mf = 720,000), is found at high concentrations (2–5 \(\mu\)M) in plasma and in extravascular spaces (8, 9). It plays a pivotal role in the clearance of proteinases from the circulation and in regulating their activity in fibrinolysis, coagulation, and complement activation (10, 11).

\(\alpha_2\)M is also a carrier of specific cytokines and various non-protolytic enzymes that include the transforming growth factor \(\beta\), the platelet-derived growth factor BB (12), the \(\beta\)-amyloid peptide (13), and recently, apolipoprotein E (7).

The binding affinities of \(\alpha_2\)M for different non-protolytic

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† The abbreviations used are: LCAT, human lecithin:cholesterol acyltransferase; HDL, high density lipoprotein; LDL, low density lipoprotein; RCT, reverse cholesterol transport; \(\alpha_2\)M, \(\alpha_2\)M-macroglobulin; LRP, lipoprotein receptor-related protein; LPL, lipoprotein lipase; rLCAT, recombinant LCAT; br, human recombinant; MEF, mouse embryonic fibroblasts; MA, methylene-activated; FPLC, fast protein liquid chromatography; CSF, cerebrospinal fluid; PC-PLC, phosphatidylcholine-specific phospholipase C.
proteins depend on its conformation, but apoM in plasma is present almost entirely in the native conformation. This form of apoM is fully functional as a proteinase inhibitor but is not recognized by the cell surface receptor, which is an apoM receptor/low density lipoprotein receptor-related protein (LRP) (8, 14). This receptor is responsible for the rapid plasma clearance of conformally transformed apoM following its reaction with proteinases or small primary amines that modify the apoM thiol ester bonds. Gonias and co-workers have documented that the binding of TGF-β isomers to apoM neutralizes the activity of TGF-β toward various cells (12, 15, 16). Indeed, apoM may be involved in controlling apoptosis (17), the immune system, and atherogenesis (18, 19) via its regulating effects on TGF-β activity.

Structural and functional evidence for several binding domains in different apoM forms led us to hypothesize that apoM modulates LCAT activity and concentration in plasma and may be involved in LCAT clearance via an LRP-mediated endocytic process. The physical association of LCAT and apoM has not been previously reported. The present study aims at providing evidence for the association of LCAT with apoM and to examine how these interactions could be affected by native and activated forms of apoM and by apolipoproteins known to bind LCAT. Cellular binding, internalization and degradation assays were used to evaluate the role of activated apoM and LRP receptor in mediating the clearance of LCAT by cells.

**EXPERIMENTAL PROCEDURES**

**Materials—**Anti-human apoM monoclonal antibody, affinity-purified rabbit anti-god IgG and affinity-purified goat anti-mouse IgG antibody were purchased from Biodesign International (Kennebunk, ME). Methyamine hydrochloride, phosphatidylcholine-specific phospholipase C and sphingomyelinase were purchased from Sigma. Partially purified, anti-human apoM polyclonal antibody and anti-human IgG polyclonal antibody were purchased from ICN Pharmaceuticals Inc. (Aurora, OH). HepG2 cells (HB-8065) were purchased from American Type Culture Collection (ATCC). Anti-RCAT goat polyclonal antibody and purified recombinant human lecithin:cholesterol acyltransferase (hrLCAT) were a gift from Dr. John S. Parks, Wake Forest University School of Medicine, Winston-Salem, NC. Anti-RCAT monoclonal antibody 2H11 was generously provided by Dr. Ross Milne, University of Ottawa Heart Institute. Mouse embryonic fibroblasts (MEF1) that express LRP and mouse embryonic fibroblasts genetically deficient in LRP (MEF2) were generously provided by Dr. Joachim Herz, Department of Molecular Genetics, University of Texas Southwestern Medical Center at Dallas.

**Blood Sampling—**Blood samples were obtained after an overnight fast from male subjects with an apoE3/3 phenotype. Blood was drawn from an arm vein into evacuated tubes containing ethylenediamine-tetraacetate (EDTA, final concentration 1.5 mm) and was kept in ice until analysis of lipids and lipoproteins.

