Analysis of Ligand Recognition by the Purified \(\alpha_2\)-Macroglobulin Receptor (Low Density Lipoprotein Receptor-related Protein)

**EVIDENCE THAT HIGH AFFINITY OF \(\alpha_2\)-MACROGLOBULIN-PROTEASE COMPLEX IS ACHIEVED BY BINDING TO ADJACENT RECEPTORS**

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The molecular basis for binding of \(\alpha\)-macroglobulin-proteinase complexes to the human two-chain 500/85-kDa \((\alpha/\beta)\) \(\alpha_2\)-macroglobulin (\(\alpha_2\)M) receptor (\(\alpha_2\)MR)/low density lipoprotein receptor-related protein was analyzed. Ligand blotting experiments showed that a 40-kDa protein, present in the affinity-purified \(\alpha_2\)MR preparation, is bound to the \(\alpha_2\)M-\(\alpha\) chain and released by heparin. Removal of the 40-kDa protein resulted in a 3-5-fold increase in binding of \(\alpha_2\)M-trypsin.

Nitrocellulose-immobilized pure two-chain \(\alpha_2\)MR was incubated with human \(\alpha_2\)M-trypsin, containing four identical subunits, and two monovalent ligands: rat \(\alpha_1\)-inhibitor-3-chymotrypsin and the 18-kDa receptor binding fragment of the \(\alpha_2\)M subunit. Binding of \(\alpha_2\)M-trypsin to the \(\alpha\)-chain of immobilized \(\alpha_2\)MR was composed of a high \((K_d = 40 \text{ pm at } 4^\circ C)\) and a low \((K_d = 2 \text{ nm})\) affinity component, \(\alpha_1\)-Inhibitor-3-chymotrypsin bound to the same sites but with one component \((K_d = 0.4 \text{ nm})\). Competition-inhibition experiments and dissociation experiments, using ligands with different valences, as well as experiments with \(\alpha_2\)MR immobilized at different densities, led to the following model. The low \((K_d = 2 \text{ nm})\) affinity of \(\alpha_2\)M-proteinase is prevalent when only one of the four domains binds to \(\alpha_2\)MR, i.e. when the receptor density is low or when neighboring receptors are occupied. The high \((K_d = 40 \text{ pm})\) affinity is achieved by binding of at least two domains to adjacent receptors.

Human \(\alpha_2\)-macroglobulin (\(\alpha_2\)M) is a tetrameric 720-kDa plasma protein composed of four identical 180-kDa subunits. \(\alpha_2\)M is capable of inhibiting a wide variety of proteolytic enzymes (reviewed in Ref. 1) involved in a broad spectrum of biological processes such as fibrinolysis, clotting, digestion, immune response, and cancer. The proteolytic cleavage of the bait region induces a series of events leading to the formation of stable \(\alpha_2\M-proteinase complexes and the exposure of previously concealed receptor-binding domains, one in each of the four subunits. The reaction with small nucleophiles such as methylamine induces a similar conformational change. \(\alpha_2\M-proteinase complexes are rapidly removed from circulation, primarily by receptor-mediated endocytosis in hepatocytes (2-4). Cytokines and growth factors such as interleukin 1/3 (5), interleukin 2 (6), interleukin 6 (7), transforming growth factor-\(\beta\) (8), and fibroblast growth factor (9) bind to \(\alpha_2\)M-proteinase complexes (reviewed in Ref. 10), but the significance of these interactions is presently unclear (11).

\(\alpha_2\M receptors (\(\alpha_2\)MR) with apparent dissociation constants \((K_d)\) varying from 40 to 540 pm at 4 \(^\circ\)C have been described in several cell types such as monocyte-macrophages (12, 13), fibroblasts (14, 15), hepatocytes (3, 4, 16, 17), and syncytiotrophoblasts (18). Low affinity binding with an apparent \(K_d\) of 2-100 nm has also been reported (3, 18-21).

