Evidence that the newly cloned low-density-lipoprotein receptor related protein (LRP) is the α_2-macroglobulin receptor

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The human placental receptor (α_2MR) for α_2-macroglobulin-proteinase complexes contains 3 polypeptides of approx. 500 kDa, 85 kDa, and 40 kDa. N-terminal sequence analysis of the 500 kDa and 85 kDa polypeptides, analysis of a random selection of peptides covering 536 residues from these polypeptides, and analysis of a 1772 bp cDNA encoding part of the 500 kDa polypeptide provide evidence that the 500 kDa and 85 kDa chains are the α- and β-subunits, respectively, of a recently cloned hepatic membrane protein, termed the 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; Herz, J., Kowal, R.C., Goldstein, J.L. and Brown, M.S. (1990) EMBO J. 9, 1769–1776). N-terminal sequence analysis of the 40 kDa polypeptide shows that it is of distinct genetic origin. It is suggested that LRP is the functional receptor for α_2-macroglobulin-proteinase complexes (α_2MR) and in addition may have as yet unsettled functions in lipoprotein metabolism.

Low-density-lipoprotein receptor related protein; α_2-Macroglobulin; α_2-Macroglobulin receptor; Placenta; Liver; Membrane protein structure

1. INTRODUCTION

Herz et al. [1] recently cloned cDNA encoding a large novel human glycoprotein with structural features suggesting a function as a recycling lipoprotein receptor. The protein, designated LRP, is expressed in hepatocytes and several other cell types [1]. The 600 kDa LRP precursor is processed into a 85 kDa C-terminal membrane spanning β-subunit (601 residues) and a 515 kDa N-terminal α-subunit (3920 residues) remaining tightly noncovalently bound to the extracellular part of the β-subunit [2].

The tetrameric human α_2-macroglobulin (α_2M) reacts with and inhibits a wide variety of proteinases, and, as a result of the formation of stable complexes, the conformation of the α_2M subunits changes so that previously concealed receptor binding domains become exposed. α_2M and other αMs participate in controlling the activity of proteinases by functioning as 'clearing vehicles' for proteinases (reviewed in [3]).

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Abbreviations: LRP, low density lipoprotein receptor related protein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; α_2M, α_2-macroglobulin; αM, α-macroglobulin (collectively); α_2MR, α_2-macroglobulin receptor; SDS-PAGE, sodium dodecylsulfate polyacrylamide gel electrophoresis; HPLC, high-performance liquid chromatography; PVDF, polyvinylidenedifluoride.

The partial protein sequences and nucleotide sequences have been deposited at EMBL/GenBank, accession number X55077

α_2MR, the receptor for α_2M complexed with proteinase, has been characterized in several cell types, notably hepatocytes [4–6], and receptor-mediated endocytosis into these cells largely accounts for the rapid removal of complexes from the circulation [7–9]. α_2MR was recently isolated by affinity chromatography from rat liver [10] and human placenta membranes [11,12]. The preparations contained three components [10,12,13]: a species judged to migrate as a 420–440 kDa polypeptide in SDS-PAGE, but possibly larger [11] (this component will be referred to below as the 500 kDa chain of α_2MR); a set of species migrating as a diffuse band corresponding to 70–85 kDa [11] (the major component of this band will be referred to as the 85 kDa chain of α_2MR); and a species of 40 kDa. High affinity binding of Ca^{2+} to the 500 kDa chain results in a conformational change necessary for binding α_2M-proteinase [13]. Electron microscopic gold immunocytochemistry using monoclonal antibodies against the 500 kDa chain revealed that the majority of the receptors in fibroblasts and monocytes are in intracellular vesicles and tubular structures [14]. Within experimental error the amino acid composition of the 500 kDa α_2MR chain [13] is identical to that calculated from the sequence of the LRP α-subunit [1,2].