**Gel Electrophoresis—**Plasma samples were separated by twodimensional, non-denaturing gradient gel electrophoresis as described previously (20, 21). In some experiments, only the apoM-containing pre-β-globular migrating segment (~2 cm) of agarose gels was separated in the second dimension.

**Immunoprecipitation Procedures—**LCAT associated with apoM was isolated from human plasma by immunoprecipitation. Plasma (120–500 μl) was incubated overnight at 4°C with 50–200 μl of anti-human apoM antibody or with control anti-human IgG antibody. The immunoprecipitates were centrifuged at 10,000 rpm for 6 min and washed three times with buffer (20 mM HEPES, pH 7.5, 0.15 M NaCl, 0.1% Triton, and 10 mM glycerol). They were analyzed by 4–22.5% SDS-polyacrylamide gel electrophoresis, together with molecular weight standards (Amersham Pharmacia Biotech). The presence of LCAT and apoM in immunoprecipitates was detected by immunoblotting. The efficiency of apoM immunoprecipitation was assessed as >95%, based on the absence of apoM in the supernatant.

**In Vitro Binding Studies—**apoM-MA and LPL were iodinated using IODOGEN® iodination reagent (1,3,4,6-tetrachloro-3′,4′-dihenylpyridiniumcoulauril, Pierce) (22). rLCAT and hrLCATH6 were iodinated as described by Bolin and Jonas (23). Free iodine was removed by PD10 column chromatography. Iodinated proteins were dialyzed extensively at 4°C against phosphate-buffered saline, pH 7.4. These 125I-labeled rLCAT preparations retained ~85% of control acyltransferase activity with rHDL substrates. LCAT activity decreased slightly over time. No difference in the binding of 125I-labeled rLCAT to apoM was observed between 125I-labeled preparations that retained 85% or 30% of control acyltransferase activity with rHDL substrates.

Preparation of apoM-MA, apoM, and binding experiments were performed as previously described (7). The effect of various apolipoproteins was measured by immunoprecipitation of the LCAT apoM-MA complex that was determined by adding these substances (as specified for each experiment) to reaction tubes prior to the addition of 125I-labeled rLCAT. In certain experiments, samples were treated with phospholipases or trypsin before addition of 125I-labeled rLCAT. Bound and unbound 125I-labeled rLCAT was separated by non-denaturing gradient gel electrophoresis 2.5–18% at 60 V (16 h, 15°C). No dissociation of 125I-labeled rLCAT bound to apoM was detected after electrophoresis. The 125I-labeled rLCAT-apoM complex was then separated from free 125I-labeled rLCAT by 500-kDa exclusion filters and isolated from preparative non-denaturing gradient gels by electroelution. The integrity of the complex was evaluated by electrophoresis under non-denaturing conditions on 3–15% gradient gels followed by autoradiography.

**Cell Culture—**HepG2 cells were cultured under standard conditions. Briefly, HepG2 cells (ATCC HB 8065) were grown in Eagle’s minimal essential medium with nonessential amino acids (12.5 μM) and with 10% fetal bovine serum. After a 24-hour incubation, serum-free medium from HepG2 (3 ml) were collected in the presence of 1 mmol/L phenylmethylsulfonyl fluoride, concentrated by using Centricon filters (Amicon) to a final volume of 200 μl, and separated by two-dimensional gel electrophoresis.

**Cell Association and Degradation Assays—**Mouse embryonic fibroblasts (MEF1) that express LRP and mouse embryonic fibroblasts genetically deficient in LRP (MEF2) were used. Cells were incubated with 125I-labeled rLCAT-apoM-MA Complex—125I-rLCAT was incubated with apoM-MA at 1:1 molar ratio for 24 h at 37°C. Radiolabeled rLCAT-apoM-MA complex was then separated from free 125I-labeled rLCAT by 500-kDa exclusion filters and isolated from preparative non-denaturing gradient gels by electroelution. The integrity of the complex was evaluated by electrophoresis under non-denaturing conditions on 3–15% gradient gels followed by autoradiography.