\(\alpha_2\)MR was recently purified by ligand affinity chromatography from rat liver (22) and human placenta (23, 24) as an approximately 500-kDa protein with associated components at 85 and 40 kDa. The 500-kDa protein contains the ligand binding site (22, 23) and high affinity Ca\(^{2+}\)-binding sites important for receptor conformation and ligand recognition (25). Sequence analyses of tryptic and cyanogen bromide fragments (26, 27) and of amino termini (27) of the 500- and 85-kDa components have recently revealed that \(\alpha_2\)MR is identical with the low density lipoprotein receptor-related protein presumed to be a receptor for chylomicron remnants and \(\beta\)-migrating very low density lipoproteins (28-31). Pulse-chase experiments with \("^{35}\)S)cysteine have shown that \(\alpha_2\)MR/low density lipoprotein receptor-related protein (here designated \(\alpha_2\)MR) is synthesized as an approximately 600-kDa precursor protein. This is cleaved in a trans-Golgi compartment to generate the two-chain molecule with the 85-kDa membrane-spanning \(\beta\)-chain anchoring the approximately 500-kDa \(\alpha\)-chain noncovalently (32). Amino-terminal sequencing of the 40-kDa protein has shown that it is of distinct genetic origin (27).

The purpose of the present study was to analyze binding of the 40-kDa protein to \(\alpha_2\)MR and to elucidate how binding of \(\alpha_2\M-proteinase to the two-chain \(\alpha_2\)MR could result in both high and low affinity binding. We performed binding experiments using \(\alpha_2\)MR immobilized onto nitrocellulose at different densities and the following ligands: human \(\alpha_2\M-trypsin, containing four binding domains; \(\alpha_1\)-inhibitor-3-chymotrypsin (\(\alpha_13\)-chymotrypsin), a rat homologue of the human \(\alpha_2\M-monomer containing one binding domain (33-35); and the 18-kDa carboxyl-terminal fragment of the \(\alpha_2\)M-subunit containing the receptor recognition domain (36, 37).

**EXPERIMENTAL PROCEDURES**

**Preparation of Macroglobulins—**Human \(\alpha_2\)M was prepared from pooled citrate plasma using Zn\(^{2+}\) chelate-affinity chromatography as...
earlier described in detail (38). Purification of the carboxyl-terminal α₁M fragment containing the receptor domain was carried out by papain digestion of methylamine-treated α₁M (37). Rat α₁I₅ was prepared from pooled EDTA plasma from 200-g male Wistar rats as described (34, 39). The ligands were iodinated with 1 mol of [¹²⁵I]Iodogen (Pierce, Rockford, IL) to 250-500 kDa α₁M subunits, as described (3). α₁I₅ was reacted with 0.2 M of methylene for 2 h at 20 °C, dialyzed, and complexed with chymotrypsin ( Worthington) by incubation of ligand and proteinase at the ratio 1:1 for 2 min at 20 °C. The enzyme activity was stopped by adding phenylmethylsulfonyl fluoride to a final concentration of 1 mM.

Purification of the carboxyl-terminal tr,M fragment containing the receptor recognition domain was carried out, as described (26). The purified proteins were iodinated according to the chloramine-T method, as previously described (3, 22). Triton was reacted with 0.2 M of methylene sulfonate to a final concentration of 1 μM of proteinase K, at 0°C for 16 h. The antibody-Sepharose CL-6B (Pharmacia LKB Biotechnology Inc., Sweden) was further purified by shaking incubation for 16 h at 4 °C, with 50 μg of heparin-Sepharose, as described (3). The purified proteins were iodinated according to the chloramine-T method, as previously described (3, 22). Triton X-114 phase separation of receptor subunits and the 40-kDa protein was performed essentially by the method of Bordier (40). The protein components were treated with 0.5% SDS for 10 min at 20 °C immediately prior to phase separation in 5 volumes of 1% Triton X-114.

