A 39-kDa protein binds to the low density lipoprotein receptor-related protein/α₃-macroglobulin receptor (LRP/α₃MR) and inhibits the binding of ligands to this receptor. We recently reported that inhibition of tissue-type plasminogen activator binding to LRP/α₃MR is mediated by both amino-terminal and carboxyl-terminal regions of the 39-kDa protein, whereas inhibition of α₃-macroglobulin-proteinase binding is mediated only by amino-terminal regions. In this report we show that amino-terminal and carboxyl-terminal regions of the 39-kDa protein bind specifically and with high affinity to LRP/α₃MR on rat hepatoma MH MC₁ cells. Following binding, these amino-terminal and carboxyl-terminal regions of the 39-kDa protein are each rapidly endocytosed and degraded with kinetics identical to the full-length 39-kDa protein. Competition binding experiments with these constructs demonstrate that amino-terminal and carboxyl-terminal regions of the 39-kDa protein compete with one another for binding to LRP/α₃MR. A model is proposed in which amino-terminal and carboxyl-terminal regions of the 39-kDa protein bind to different sites on LRP/α₃MR in order to inhibit ligand binding.

The low density lipoprotein receptor-related protein/α₃-macroglobulin receptor (LRP/α₃MR) binds a diverse array of ligands including α₃-macroglobulin-proteinase (α₃M*) (1, 2), apolipoprotein E-enriched βVLDL (3–6), lipoprotein lipase (7), lactoferrin (8), urokinase-type plasminogen activator (u-PA); plasminogen activator inhibitor type-1 (PAI-1) complexes (9), tissue-type plasminogen activator (t-PA), and t-PA:PAI-1 complexes (10–12), *Pseudomonas* exotoxin A (13), and a 39-kDa heparin-binding protein that copurifies with LRP/α₃MR (14, 15). This 39-kDa protein, also termed receptor-associated protein (RAP), blocks the binding and/or uptake of all known ligands to LRP/α₃MR (1, 10–13, 16, 17). LRP/α₃MR is a widely expressed plasma membrane glycoprotein of 4525 amino acids that structurally resembles four LDL-receptor molecules arranged in series. The domain structure of LRP/α₃MR includes 22 epidermal growth factor repeats and 31 cysteine-rich ligand-binding complement-type repeats (18). The 31 complement-type repeats are organized into four clusters of 2, 8, 10, and 11 repeats. Recently the binding of RAP, α₃-macroglobulin light chain, and u-PA:PAI-1 complexes was identified within a 624-amino acid region of LRP/α₃MR containing a cluster of 8 complement-type repeats (19). It seems likely that α₃-macroglobulin light chain and u-PA:PAI-1 complexes bind to different sites within this 624-amino acid region because LRP/α₃MR-specific ligands have been demonstrated to not cross-compete for binding (8, 10). The mechanism of RAP inhibition of α₃-macroglobulin light chain and u-PA:PAI-1 binding within this 624-amino acid region has not been elucidated.

We recently utilized glutathione S-transferase (GST)-fusion proteins encoding different regions of the 39-kDa protein to demonstrate that multiple regions on the 39-kDa protein can interact with LRP/α₃MR to inhibit ligand binding (20). GST-fusion proteins encoding amino- and carboxyl-terminal regions of the 39-kDa protein inhibited t-PA binding to LRP/α₃MR on rat hepatoma MH MC₁ cells, whereas only amino-terminal constructs inhibited α₃M* binding. Inhibition of t-PA and α₃M* binding to LRP/α₃MR required residues 18–24 and 100–107 within amino-terminal constructs. Inhibition of t-PA binding by carboxyl-terminal constructs required residues 200–225 and 311–319 of the 39-kDa protein (20).

The purpose of the present study was to examine the mechanism by which amino- and carboxyl-terminal regions of the 39-kDa protein interact with LRP/α₃MR on MH MC₁ cells to regulate ligand binding. We found that residues 1–114, 115–319, and 200–319 of the 39-kDa protein, generated as fusion proteins with GST, behaved identically to the full-length protein, GST/1–319, in terms of binding, uptake, and degradation by MH MC₁ cells. We also found that amino- and carboxyl-terminal constructs competed with one another for binding to LRP/α₃MR. We propose that amino- and carboxyl-terminal regions of the 39-kDa protein bind to separate sites on LRP/α₃MR to inhibit ligand binding.

**EXPERIMENTAL PROCEDURES**

**Materials**—Carrier-free sodium [¹²⁹]iodide [¹²⁹]Iodide was purchased from Amersham Corp. IODO-GEN was from Pierce Chemical Co. Pronase was obtained from Calbiochem.

**Cloning, Expression, and Purification of 39-kDa Protein Constructs**—The GST-fusion proteins encoding residues 1–114, 12–107, 15–114, 115–319, 151–319, 200–319, and 1–319 of the 39-kDa protein were prepared and purified as described previously (20).

