Sequence Identity between the α2-Macroglobulin Receptor and Low Density Lipoprotein Receptor-related Protein Suggests That This Molecule Is a Multifunctional Receptor*

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Ten peptides, derived from human α2-macroglobulin (α2M) receptor by chemical or proteolytic digestion, were sequenced. Comparative analysis revealed that all of the resulting sequences were present within the cDNA-deduced structure of low density lipoprotein receptor-related protein (LRP) (Herz, J., Hamann, U., Rogne, S., Myklebost, O., Gausepohl, H., and Stanley, K. K. (1988) EMBO J. 7, 4119–4127). The findings provide evidence that the α2M receptor and LRP are the same molecule. Further evidence comes from immunoprecipitation experiments using a monoclonal antibody specific for the α2M receptor that show this molecule, like LRP, to contain two polypeptides of approximately 420 and 85 kDa that are covalently associated. An additional component of this receptor system is a 39-kDa polypeptide that co-purifies with the α2M receptor during affinity chromatography. Solid phase binding studies reveal that the 39-kDa polypeptide binds with high affinity (Kₐ = 18 nM) to the 420-kDa component of the α2M receptor. The apparent identity of LRP and the α2M receptor suggests that this molecule is a multifunctional receptor with the capacity to bind diverse biological ligands and highlights a possible relationship between two previously unrelated biological processes, lipid metabolism and proteinase regulation.

α2-Macroglobulin (α2M) is a plasma glycoprotein that binds and inhibits proteinases from all subclasses (1). Proteinases “activate” α2M by cleaving (2) it at a specific “bait” region which initiates a series of conformational changes within the molecule (3–5). These conformational alterations not only inhibit the proteinase but enable the α2M molecule to interact with specific cell surface receptors. The resultant complex is then internalized by α2M receptor-mediated endocytosis (6, 7).

Ultimately, the α2M receptor has been purified by ligand affinity chromatography from detergent extracts of placenta (8) and liver (10, 11). This procedure identified the receptor as a large glycoprotein with an approximate molecular mass of 420 kDa. This molecule binds to the activated form of α2M with an affinity (8) similar to that observed for the binding of α2M to fibroblasts (7), hepatocytes (12), macrophages (13, 14), and monocytes (15). In addition to this large polypeptide, two additional components were observed to co-purify. One of these, with an apparent molecular mass of 85 kDa, was thought to originate from proteolysis of the intact receptor (8). The second molecule has an approximate molecular mass of 39 kDa and does not appear to bind to α2M.

The present investigation was undertaken to determine the primary structure of the α2M receptor. The results establish that the sequence of the α2M receptor is probably identical to the deduced sequence of a recently described (16) cell surface protein termed low density lipoprotein receptor-related protein (LRP), so named due to its structural similarity to the LDL receptor.

EXPERIMENTAL PROCEDURES

Proteins—The α2M receptor was purified by affinity chromatography from detergent extracts of placenta as described (8). Its purity was confirmed by analysis on SDS-PAGE. The 39-kDa polypeptide was further purified by cation exchange chromatography on a Mono S column (Pharmacia LKB Biotechnology Inc.). The column was equilibrated with 20 mM Tris, 20 mM octyl β-D-glucopyranoside, pH 8.0, and the 39-kDa polypeptide was eluted with 100 mM NaHCO₃, 2 mM NaCl, 20 mM octyl β-D-glucopyranoside, pH 10.5. Concentrations of the 420- and 39-kDa polypeptides were determined by absorbance measurements assuming that E₁%₄₀₀ = 10.