Preparation of Polyclonal and Monoclonal Antibodies—Polyclonal rabbit antibodies against the α₁MR and 40-kDa protein were raised by immunizing a rabbit with 0.1 mg of α₁MR in 50 μl of Freund’s incomplete adjuvant with 4-week intervals (2-week interval for the first 8 weeks). For production of monoclonal antibodies, mouse myeloma cells (NS-1) were fused with spleen cells from mice immunized with affinity-purified human α₁MR, as described earlier (41). Screening for positive clones was carried out after 7 days by an indirect enzyme-linked immunosorbent assay. Positive clones were isolated twice by limiting dilution. Class and subclass specificity of antibodies were determined using an anti-mouse antibody typer kit (Bio-Rad, 172–2055). The novel antibodies A2MR-J₁ (against α₁MR β-chain) and S4-D5 (against the 40-kDa protein) belonged to the IgG₁ subclass and IgM class, respectively. The IgG antibody, A2MR-A₂, against the α₁MR α-chain has previously been described as A2MR-2 (25). The 40-kDa protein was purified by affinity chromatography according to the instructions by the manufacturer (Pharmacia) of protein G-Sepharose. Neither the monoclonal antibodies directed against the α₁-chain nor the β-chain interfered with binding of α₁M-protein (Ref. 41, data not shown).

Measurement of α₁MR and the 40-kDa Associated Protein—Protein concentrations were determined by measuring absorbance at 280 nm (25, 26) and by the following indirect double-layer (“sandwich”) enzyme-linked immunosorbent assay. Microtiter wells from NUNC (Denmark) were coated for 16 h at 4 °C with 100 μl of polyclonal rabbit anti-α₁MR antibodies (40 mg of IgG/ml in 50 mM NaHCO₃, pH 9.5) digested against the affinity-purified α₁MR preparation. After washing three times and blocking with 0.2% nonfat milk, α₁MR preparation was applied at varying concentrations. The different constitutents in the α₁MR preparation were then detected with mouse monoclonal antibodies (A2MR-R₂, A2MR-R₁, or S4-D5, 2 μg/ml) and peroxidase-conjugated rabbit anti-mouse antibody (diluted 1:1000 from Pierce, Rockford, IL). The incubations with antibodies and antigen were performed as described earlier (3). For immunoprecipitation, the sample buffer for SDS-PAGE.
Fig. 2. Separation of the constituents in the ligand affinity-purified α₂MR preparation. Lane 1, nonreducing SDS-PAGE of the ¹²⁵I-α₂MR preparation after precipitation with the α-chain-specific monoclonal antibody, A2MRα-2. Lanes 2 and 3, hydrophilic phase (lane 2) and detergent phase (lane 3) after Triton X-114 (1%) precipitation of SDS (0.2%)-treated ¹²⁵I-α₂MR preparation. Lane 4, 100 pm ¹²⁵I-α₂MR preparation incubated for 16 h at 4°C with 25 mg of heparin-Sepharose CL-6B/ml. Lane 5, α₂MR preparation was applied to a Mono-S cation exchange column; the 40-kDa protein retained on the column was eluted by 2 M NaCl, 100 mM NaHCO₃, pH 10.5, iodinated, and applied to the gel.

preparation using A2MRα-2. The coprecipitation of the constituents confirms the tight binding between two-chain α₂MR and the 40-kDa associated protein. Lanes 2 and 3 show that the membrane-spanning β-chain, in contrast to the α-chain, was extracted by Triton X-114, whereas the 40-kDa protein was not, indicating a hydrophilic nature for this molecule. The 40-kDa protein was removed from the α₂MR preparation by incubation with heparin-Sepharose (lane 4). Control experiments with incubation of Sepharose alone showed no removal (data not shown). Lane 5 shows purification of the 40-kDa protein using retention on a Mono-S cation exchange column, followed by elution at high salt. Two-chain α₂MR was retained on an Mono-Q anion exchange column (data not shown).