Here we provide evidence from partial sequence analysis (protein and DNA level) that the LRP α-subunit is the 500 kDa chain of α_2MR, and that the LRP β-subunit is the 85 kDa chain of α_2MR. The 40 kDa polypeptide is not related to LRP. The role of α_2MR for clearance of α_2M-proteinase complexes seems beyond doubt, whereas a possible role for bind-

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ing and clearance of lipoproteins is under debate [15–19]. We therefore suggest that the originally cloned protein functions as the receptor for α_2M-proteinase complexes and in addition may have as yet unsettled functions in lipoprotein metabolism.

2. MATERIALS AND METHODS

2.1. *Proteins*

Human placental α_2MR was isolated by affinity chromatography using immobilized methylamine-treated α_2M as described earlier [11].

2.2. *Peptide separation and protein sequence analysis*

Peptides were separated by reverse phase HPLC on columns of Nucleosil C18, 5 μm (4·250 mm) and Aquapore (2.1·200 mm) using 0.1% trifluoroacetic acid as the aqueous solvent, and gradient elution with ethanol or acetonitrile. Most peptide pools were rechromatographed using shallow gradients of organic solvent. Large CNBr fragments (>8 kDa) were separated by SDS-PAGE using standard 15% or 20% Tris/glycine gels and electroblotted onto PVDF membranes [20] (ProBlott, Applied Biosystems). Automated Edman degradation of 10–50 pmol samples was performed using an Applied Biosystems model 477A sequencer equipped with a model 120A on-line HPLC operated according to the manufacturer's instructions.

2.3. *Materials for cDNA cloning*

Restriction endonucleases and DNA modifying enzymes were purchased from Boehringer Mannheim. A Sequenase kit was from United States Biochemicals. mRNA purification kits were from Pharmacia, and cDNA synthesis and cloning kits were from Amersham. γ-[^{32}P]ATP was from ICN.

2.4. *Construction of cDNA library*

Total RNA was purified from freshly obtained placentas by the guanidine thiocyanate method [21], and poly(A)$^+$ mRNA was selected by oligo-dT-Sepharose chromatography. The poly(A)$^+$ selected mRNA was subjected to ultracentrifugation in a sucrose gradient, and RNA larger than approx. 4800 nucleotides was used in constructing a randomly primed cDNA library [22,23] according to the manufacturer's instructions.

2.5. *cDNA cloning and sequence analysis*

Degenerate oligo-deoxynucleotide probes corresponding to partial peptide sequences (6–8 residues) were synthesized on an Applied Biosystems model 381A DNA synthesizer and used for screening the cDNA library. Screening, colony purification, cDNA sequencing of restriction fragments and compilation of sequence data were performed as reported earlier [24]. From the probe based on the sequence Gln-Cys-Asp-Tyr-Asp-Asn-Asp-Cys several hybridizing colonies were identified, and one having an insert of approx. 1700 bp was further characterized. The cDNA sequence and the derived protein sequence were compared to sequences contained in the GenBank (release 23) and MIPSX (release 16) databases [25].

3. EXPERIMENTAL

For structural studies 800–1000 pmol α_2MR was freezedried, redissolved in 50% formic acid and subjected to gel chromatography on a 24-ml column of Superose 12 eluted with the same solvent. The 500 kDa chain eluted as a sharp peak followed by a broad peak containing the 85 kDa and 40 kDa species [13]. The material in each peak was pooled, freezedried, reduced with dithioerythritol, and alkylated with [^{14}C]iodoacetic acid. The *S*-carboxymethylated preparations were recovered by passage through a 6-ml Sephadex G-25F column eluted with 50% formic acid. Four digests were investigated. (i) Following treatment of the 500 kDa chain with CNBr the material was fractionated by Superose 12 gel chromatography using 50% formic acid as solvent. The large fragments were collected in two pools, separately digested with trypsin, and the peptides separated by HPLC. The small CNBr fragments were directly fractionated by HPLC. (ii) Peptides from a tryptic digest of the 500 kDa chain were initially fractionated on a Mono Q column (HR5/5) using gradient elution with ammoniumbicarbonate at pH 8.5 (10–500 mM). The effluent was collected in 25 pools, and the peptides in 14 of these were further separated by HPLC. (iii) A second CNBr digest of the 500 kDa chain was fractionated on Superose 12 as described above, but the effluent was collected in 8 pools. Two pools containing the majority of the large CNBr fragments were separated by SDS-PAGE and electroblotted. Three pools containing the small CNBr fragments were separated by HPLC. (iv) The pools from the initial separations containing the 70–85 kDa and 40 kDa species were either treated with CNBr and separated by SDS-PAGE or directly separated by SDS-PAGE followed by electroblotting. In the latter case the 85 kDa and the 70–75 kDa species were analyzed separately.