Cleavage of the α2M/LRP receptor and Protein Sequencing—For LyS C digestion, 176 μg (350 pmol) of purified 420-kDa receptor was concentrated to 0.5 ml, 0.86 g of guanidine HCl was added, and the pH was adjusted to 8.5 by the addition of 0.038 g of Tris base. 7 μl of β-mercaptoethanol was added and the reduction extended for 2 h at room temperature prior to digestion by 35 μl of 4-vinylpyridine. Alkylated carrier was carried out for 2 h at room temperature. The reduced and alkylated receptor was applied to a Sephadex G-25 column equilibrated with 2.5 mM Tris, 0.1 mM EDTA, pH 7.4, containing 5% acetonitrile (v/v). Protein fractions were pooled, lyophilized, and resuspended in 25 mM Tris, 1 mM EDTA, pH 8.5, containing 5% acetonitrile (v/v). 4.2 μg of LyS C (Boehringer Mannheim) was added, and the digestion was carried out for 20 h at 37°C with shaking. Following digestion, the mixture was centrifuged, and the peptides were resolved using an Applied Biosystems model 130 microbore HPLC and RP300 L8 cartridge. Major peaks were collected and used for sequencing on an Applied Biosystems model 477A protein sequencer with an on-line Applied Biosystems model 130 phenylthiobutyryl degradation monitor. For CNBr digestion, the purified receptor was reduced and alkylated as above except that reduction was performed at 37°C for 2 h. Following alkylation and desalting, 100 μg (200 pmol) of receptor was dissolved into 80 μl of 70% formic acid. 0.5 mg of CNBr was added and incubated at room temperature for 20 h in a nitrogen atmosphere. Following extensive lyophilization, the sample was dissolved in 5% acetonitrile, 0.1% trifluoroacetic acid and the peptide isolated as above. Major peaks were used for sequencing analysis or used for further proteolytic digestion.

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The abbreviations used are: α2M, α2-macroglobulin; LRP, low density lipoprotein receptor-related protein; LDL, low density lipoprotein; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; HPLC, high pressure liquid chromatography; TBS, Tris-buffered saline; PMSF, phenylmethylsulfonyl fluoride; VLDL, very low density lipoprotein.

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**Transfer**—Human gingival fibroblasts were grown in 100 × 20-mm culture dishes until almost confluent and washed with cysteine-free RPMI. The cells were then incubated overnight with 10 ml of 25 μCi/ml [35S]cysteine in cysteine-free RPMI, washed with TBS containing 1 mM PMSEF and 20 μg/ml leupeptin, and extracted with TBS containing 0.1% Triton X-100, 1 mM PMSEF, 20 μg/ml leupeptin. The remainder of the steps were carried out as previously described (17), except that protein G-Sepharose was used in place of protein A-Sepharose.

**ELISA**—An ELISA (18) was performed as previously described (8), except that the blocking step included 1 mg/ml rabbit IgG. The Kd was estimated from the data as previously described (8).

**Monoclonal Antibody Production**—The immunization protocol and fusion were carried out as previously described (19). Mice were immunized with polypeptides eluted from the αM affinity column. Solid-phase screening assays (20) using purified components were utilized to identify antibodies of interest.

**S-PAGE and Western Blotting**—A discontinuous pH gel system (Laemmli) was used with a 4% polyacrylamide stacking gel and a 5–15% separating gel. For immunoblotting experiments, the gels were run at 4°C. Prior to transfer, the gel was soaked in TBS, 5 mM CaCl2, 0.1% Tween 20, 2 mM CaCl2, and 10% fetal calf serum (Buffer A). Following blocking, the membranes were incubated with various antibodies diluted into buffer A. Antibody binding was detected with an anti-mouse alkaline phosphatase conjugate.

**RESULTS**

**Sequencing Analysis**—To determine the primary structure of the αM receptor, protein sequencing was performed. The receptor was purified by affinity chromatography, followed by anion exchange chromatography (8). By utilizing these procedures it is possible to obtain highly purified preparations that migrate as a single 420-kDa band upon SDS-PAGE (8).

The amino terminus of the receptor was blocked, and therefore, peptides were generated from this molecule by either chemical or proteolytic digestion. This resulted in the sequence of 10 peptides (Fig. 1). The identity of a total of 146 residues was determined. All resulting sequences were found at various locations throughout the cDNA-deduced sequence of LRP (16). Only 5 out of the 146 residues differed between the sequence deduced from cloning and that determined from chemical sequencing; the differences could represent either sequencing errors or polymorphisms. Overall, these findings provide convincing evidence that LRP and the αM receptor are the same molecule.

**Immunoprecipitation of the αM Receptor**—Hers et al. (21) examined the biosynthesis of LRP and found this molecule to be synthesized as a 600-kDa precursor that is cleaved in the Golgi to form a heavy and light chain, with approximate molecular masses of 500 and 85 kDa, respectively. These two chains are proposed to associate noncovalently to form the functional receptor (21). Immunoprecipitation experiments, using a monoclonal antibody, were employed to determine if both the 420- and 85-kDa components of the αM receptor co-precipitated.