Fig. 3, lane 1, shows by ligand blotting technique that the α-chain bound ¹²⁵I-labeled 40-kDa protein. Addition of heparin completely abolished this binding (lane 2), whereas EDTA had no effect (lane 3). This is in contrast to the complete inhibition of the Ca²⁺-dependent α₅M-trypsin binding by EDTA (25). Reduced α₂MR did not bind the 40-kDa protein (lane 4).

The question arose whether the 40-kDa protein might interfere with α₅M-proteinase binding to the α₂MR α-chain. Fig. 4 shows that its removal from the α₂MR preparation with heparin-Sepharose resulted in a high increase in binding of 5 pm ¹²⁵I-α₅M-trypsin. In four experiments, using different α₂MR preparations, binding of ¹²⁵I-α₅M-trypsin was increased 3–5-fold. Incubation of the pure two-chain α₂MR with 40-kDa protein reduced ¹²⁵I-α₅M-trypsin binding by up to 90% with half-maximal inhibition at approximately 200 pm, and heparin abolished this inhibition. Conversely, α₅M-trypsin (200 nm) reduced the binding of ¹²⁵I-labeled 40-kDa protein, although by only about 50% (data not shown). Thus, the 40-kDa protein is a ligand of the α-chain that clearly interferes with binding of α₅M-proteinase. The affinity-purified α₂MR preparation is, to a large extent, occupied with the 40-kDa associated protein. The experiments described below were therefore performed using α₂MR preparations further purified by incubation with heparin-Sepharose and essentially devoid of the 40-kDa associated protein (cf. Fig. 2, lane 4).

Properties of the Ligands—Fig. 5, lane 1, shows that α₅M-trypsin migrates both as a dimeric (approximately 360 kDa) and a tetrameric species when subjected to nonreducing SDS-PAGE. The reason is that the proteinase molecules establish covalent cross-links between lysine residues of the proteinase and glutamyl residues of the cleaved α₅M thiol esters, either within or between α₅M half-molecules (42). α₅M-trypsin migrates as a single band in nondenaturating gels (43). Moreover, electron microscopic examination of the present preparation demonstrated a homogeneous population of the typical H-shaped complexes (data not shown). Thus, the α₅M-trypsin complexes present in the incubation buffers all had four receptor-binding domains. However, in view of the shape of the complex and the location of the receptor-binding domains at the tip of each "leg" (44), it seems likely that α₅M-trypsin is functionally davalent when interacting with receptors on a
cell surface or immobilized onto nitrocellulose. Lane 2 shows SDS-PAGE of the 200-kDa α1L-chymotrypsin complex. This is homologous to the α2M subunit and has one binding domain. Lane 3 shows the 18-kDa carboxyl-terminal fragment of α2M previously shown to possess receptor binding activity (36, 37), i.e. a monovalent analogue. The slightly faster migrating band is due to carbohydrate heterogeneity (42). Receptor Binding Affinities of α1L-Chymotrypsin and α2M-Trypsin—Fig. 6 shows the Scatchard transformations of binding of α1L-chymotrypsin and α2M-trypsin, respectively, to nitrocellulose-immobilized two-chain α2MR. The curve representing α1L-chymotrypsin binding is linear, in agreement with binding to a single class of receptors, whereas the curve representing α2M-trypsin binding is concave upward, giving the illusion of at least two classes of receptors or negative cooperativity. The intercepts with the abscissa show binding of about 0.6-0.7 mol of ligand molecule/mol of receptor. The dissociation constant (Kd) for binding of α1L-chymotrypsin was determined as 440 ± 65 pM in five experiments. By fitting a two-receptor model to the data for α2M-trypsin, the apparent Kd values were calculated to 37 ± 9 pM and 2.2 ± 0.4 nM (n = 5). Indistinguishable results were obtained when α2M-chymotrypsin or α2M-methylamine were used as ligands (data not shown). Results closely similar to those shown in Fig. 6 were obtained when measurements were performed with α2MR bound to immobilized monoclonal antibody A2MRa-2 and when experiments were performed using placental membrane fractions (data not shown).