4. RESULTS

The SDS-PAGE pattern of the α_2MR preparation is shown in Fig. 1. The size of the largest chain of α_2MR is compatible with that of a 500 kDa species. The size of heavy chain of laminin has been estimated at 400 kDa or 440 kDa (cf. [10]), and 400 kDa was previously chosen as the reference value [10–13]. Thus, 420 kDa [12] or 440 kDa [10,11,13] previously published for the size of the large chain of α_2MR are likely to be underestimates. As judged from the staining intensities of the bands the 40 kDa peptide could be present in a 1:1 stoichiometry with the 500 kDa and the 85 kDa chains. Since ligand [10–12] and Ca^{2+} [13] binding activities are found in the 500 kDa chain this was initially selected for sequence studies.

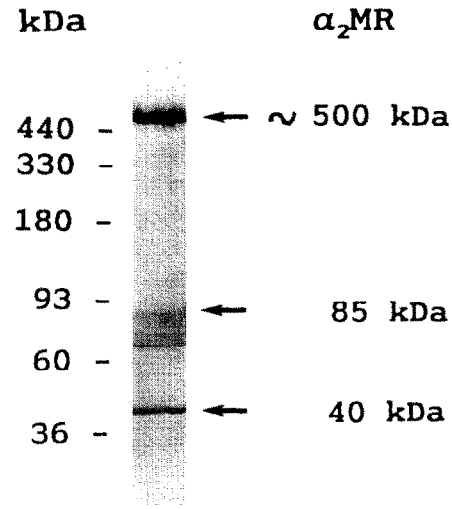

Fig. 1. SDS-PAGE of α_2MR purified by affinity chromatography. The gel contained 4–16% polyacrylamide and was run at reducing conditions. The size (kDa) and position for the following protein standards: lactate dehydrogenase (36), catalase (60), phosphorylase b (93), α_2M subunit (180), thyroglobulin (330), and laminin heavy chain (440) are indicated.