**Lipid and Lipoprotein Assays—**Cholesterol and triglyceride concentrations were determined enzymatically on an autoanalyzer (Cobas Mira, Roche Molecular Biochemicals). HDL-cholesterol concentration was determined by measuring cholesterol in the supernatant after heparin-manganese precipitation of apoB-containing lipoproteins in the d > 1.006 g/ml fraction of plasma prepared by ultracentrifugation with heparin-manganese. Plasma apoA-I and apoA-I concentrations were measured by nophelometry (Behring Nephelometer 100 Analyzer). ApoE phenotypes were determined by immunoblotting of plasma separated by minigel electrophoresis (28). Low density lipoprotein (LDL) (1.019 < d < 1.063) and HDL (1.125 < d < 1.21 g/ml) were isolated from normal-dense plasma by sequential ultracentrifugation using a Beckman ultracentrifuge. The total HDL fraction was isolated from plasma by automated gel filtration chromatography (FPLC).
RESULTS

We used two-dimensional gel electrophoresis to separate plasma apolipoprotein complexes in the HDL size range (Fig. 2, lower panel) by agarose gel electrophoresis and then according to size in the second dimension (Fig. 2, right panel). LCAT protein co-migrated in approximately equal proportions of plasma LCAT dissociated from native plasma and plasma LCAT isolated by immunoprecipitation. The amount of plasma LCAT associated with apoM was detected by immunoblotting with the polyclonal rabbit anti-human apoM antibody (lane a, respectively). LCAT was also present in commercial preparations of apoM separated by SDS-polyacrylamide gradient gel electrophoresis under nonreducing conditions and in the presence of a reducing agent (b-mercaptoethanol) (lanes a and b, respectively). Purified rLCAT (5 μg) (lane c) was used as control. 125I-labeled molecular mass standards are also shown in panels A, B, and C.

Fig. 2. Association of plasma LCAT with apoM isolated by immunoprecipitation (A) and association of LCAT with commercially available apoM (B and C). A, plasma from three normolipidemic subjects (200 μl) was immunoprecipitated with anti-human-apoM (lanes b, c, and d) or with nonspecific anti-human-IgG antibodies (lane a, one subject only). Immunoprecipitates were separated by SDS-polyacrylamide gradient-gel electrophoresis. LCAT and apoM were detected by immunoblotting with polyclonal goat anti-human LCAT antibodies and then with 125I-labeled rabbit anti-goat IgG antibody (A, upper panel). apoM was detected by 125I-labeled anti-apoM antibody (lower panel). B, Immunodetection of LCAT in native and methylamine-treated commercial apoM (100 μg) separated by nondenaturing gel electrophoresis (lanes a and b, respectively). C, commercial apoM separated by SDS polyacrylamide gradient gel electrophoresis under nonreducing conditions and in the presence of a reducing agent (b-mercaptoethanol) (lanes a and b, respectively). Purified rLCAT (5 μg) (lane c) was used as control. 125I-labeled molecular mass standards are also shown in panels A, B, and C.

Revealed one IgG band detected by the second antibody used in the LCAT detection. Lanes b, c, and d demonstrate, in addition, an LCAT band with molecular mass of ~66 kDa. The absence and presence of apoM in respective samples are shown in the (Fig. 2A, lower panel). Removal of apoM from plasma by affinity chromatography reduced most of the LCAT associated with apoM but not α-migrating LCAT (data not shown). The amount of plasma LCAT associated with apoM was estimated to be ~40% of total plasma LCAT in the HDL size range (see later, Fig. 6A).

LCAT was also present in commercial preparations of apoM, purified by metal chelate chromatography. Lane a of the left panel of Fig. 2B shows that a significant amount of LCAT was immunodetectable in commercial apoM separated, in this case, by non-denaturing gradient gel electrophoresis; the complex had an apparent diameter of 18.5 nm. The specificity of anti-LCAT antibody was confirmed by SDS-polyacrylamide gel electrophoresis under reducing conditions followed by immunodetection of LCAT in commercial preparations of apoM.

LCAT was present with molecular masses of 66 kDa, as shown in lanes a and b of Fig. 2C. In this experiment purified rLCAT was used as control, lane c. The difference in apparent molecular mass between plasma LCAT, as shown by the similar amount of LCAT...
associated with α2M under non-denaturing conditions (Fig. 2B, lanes a and b, respectively). Under non-reducing conditions, SDS gel electrophoresis caused a part of LCAT to dissociate from commercial α2M, as shown in lane a of Fig. 2C. LCAT-α2M complex was resistant to boiling in the presence of SDS. In addition, the in vitro formation of 125I-labeled rLCAT-α2M was not inhibited by iodoacetic acid, indicating that free sulfhydryl groups are not required for complex formation, providing evidence for the non-covalent association of LCAT with α2M (data not shown).