Fig. 7A shows that α2M-trypsin at high concentrations inhibited binding of 10 pM 125I-α1L-chymotrypsin with half-maximal inhibition at 2 nM, i.e. equivalent to Kd for the apparent low affinity α2M-trypsin binding. The 18-kDa fragment inhibited binding of 125I-α1L-chymotrypsin according to a one-receptor model, and about 100 nM was required for half-maximal inhibition. Fig. 7B shows that 0.4 nM α2M-trypsin (or 100 nM 18-kDa fragment) was required to inhibit binding of 5 pM 125I-α2M-trypsin half-maximally.

Dissociation of α1L-Chymotrypsin and α2M-Trypsin—Dissociation of prebound 125I-α1L-chymotrypsin followed a single exponential curve (0-7 h) in the absence or presence of unlabeled ligands at large concentrations (Fig. 8A). On the other hand, most of the bound 125I-α2M-trypsin dissociated very slowly in the absence of unlabeled ligand, and the process was markedly accelerated by excess unlabeled ligand (Fig. 8B). Experiments with placental membranes gave similar results. Dissociation of α2M-trypsin was markedly accelerated, whereas dissociation of α1L-chymotrypsin was not (data not shown).

**FIG. 6.** Concentration dependencies of α1L-chymotrypsin and α2M-trypsin binding to immobilized α2MR. The data are plotted according to Scatchard. 125I-α1L-chymotrypsin (○) or 125I-α2M-trypsin (●) were incubated for 16 h at 4°C with 125 pM immobilized α2MR and homologous unlabeled ligand. The points represent mean values of triplicates. The curve representing α2M-trypsin is the best least squares fit to a two-receptor model, and the curve representing α1L-chymotrypsin is the fit to a one-receptor model. The intercepts at 75-85 pM show that 60-70% of the immobilized receptors are capable of binding α2-macroglobulin-proteinase.

**FIG. 7.** Competition of α1L-chymotrypsin, α2M-trypsin, and receptor binding fragment of α2M. A, displacement of 10 pM 125I-α1L-chymotrypsin bound to 125 pM α2MR by unlabeled α1L-chymotrypsin (○), α2M-trypsin (●), and the 18-kDa carboxyl-terminal α2M fragment (●). The result of Scatchard analysis of the latter curve was compatible with binding to one receptor B, displacement of 5 pM 125I-α2M-trypsin bound to 125 pM α2MR by unlabeled α2M-trypsin (○), α1L-chymotrypsin (●), and the 18-kDa carboxyl-terminal α2M fragment (●). Values are means of four replicates.

**DISCUSSION**

The present data show that the 40-kDa molecule is a heparin-binding protein that binds to the α2MR α-chain and inhibits binding of 5 pM 125I-α2M-trypsin by up to 90%. Ligand (α2M-methylamine) affinity-purified α2MR preparations contained enough associated 40-kDa protein to reduce α2M-trypsin binding by up to 80%. The nature of the mutual interaction between binding of the two ligands remains to be elucidated; they may interact with overlapping sites or binding of the 40-kDa protein may change the overall affinity of α2MR to α2M-proteinase by an allosteric mechanism.
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**Fig. 8.** Dissociation of \( {}^{125}\text{I}-\alpha_1\text{-chymotrypsin} \) and \( {}^{125}\text{I}-\alpha_2\text{M-trypsin} \). A, dissociation at 4 °C of \( {}^{125}\text{I}-\alpha_1\text{-chymotrypsin} \) bound to 125 pm immobilized \( \alpha_2\text{MR} \) in buffer without (\( \Delta \)) or with 100 nM \( \alpha_1\text{-chymotrypsin} \) (\( \bigcirc \)) or \( \alpha_2\text{M-trypsin} \) (\( \bigcirc \)). B, dissociation of prebound \( {}^{125}\text{I}-\alpha_2\text{M-trypsin} \) without (\( \bigcirc \)) or with 100 nM \( \alpha_1\text{-chymotrypsin} \) (\( \square \)), \( \alpha_2\text{-chymotrypsin} \) (\( \triangle \)), or 10 \( \mu \)M 10-kDa \( \alpha_2\text{M} \) fragment (\( \Box \)). B, bound at time \( t \); \( B_0 \), bound at time 0. The insets are linearized values of the data from 0–7 h. Mean values of triplicates.