Table I

Stretches of amino acid sequence from α_2MR

Partial sequence	Position in proLRP sequence
AIDAPKTC[a]	1-8
GKVFFTDYGQIPKVERCD[b]	316-333
DGQNRTKLVDSKIV[c]	335-348
IPIENLM[b]	534-540
YWTDWEEDPKDSRRGRLERAW[b]	644-664
SERPPIFEIR[d]	765-774
YDAQQQQVGTNK[c]	776-787
CISKAWVCDGDND[e]	1099-1111
ELGPDNHTCQIQ[c]	1194-1205
EVLRGHEFLSHPF[c]	1451-1463
EIRGVDLDAPYYNYIISF[c]	1573-1590
VYWSDVR[d]	1610-1616
NLFWTSYDTNKK[d]	1652-1663
GDKLWWADQVSEK[b]	1777-1789
GLSTISRAKRDQTWREDV[c]	1923-1940
AITVHPEKGYLFWTEWGQYPR[d]	1993-2013
FSVSVFEDFIYWSD[c]	2080-2093
KNVIALAFDYRA[c]	2215-2226
IFFSDIHFGNIQQINDDGS[e]	2236-2254
FWTNWNEQHPSI[b]	2328-2339
RAALSGANVLTLIE[c]	2341-2354
GIIAVANDTN[c]	2447-2456
GVLFQPCERTSLCYAPSW[e]	2635-2652
TSLCYAPSWVCDGANDCGDY[e]	2644-2663
CIPMSWTCDKE[e]	2689-2699
CISKQWLCDGSDDCGDGSDEAA[e]	2727-2748
CQNRQCIPKHFVCD[c]	2806-2819
CLSSRQWECDGEND[e]	2851-2864
IYWTDVTTQG[b]	3052-3061
HLNGSNVQVL[c]	3068-3077
IYWADAREDYIEFASLDGSN[d]	3184-3203
DLHVFHALRQPDVP[c]	3258-3271
CIPGIFRCNGQDNCGDGEDE[e]	3407-3426
CIPARWKCDGEDD[e]	3488-3500
CVPGRWQCDYDNDCGDNSDEE[e]	3529-3549
FVCPPNRPFR[d]	3674-3683
KTHNTCKAEGSEYQVLY[b]	3836-3852
QIDRGV[f]	3925-3930
DGTLRETLVQDN[g]	4013-4024
YSREKNQPKP[h]	

[a]N-terminal sequence of *S*-[^{14}C]carboxymethylated 500 kDa chain. A des-Ala sequence was seen in equimolar yield. The Cys-residue was assigned from a 25% increase in radioactivity in steps 7 and 8.
[b]CNBr fragment from 500 kDa chain, purified by HPLC.
[c]CNBr fragment from 500 kDa chain, purified by SDS-PAGE.
[d]Tryptic peptide from a pool of large CNBr fragments originating from 500 kDa chain.
[e]Tryptic peptide from 500 kDa chain.
[f]N-terminal sequence of 85 kDa chain.
[g]Large CNBr fragment originating from pool containing 85 kDa and 40 kDa species.
[h]N-terminal sequence of 40 kDa species.

Partial sequences of a random selection of 36 peptides accounting for 524 residues from the 500 kDa chain are shown in Table I. In every case these sequences matched with segments located between residues 316 and 3852 in the amino acid sequence of proLRP [1] (Fig. 2). The 500 kDa chain of α_2MR contained two N-terminal sequences (8 residues determined) in approx. equimolar yield, the first corresponding to that predicted for proLRP [1], the second to its des-Ala sequence. In addition, the sequence of a 1772 bp cDNA hybridizing with a degenerate oligo-deoxynucleotide probe based on the peptide sequence Gln-Cys-Asp-Tyr-Asp-Asn-Asp-Cys (Fig. 2) revealed complete identity with nucleotides 10289–12060 of proLRP[1] [1].

No partial peptide sequences matching residues downstream of residue 3852 of proLRP were obtained from the α_2MR 500 kDa chain. However, sequenator analysis of the 85 kDa chain revealed a six-residue match starting from residue 3925 of proLRP (Table I). Furthermore, the N-terminal sequence of an approx. 40 kDa CNBr fragment obtained from the pool containing the 85 kDa and 40 kDa species matched with residues 4013–4024 of proLRP (Table I). Hence, the results of partial sequence analysis of α_2MR show that its 500 kDa chain as well as its 85 kDa chain are contained within the sequence of proLRP (Fig. 2). The random sequence data obtained from the large chain of α_2MR and the presence of approx. 17% carbohydrate [13] are in agreement with the size of at least 500 kDa.