The binding of LCAT to activated (α2M-MA) and native α2M was investigated in vitro by incubation of 125I-labeled rLCAT at 37°C with different concentrations of α2M for various times. Bound and unbound 125I-labeled rLCAT were separated by non-denaturing gel electrophoresis, and 125I-labeled rLCAT was quantified by direct scintillation counting. LCAT binding was found to occur in a time- and concentration-dependent manner. Maximum binding was reached after 6 h for both α2M and α2M-MA and remained constant for the remaining 18 h of the experiment (data not shown). α2M-MA had a 2-fold greater capacity to bind LCAT relative to α2M (Fig. 3, upper and left panels). The affinity of LCAT binding to both forms of α2M was assessed from a double-reciprocal plot of the binding data (right panel). Dissociation constants (Kd) calculated from the slope of the regression lines were 3.93 and 1.9 μM for the non-activated and activated forms of α2M, respectively, indicating that LCAT had a greater affinity to bind to α2M-MA than to α2M.

We have previously reported that phosphatidylcholine-specific phospholipase C (PC-PLC) activate native α2M (7). To verify that LCAT does not bind to α2M through its well characterized proteinase-trapping mechanism, we determined whether the conformation of native α2M could be affected by LCAT treatment. Native α2M isolated by metal chelate chromatography (having detectable quantities of bound LCAT (Fig. 2B, lane a)) was incubated for 12 h at 37°C with sphingomyelinase (SM-ase), PC-PLC, or rLCAT. α2M-MA and trypsin-α2M (α2M-T) were used as activated forms of α2M in this
and purified plasma had significant LCAT activity in either conformation of commercial LCAT tested for LCAT activity. Under these conditions, there was no possibility of inactivating LCAT by the purification process (as evidenced by the smaller size and increased mobility of α₃M separated by nondenaturing gradient gel electrophoresis) as shown (Fig. 4).

To verify that the association of LCAT does not depend on the presence of lipid, we investigated whether the binding of ¹²⁵I-rLCAT could be prevented by prior treatment of α₃M with phospholipases (sphingomyelinase and phosphatidylcholine-specific phospholipase C) or delipidating solvents. No decrease was observed in the binding of ¹²⁵I-rLCAT to native α₃M (data not shown).

We next investigated whether HDL particles would affect the association of LCAT with native α₃M. Native α₃M (50 μg) was incubated with increasing amounts of reconstituted HDL, r(LpA-I), or native HDL₃ (Fig. 5). 50 μg of r(LpA-I) or HDL₃ inhibited the association of LCAT with native α₃M by 50 and 30% respectively. At all concentrations used in this experiment, r(LpA-I) particles were more efficient inhibitors than native HDL₃.

We measured the ability of LCAT-α₃M complex to esterify cholesterol in vitro by examining the effect of removing the LCAT-α₃M complex from plasma by immunoprecipitation of α₃M using a purified anti-α₃M IgG fraction and nonspecific anti-human IgG. The fractional esterification rate of cholesterol in plasma was the same in IgG-depleted plasma samples as in IgG-depleted plasma samples (Fig. 6B). As ~40% of the plasma LCAT was associated with α₃M (Fig. 6A), this result shows that LCAT associated with α₃M in plasma is inactive in the esterification of cholesterol. To investigate this observation further, we assayed for LCAT activity using a proteoliposome substrate. The same amount of LCAT (1 μg) present in commercial α₃M preparation (native α₃M and α₃M-MA) purified plasma α₃M (isolated by ultracentrifugation d’ > 1.25) and separated from other plasma proteins by FPLC, thus reducing the possibility of inactivating LCAT by the purification process and the IgG-depleted HDL fraction (isolated by FPLC) was tested for LCAT activity. Under these conditions, there was no significant LCAT activity in either conformation of commercial and purified plasma α₃M in contrast to the activity associated to HDL fraction (Fig. 6C).