**Fig. 9.** Binding of \( {}^{125}\text{I}-\alpha_1\text{-chymotrypsin} \) and \( {}^{125}\text{I}-\alpha_2\text{M-trypsin} \) to \( \alpha_2\text{MR} \) immobilized at varying densities. Receptor (4-ml solution containing 0–80 fmol) was immobilized to nitrocellulose to give the indicated \( \alpha_2\text{MR} \) concentrations and incubated in 200 ml with 5 pm \( {}^{125}\text{I}-\alpha_2\text{M-trypsin} \) (\( \bigcirc \)) or 10 pm \( \alpha_2\text{-chymotrypsin} \) (\( \bigcirc \)) at 4 °C. Values are means of triplicates.

The function of the 40-kDa protein is presently not known. Two-dimensional gel electrophoresis and immunoblotting of cell lysates have revealed\(^2\) that the monoclonal antibody, S4-D5, cross-reacts with another heparin-binding protein, a 70-kDa nuclear protein that recognizes the 3' splice site of premessenger RNA (47). Electron microscopic immunocytochemistry has shown antigen recognition in the nucleus and cytoplasm, but not on the cell surface.\(^2\) Thus, the 40-kDa protein may have functions normally unrelated to the \( \alpha_2\text{MR} \) and may be associated with the \( \alpha \)-chain in the process of \( \alpha_2\text{MR} \) purification.

The heparin-releasable association of the 40-kDa protein is interesting in view of the reported binding of apolipoprotein E-rich lipoproteins and liposomes to \( \alpha_2\text{MR} \) (under the name low density lipoprotein receptor-related protein (29–31)). Clusters of basic amino acids in apolipoprotein E (residues 142–158) are essential for heparin-releasable binding to the cysteine-rich repeats of the low density lipoprotein receptor (48), which is highly homologous to the cysteine-rich repeats of \( \alpha_2\text{MR} \) (28). Thus, the 40-kDa protein and apolipoprotein E-rich lipoproteins might share binding domains in the \( \alpha_2\text{MR} \).

The present results provide the first data for binding of \( \alpha\text{M-proteinase} \) complexes to two-chain \( \alpha_2\text{MR} \) without interference of other proteins. The distinctive high affinity binding of tetrameric \( \alpha_2\text{M-proteinase} \) is explained according to the model shown in Fig. 10 A–C. Due to the H-shaped structure of the complex, dimeric binding of \( \alpha_2\text{M-proteinase} \) seems most likely, but binding of some complexes to more than two receptors cannot be excluded. It is important to note that the monomeric \( \alpha_1\text{-chymotrypsin} \) (Fig. 10A) binds according to a “simple” model, i.e., a linear Scatchard plot with a stoichiometry indicating that most of the receptors are in a conformation capable of binding the ligand (see Fig. 6 and its