Intracellular processing of proLRP occurs at the tetrabasic sequence -Arg-His-Arg-Arg (3921–3924) [2]. Since the amino acid composition of the 500 kDa chain [13] is in excellent agreement with that calculated for residues 1–3920 of LRP, we conclude that it most likely is identical with the 515 kDa α-subunit of LRP [2]. Although the sequence data of the 85 kDa chain of α_2MR containing the membrane spanning segment are less extensive, this chain is likely to be the β-subunit of LRP. The 85 kDa chain contains seven Asn-residues which can potentially by glycosylated. The smearing of the 85 kDa chain in SDS-PAGE (Fig. 1) indicates a heterogeneous pattern of glycosylation. As discussed in [12] the species of approx. 70–75 kDa, present in variable amounts, possibly results from a slight degradation of the 500 kDa chain, but their position could not be determined by sequence analysis. Fig. 2 shows a schematic representation of the domain structure of LRP [1,2] and the localization of the stretches of partial sequence determined in α_2MR.

The N-terminal sequence (10 residues determined) of the 40 kDa species in the α_2MR preparation (Table I) neither matched with any segment of LRP nor with protein sequences contained in the PIR/GenBank databases, indicating that it is a protein of distinct genetic origin.

5. DISCUSSION

In view of the results of sequence analysis, the 3 proteins in the affinity purified α_2MR complex are called α_2MR α-subunit, α_2MR β-subunit, and 40 kDa associated protein. These species are tightly associated in detergents such as cholamidopropyldiethyl am-