To evaluate the ability of ¹²⁵I-rLCAT-α₃M complex to interact with the LRP receptor, we combined ¹²⁵I-rLCAT with unlabeled α₃M-MA, and the resulting complex was purified. The inset of Fig. 8, shows gel electrophoretic separation of free ¹²⁵I-rLCAT (lane a) and ¹²⁵I-rLCAT-α₃M-MA complex (lane b). Equivalent amounts of ¹²⁵I-rLCAT-α₃M-MA, ¹²⁵I-rLCAT-α₃M-MA, ¹²⁵I-rLCAT, and ¹²⁵I-LPL were incubated with LRP-expressing cells LRP (+/+) and control cells that did not express LRP (−/−) at 4 °C for 5 h, and the levels of specific binding were measured. As shown in Fig. 7B, incubation with cells expressing the LRP receptor resulted in the binding of ¹²⁵I-rLCAT-α₃M-MA complex, but cells deficient in this receptor (MEF2, LRP −/−) were unable to bind significant amounts of ¹²⁵I-rLCAT-α₃M-MA complex. In separate experiments (Fig. 7A) we have confirmed the absence of LRP from MEF2 (LRP −/−) cells by measuring the specific binding of ¹²⁵I-α₃M-MA. To determine whether ¹²⁵I-rLCAT alone might interact with LRP-receptor, we added equivalents amount of ¹²⁵I-rLCAT to cultured MEF1 (LRP+/+) and control MEF2 (LRP−/−), and the level of specific binding was measured. As shown in Fig. 7C, minimal LCAT binding to LRP+/+ and LRP−/− cells is observed. The control protein in this experiments was purified LPL, which bind to LRP +/+ but not significantly to LRP −/− (Fig. 7D).

To evaluate the ability of ¹²⁵I-rLCAT-α₃M-MA complex to interact with the LRP receptor, equivalent amounts of ¹²⁵I-rLCAT-α₃M-MA were incubated with LRP (+/+) and control LRP (−/−) cells, and the level of cell association and degradation of this complex was measured. As shown in Fig. 8, incubation with cells expressing the LRP receptor resulted in the internalization and degradation of the complex, but cells deficient in this receptor (LRP −/−) were unable to internalize or degrade significant amounts of ¹²⁵I-rLCAT-α₃M-MA complex.

**DISCUSSION**

We have shown that LCAT, co-migrates with α₃M in human plasma in an intermediate-sized complex (18.5 nm in diameter) between LDL and HDL lipoproteins (Fig. 1A). Plasma LCAT associated with α₃M could be specifically immunoprecipitated using antibodies directed against α₃M. Another experiment demonstrated that purified native α₃M (isolated by metal-chelate chromatography) consistently contained detectable amounts of LCAT (Fig. 2B). LCAT became bound to α₃M in a
may be recognizing a protein motif, such as an amphiphatic α-helix, that is common to apolipoproteins and αM and is independent of lipid content. The association of LCAT with apolipoprotein complexes may thus be due to direct protein-protein interaction, suggesting that LCAT binding and activation by apolipoprotein are independent events. It was documented that the association of LCAT to lipoprotein surfaces essentially was independent of their composition (31).

We have previously shown (7) that the binding of apoE to αM depends on the apoE phenotype. Charge or conformational differences in LCAT mutants might similarly affect their binding to αM species in plasma. Adimoolam et al. (32) have shown that naturally occurring mutants of human LCAT, T123I and N228K, expressed in COS-1 cells, bind to a lipoprotein particle (rHDL) with about half the affinity of wild-type LCAT. Again, activated αM-MA binds two times as much LCAT as native αM (Fig. 3), and various cytokines and growth factors (e.g. TGF-β1, NGF-β, PDGF, bFGF, and TNF-α) also bind to αM-MA with greater affinity than to native αM (12).

LCAT shares several sequence regions with other lipases (33). Phosphatidylcholine-specific phospholipase C activates αM (7), but rLCAT does not (Fig. 4). This suggests that LCAT does not bind to αM through its well characterized proteinase-trapping mechanism (34).