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\(^2\) S. K. Moestrup, J. Celis, E. I. Christensen, and K. Kaltoft, manuscript in preparation.
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Legend, dissociation following an exponential course with or without excess unlabeled ligand in the medium (Fig. 8A), and binding proportional with the receptor density (Fig. 9). These are necessary premises for drawing conclusions from the more complicated binding of α₂-M-trypsin. The upward concave Scatchard plot (Fig. 6) is explained as the result of primarily dimeric and, thus, high affinity binding at low occupancies and primarily monomeric and low affinity binding at high occupancies. The acceleration of 125I-labeled α₂M-trypsin dissociation in the presence of excess monomeric or dimeric ligand (Fig. 8B) is explained as the result of frequent dissociation of either one of the two legs, followed by binding of unlabeled ligand to the exposed receptor; the labeled molecule is now bound in the low affinity form and will dissociate rapidly. The low binding of α₂M-proteinase at low receptor density (Fig. 9) is explained as the result of infrequent occurrence (Fig. 10B) of pairs of receptors capable of binding two legs (Fig. 10C). Finally, the model is also supported by the finding that α₂M-trypsin is a poor competitor of α₁I-chymotrypsin binding (Fig. 1A), i.e. corresponding to its low affinity constant.

The findings that the 40-kDa protein interferes with α₂M-trypsin binding and that the overall affinity depends on the density of immobilized α₂MR could explain previously reported apparent affinities for α₂M-proteinase in the nanomolar range (24) and around 500 pm (22, 23). The dimeric binding model also helps to explain previous observations with the receptor binding domain (18-kDa fragments) of α₁I-chymotrypsin. Enghlid et al. (49) reported a Kᵦ for the α₁I-derived fragment of 10 nM, i.e. an affinity of 2-3% of that of the native molecule (Fig. 6). The Kᵦ for the α₂M-derived fragment is about 100 nM (Fig. 7) (37, 49), i.e. about 2% of the affinity of α₂M-proteinase when binding in the monomeric mode. Thus, the higher intrinsic affinity of α₁I-chymotrypsin appears to be reflected in its receptor-binding domain.

When α₂MR is in a solubilized state, α₂M-methylamine should theoretically not be able to gain the affinity advantage of divalent binding characteristic of α₂MR immobilized to a solid support. α₁I-chymotrypsin should therefore be more suitable for affinity chromatography due to its higher intrinsic affinity. Accordingly, we have found that the receptor output from a α₁I-chymotrypsin-Sepharose column was more than twice as high as that from a parallel column with the same amount of immobilized α₂M-methylamine. The dimeric high affinity mode of α₂M-trypsin binding is important also in cell membranes, as judged from the nearly identical Kᵦ values obtained when using human placental membranes. Moreover, high and low affinity binding of α₂M-trypsin (3) and a relatively poor competition of α₂M-trypsin with 125I-α₁I-chymotrypsin (34) have been reported in rat hepatocytes. The dimeric binding mode requires a high density of receptors, and electron microscopic gold immunocytochemistry has revealed high concentrations of α₂MR in coated pits of human fibroblasts (41). Thus, the high affinity may be a mechanism for modulating clearance of α₂M-proteinase complexes that may bind preferentially to receptors located in coated pits.

The dimeric mode of binding resembles that of IgG molecules obtaining a "bonus" of high affinity through binding of the two Fab fragments to two pairs of surface antigens (50). It is also known that interleukin-2 obtains a high affinity through binding to different neighboring receptors. Tac and non-Tac proteins (51). The α₂M-proteinase complex is an unusual ligand, with four exposed receptor binding domains, at least two of which are capable of binding to receptors suitably distributed at the cell surface. Its dimeric binding is the basis for nonlinear Scatchard plots and for ligand-induced accelerated dissociation. This phenomenon is frequently observed in receptor biology and explained as negative cooperativity among receptors. It seems possible that cobinding of two (or more) sites at one ligand to distinct sites at cell surface molecules may explain accelerated dissociation in other receptor-binding systems.

In conclusion, we have provided evidence that α₂M-proteinase can bind both with high and low affinity to α₂MR; high affinity when binding is dimeric to adjacent receptors, and low affinity when binding is monomeric due to scattered receptors or high receptor occupancy.

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