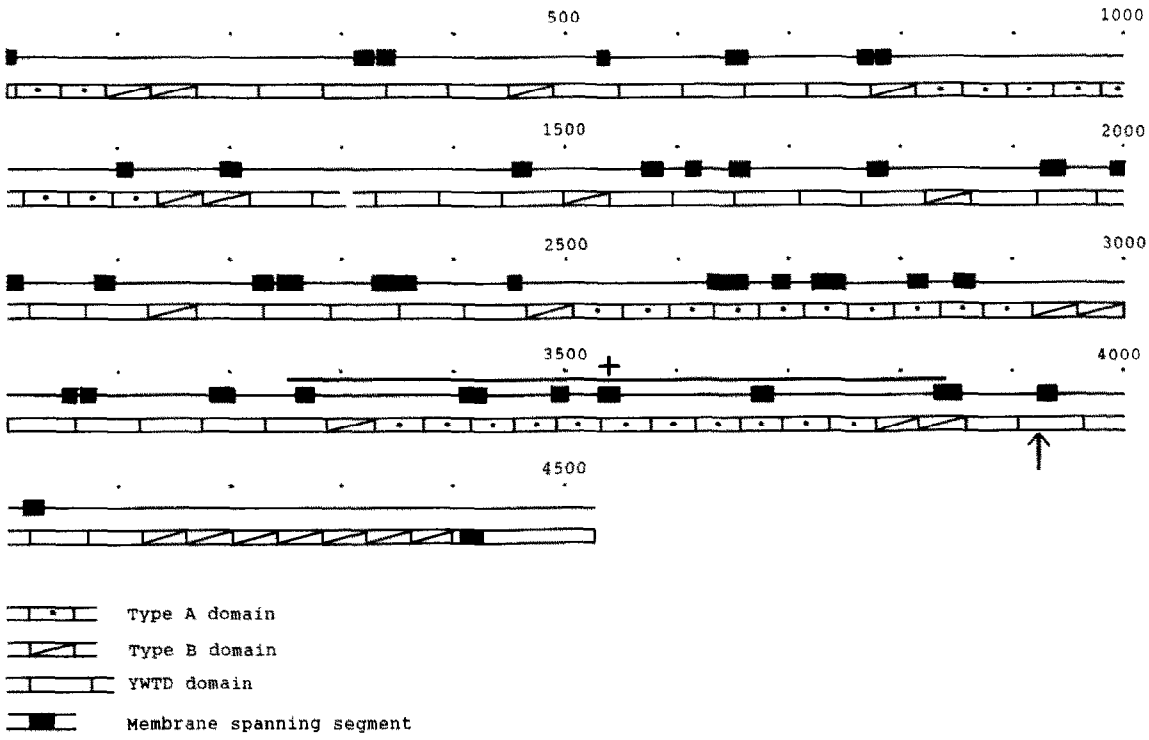

Fig. 2. Schematic representation of LRP/α_2MR. The model is modified from Refs [1,2] and shows the assembly in LRP/α_2MR of type A domains (also present in the LDL receptor and several complement proteins), type B domains (also present in the LDL receptor and epidermal growth factor precursor) and YWTD domains (also present in the LDL receptor). The membrane spanning segment and the site of processing (arrow) are indicated. The α-subunit contains residues 1–3920, and the β-subunit contains residues 3925–4525 of proLRP. The C-terminal approx. 100 residues of the β-subunit are located intracellularly. Stretches of partial amino acid sequence of α_2MR 500 kDa and 85 kDa chains matching stretches of LRP are indicated by solid boxes in the upper set of lines, and the part determined from a partial cDNA is indicated by a continuous line. The probe site is indicated by a cross.

moniopropane sulfonate or octyl-β-D-glucopyranoside, and separation may be achieved by high salt or SDS at low concentrations [10–12]. Previous work has shown that binding of α_2M-proteinase occurs to the α_2MR α-subunit [10–12]. Binding occurs with similar affinities to immobilized α_2MR complex and to α_2MR in intact cells and membranes [10,11,5,6]. In fact, binding profiles are indistinguishable when experiments are performed under comparable conditions (divalent binding to pairs of receptors at 4°C, $K_{diss.}$ approx. 40 pM (S.K.M. and J.G., unpublished)). Thus, α_2M-proteinase binds to the α-subunit which is non-covalently associated with the extracellular part of the membrane-anchored β-subunit.

The proposal that LRP functions as a receptor for lipoproteins was reviewed by Soutar [15] who concluded that LRP may have other functions as well, or even instead. Uptake of lipid from rabbit β-VLDL artificially enriched with human apolipoprotein E occurred into LDL receptor deficient human fibroblasts and was blocked by antibodies against proLRP [16]. The enriched β-VLDL showed Ca^{2+} dependent binding to LRP in a ligand blotting assay, and the binding was blocked by the inclusion of human apolipoprotein C [19]. Apolipoprotein E incorporated into liposomes has also been reported to bind to a receptor having the characteristics of LRP in human hepatic membranes, although in a Ca^{2+} independent way [18]. Others have found Ca^{2+} dependent, but apolipoprotein E independent binding of rabbit LDL to a rat hepatic receptor compatible with LRP [17]. Taken together with the structural features of LRP, the binding and uptake experiments, in spite of their apparent mutual inconsistencies, do suggest that LRP, besides being the functional receptor for α_2M-proteinase complexes, has as yet unsettled functions in lipoprotein metabolism.

Binding of apoliproteins B and E to the LDL receptor depends in part on a determinant containing the conserved sequence -Arg-X-X-Arg-Lys-Arg-X-X-Arg/Lys- located within residues 3345–3381, and 140–150, respectively, of the two proteins [26,27]. The receptor recognition site in α_2M and other αMs is located in the C-terminal 138-residue domain (residues 1314–1451) [28,29]. In view of the possibility that α_2MR may also bind and internalize lipoproteins we examined whether the sequence shown above is present in the αMs. The search proved negative; but, intriguingly, an 81-residue segment of apolipoprotein B (residues 3342–3422) aligned with residues 1314–1394 of human α_2M (18% identities); likewise a 68-residue segment of apolipopro-

tein E (residues 95–158) aligned with residues 1314–1372 of α_2M (17% identities). These alignments had the highest scores when the sequences of the entire proteins were compared, but their significance is presently unclear.

In conclusion we have provided evidence that LRP, thought to be a novel recycling lipoprotein receptor, is the receptor for α_2M-proteinase complexes. Its possible role in lipoprotein metabolism needs to be elucidated. The 40 kDa peptide in the α_2MR complex, not noticed in previous work on LRP [1,2], is of distinct genetic origin, and its possible role in the function of α_2MR for clearance of α_2M-proteinase complexes remains to be clarified.

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