The specificity of the monoclonal antibody was evaluated by immunoblotting. The results shown in Fig. 2 (lane 2) demonstrate that antibody 8G1 binds specifically to the 420-kDa polypeptide but not to the 85- or 39-kDa polypeptides, while two other monoclonal antibodies, 5A6 and 8B8, were selective for the 85-kDa polypeptide (Fig. 2, lanes 4 and 6). The immunoblotting results are also in agreement with solid phase binding experiments demonstrating that 8G1 binds selectively to microtiter wells coated with the 420-kDa polypeptide. In immunoprecipitation experiments, 8G1 precipitated not only the 420-kDa polypeptide but a second polypeptide with an apparent molecular mass of 85 kDa (Fig. 3). These data suggest that the 420- and 85-kDa components are associated by relatively strong non-covalent interactions, as has been suggested for LRP, and provide additional evidence that these two molecules are similar. Parallel immunoprecipitation of media fractions indicates that neither the 420- nor 85-kDa polypeptides are secreted.

**Interaction of the 39-kDa Polypeptide with the αM Receptor**—A third protein, with an apparent molecular mass of 39

![Fig. 1. Comparison of the sequence of peptides derived from the purified 420-kDa αM receptor with the corresponding sequences in LRP (16). Lys C peptides were generated by Lys C digestion of the reduced and alkylated receptor. Three of the peptides (CNBr 17/Lys C 29, CNBr 19/Glu C 2, and CNBr 19/Glu C 10) were first digested with CNBr, purified by HPLC, and further digested with Glu C or Lys C. Amino-terminal residues enclosed in parentheses represent sequences based on the method used to generate the peptide. The boxed residues represent differences between the chemical sequence and that deduced from the cDNA for LRP (16). An X indicates a cycle in which no amino acid could be identified. In the case of Lys C 39 (peptides 1 and 4), two sequences with similar yields were obtained. The yield from the first cycle for peptides 1–10 and the repetitive yield for each sequence run was: 1) 36 pmol, 85%; 2) 14 pmol, 94%; 3) 16 pmol, 90%; 4) 35 pmol, 93%; 5) 40 pmol, 86%; 6) 40 pmol, 88%; 7) 15 pmol, 88%; 8) 47 pmol, 89%; 9) 15 pmol, 91%; 10) 8 pmol, 94%.](http://www.jbc.org/)

![Fig. 2. Immunoblot of the polypeptides of the αM receptor.](http://www.jbc.org/)
to LRP. The sequences of a total of 10 peptides were obtained. These peptides were prepared from different preparations of the α2M receptor by either chemical or proteolytic cleavage, or both. The fact that all of the peptides that were sequenced are located within the deduced sequences of LRP strongly argues against the possibility that LRP represents a minor contaminant of the α2M receptor preparation. In addition to this structural evidence, biochemical analysis reveals additional similarities between LRP and the α2M receptor. This includes identification of an 85-kDa polypeptide that associates with the 420-kDa component of the α2M receptor during affinity purification and immunoprecipitation experiments. These results are consistent with the proposal that the α2M receptor, like LRP, consists of at least two polypeptides that are non-covalently associated to form a functional receptor. The relationship between these two polypeptides was established by Herz et al. (1990) who demonstrated that the 85-kDa polypeptide originates from the carboxyl-terminal region of a single chain precursor LRP molecule following cleavage in the Golgi during receptor processing. Interestingly, blotting experiments in the present investigation, using monoclonal antibodies specific for the 85-kDa component, suggest that the active α2M receptor (i.e., receptor eluted from the ligand affinity column) is present exclusively in the two-chain form. Whether or not the single chain form of the receptor retains the ability to bind to ligand remains to be determined. Discrepancies between our estimate of 420 kDa for the molecular mass of the heavy chain of the α2M receptor and the estimate of 500 kDa for the heavy chain of LRP are likely due to uncertainties in determining the mass of large proteins from their mobility in SDS-PAGE. Thus all of the biochemical evidence currently available indicates that the α2M receptor and LRP are the same molecule.