The binding studies shown in Fig. 3 predict that LCAT bound with low affinity to native αM may dissociate from the complex in the presence of LpA-I particles. We postulate that native discoidal HDL particles affect the availability of active LCAT, which is crucial for their maturation, by modulating its dynamic distribution between α-migrating HDL and αM. When 125I-labeled rLCAT was incubated with αM in the presence of a 4-fold excess of r(LpA-I) over αM, the association of rLCAT with αM was almost completely inhibited. Actually, r(Lpa-I) inhibited the binding of 125I-rLCAT with αM more efficiently than did native LDL (Fig. 5).

Studies by Adimoolam et al. have documented that normal LCAT dissociates from rHDL after one catalytic cycle (32). It is possible that the fraction of LCAT bound preferentially to αM reflects a catabolic compartment. We may say that an important proportion of HDL-sized LCAT (40%) is sequestered into an inactive pool by αM, which does not react with cholesterol of lipoprotein origin (Fig. 6) and leads to the concept that plasma αM inhibits the enzymatic activity of LCAT. It is not possible to use a water-soluble substrate such as p-nitrophenylvalerate to provide evidence that the LCAT associated with αM is catalytically active; the latter being a substrate for serine proteases and esterases, it might also interact with chymotrypsines (30), which binds to native purified αM.

LCAT with low activity has been demonstrated in CSF (35). LCAT may be synthesized in the brain (36). Recently, Collet et al. (37) reported that cultured nerve cells secreted a functional LCAT protein. The present study shows that most of the LCAT in CSF was associated with αM (Fig. 1B). Although the functional significance of LCAT binding to αM in CSF remains to be determined, this complex may be involved in mediating the clearance of LCAT by the LRP receptor in the brain.

One can also speculate that native αM could be involved in the stabilization, the decreased plasma clearance, and the resistance of plasma LCAT to proteinase cleavage. Indeed, we have observed that significant amounts of rLCAT resisted trypsin digestion when bound to αM, whereas unbound rLCAT was readily degraded (data not shown).

The results presented in this study provide direct evidence that LRP is a receptor for the LCAT-αM-MA complex (Figs. 7 and 8), thus indicating that it could mediate LCAT-αM-MA binding, endocytosis, and degradation in any tissue that ex-
presses LRP. This work provides evidence for the first time that the LRP receptor may play an important role in the catabolism of LCAT.

Further study of the interaction between LCAT and α₂M may provide new insights into plasma factors affecting HDL particles remodeling, the mechanism of reverse cholesterol transport, and the clearance of LCAT via an α₂M/LRP receptor that is dependent on the conformation of α₂M in vivo.

**FIG. 7.** Cells expressing LRP receptor bind ¹²⁵I-rLCAT-α₂M-MA complex. MEF1 (LRP +/-) or MEF2 (LRP −/−) cells were incubated for 5 h at 4 °C with either ¹²⁵I-α₂M-MA (2 μg/ml, 6000–7000 cpm/ng) as shown in A, purified ¹²⁵I-rLCAT-α₂M-MA complex (4 μg/ml, 4500–5000 cpm/ng) as shown in B, purified ¹²³I-rLCAT (1 μg/ml, 14000 cpm/ng) as shown in C, and purified ¹²⁵I-LPL (0.5 μg/ml, 5000 cpm/ng) as shown in D. To determine the specific binding, the following unlabeled competitors concentration were used: α₂M-MA, 150 μg; rLCAT, 50 μg; and LPL, 20 μg. Plotted values are means ± S.D. of triplicate values. One experiment, representative of three, is shown.

**FIG. 8.** Cells expressing LRP receptor internalize and degrade ¹²⁵I-rLCAT-α₂M-MA complex. MEF1 (LRP +/-) or MEF2 (LRP −/−) cells were incubated for 5 h at 37 °C with purified ¹²⁵I-rLCAT-α₂M-MA complex (4 μg/ml, 4500–5000 cpm/ng). The inset shows an autoradiograph of purified ¹²³I-rLCAT-α₂M complex (lane b) and free ¹²³I-rLCAT (lane a). The amount of specific cell-associated radioactivity (bound plus internalized and degraded) is shown. Plotted values are means ± S.D. of triplicate values. One experiment, representative of three, is shown.
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