The present investigation has identified a third molecule that associates with this receptor. This polypeptide, which has an apparent molecular mass of 39 kDa, co-purifies with the α2M receptor during affinity chromatography and binds to the 420-kDa polypeptide of the α2M receptor with high affinity. This polypeptide would not be detected by immunoprecipitation of [35S]cysteine-labeled cell extracts due to the absence of cysteine residues within the protein. Whether this molecule represents a receptor subunit or an additional ligand for this receptor remains to be determined, and studies are currently under way to delineate the function of this unique molecule.

The apparent identity between LRP and the α2M receptor raises important questions about the ligand for this receptor. Ligand binding studies with the purified α2M receptor clearly establish that the activated form of α2M is a ligand. The interaction is of high affinity (Kd = 0.6-12 nM) (8-10), is specific for the activated form of α2M (8), depends upon calcium (8-10), and is inhibited when the pH is reduced to 5.0 (8-10) as expected for this interaction (22). Furthermore, cross-linking studies (9, 10) and purification of the heavy chain (8) of the α2M receptor provide additional evidence that the interaction between activated α2M and the receptor involves the larger 420-kDa polypeptide chain. It has also been suggested that LRP might function as a receptor for lipoproteins that contain apoE (23-26) or apo B (26). Indirect evidence for binding of LRP to lipoproteins that contain apoE comes from studies examining the incorporation of [14C]oleate into cholesterol [14C]oleate using a mutant fibroblast cell line that lacks the LDL receptor (23). These studies demonstrated that β-VLDL, enriched with apoE, stimulated such incorpora-

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ration and that the stimulation could be blocked by an antibody against LRP. However, it was not possible to measure directly the binding, uptake, and degradation of apoE-enriched 125I-labeled VLDL by these cells. More direct evidence for an interaction between apoE- and apoB-containing lipoproteins and LRP was derived from cross-linking studies (24) and ligand blotting studies (25, 26) which demonstrated a Ca2+-dependent binding of various lipoproteins to LRP immobilized on nitrocellulose. Thus, while it is not possible to estimate the affinity of these interactions from studies of this kind, there is considerable evidence for the interaction of certain lipoproteins with LRP.

It is likely that the ability of this receptor to bind to apparently structurally unrelated ligands is a unique feature of the “modular” structure of this molecule. LRP was originally identified (16) by screening a murine lymphocyte cDNA library with an oligonucleotide derived from the “class A cysteine-rich” motif of low density lipoprotein receptor. The complete amino acid sequence of LRP (16) reveals that the extracellular domain contains 31 copies of the “cysteine-rich” motif that is also found in the LDL receptor and terminal complement components, as well as 22 copies of repeats with homology to the epidermal growth factor precursor. The involvement of the various “modules” in binding to different ligands has been confirmed by an investigation of the regions on the LDL receptor responsible for binding to apolipoprotein E and apolipoprotein B (27). This analysis substantiated that these two ligands bind to different regions of LDL receptor. Interestingly, another receptor, the insulin-like growth factor II receptor, also binds to diverse biological ligands. The receptor, which contains 15 repeat sequences and a domain homologous to the type II repeat found in fibronectin, is identical for this receptor (i.e. insulin-like growth factor II and lysosomal enzymes containing phosphomannosyl residues) bind to different regions of the molecule (30). Thus, it appears that certain receptors are able to bind structurally diverse ligands through distinct binding sites on the molecule.

The apparent ability of the α2M receptor to bind distinct ligands also raises questions regarding the overall function of this receptor. Studies of the binding of α2M-proteinase complexes or methylamine-activated α2M to macrophages suggest that this binding regulates many cell functions such as protease secretion, antigen processing, and activation of the respiratory burst by phorbol esters (23-33). These observations suggest an expanded role for this receptor beyond its catabolic function to modulate levels of protease activity. It is conceivable that various ligands (e.g., α2M-proteinase complexes, lipoproteins) may have a concerted role in the process of modulating receptor function. For example, it is certainly possible that α2M may in some manner facilitate the binding of various lipoproteins to this receptor. In this regard, it is interesting to note that several vitamin K-dependent zymogens and proteinases, such as thrombin, have been reported to bind to VLDL (34, 35). The potential cooperative role that ligands may have in the process of modulating the levels of proteinases and lipoproteins and how the different functions of the receptor are integrated remain to be investigated.

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