**Protein Iodination**—Recombinant GST-39-kDa fusion proteins (50 μg) were labeled with [¹²⁹]Iodide using the IODO-GEN method as
described previously (21). Specific activities were generally 2-10 pCi/μg of protein.

Ligand Binding Assays—Rat hepatoma MHIC1 cells were cultured as described previously (21). MHIC1 cells were seeded into multwell (12 wells/plate) culture plates 2 days before the assay. Ligand binding buffer was phosphate-buffered saline containing 1 mM CaCl2 and 0.5 mM MgCl2 (PBSc). Cell monolayers were washed three times with 4 °C PBSc. Binding was initiated by adding 0.5 ml of PBSc containing the indicated 125I-labeled ligand in the absence or presence of competitor protein. After incubation at 4 °C for 2 h, unbound ligand was removed by washing the cells three times with PBSc. Cells were lysed in 62.5 mM Tris-HCl, pH 6.8, containing 2% (w/v) SDS and 10% (v/v) glycerol. Radioactivity of cell lysates was quantified by γ scintillation spectrometry (Packard model C5304). Nonspecific binding was determined in the presence of excess unlabeled ligand.

Ligand Uptake, Internalization, and Degradation—MHIC1 cells were cultured as described above. MHIC1 cells were seeded into 6-well dishes and 125I-GST/1-114 (5 nm), 125I-GST/200-319 (8 nm), or 125I-GST/1-319 (5 nm) was allowed to bind for 2 h at 4 °C. Nonspecific binding (less than 0.5% total binding) was determined in the presence of excess unlabeled ligand (500 nm GST/1-114, 300 nm GST/115-319, 500 nm GST/200-319, 500 nm 125I-GST/1-319). After binding, cells were washed three times with 4 °C PBSc to remove unbound ligand. To initiate uptake, cells were warmed rapidly to 37 °C by adding prewarmed PBSc containing 200 μM unlabeled ligand. Following incubation at 37 °C for selected intervals, the overlaying medium was removed and precipitated by the addition of bovine serum albumin to 10 mg/ml and trichloroacetic acid to 20%. The cell monolayers were washed three times with 4 °C PBSc and incubated with PBSc containing 0.25% Pronase for 30 min at 4 °C. Cells were detached from the dishes by gentle pipetting and separated from the buffer by centrifugation. Radioactivity of cell pellets (defining internalized ligand) and supernatant fractions (defining cell surface radioactivity) were determined separately. Degradation of ligand was defined as the appearance of radioactive ligand fragments in the overlaying media that were soluble in trichloroacetic acid.

RESULTS

Specific Binding of 125I-GST-39-kDa Protein Constructs to MHIC1 Cells—We previously demonstrated that GST-fusion protein constructs encoding residues 1-114 (GST/1-114), 115-319 (GST/115-319), and 200-319 (GST/200-319) of the 39-kDa protein bound to purified LRP/α2MR immobilized on nitrocellulose (20). To characterize the binding of these constructs to LRP/α2MR on rat hepatoma MHIC1 cells, saturation binding experiments were performed. As shown in Fig. 1, the 125I-radiolabeled constructs GST/1-114 (Fig. 1A), GST/115-319 (Fig. 1B), and GST/200-319 (Fig. 1C), as well as the full-length protein, GST/1-319 (Fig. 1D), each bound specifically to MHIC1 cells over the concentration ranges of 1-30 nm. Saturation of specific binding was observed at concentrations in excess of 12 nm for each of the radiolabeled proteins. Scatchard (22) analysis of the binding data (insets of Fig. 1) yielded equilibrium dissociation constants (Kd) of 5.2, 6.9, 15, and 15 nm with 6.2 x 106, 2.2 x 106, 1.7 x 106, and 1.2 x 106 binding sites/cell for the 125I-radiolabeled constructs GST/1-114, GST/115-319, GST/200-319, and GST/1-319, respectively. Table I shows the average Kd values and number of binding sites/cell from four to five such binding experiments. Independent and simultaneous binding experiments yielded an average of four times more binding sites for carboxyl-terminal constructs than amino-terminal constructs (see Table I).

Uptake and Degradation of 125I-GST-39-kDa Protein Constructs by MHIC1 Cells—We previously demonstrated that the full-length 39-kDa protein (i.e. residues 1-319) is rapidly endocytosed and degraded via LRP/α2MR on MHIC1 cells (23). To investigate whether MHIC1 cells could similarly endocytose and degrade amino- and carboxyl-terminal regions of the 39-kDa protein, single cycle uptake and degradation experiments were performed. MHIC1 cells were incubated with 8 nm 125I-GST/1-114 (Fig. 2A), 8 nm 125I-GST/115-319 (Fig. 2B), 5 nm 125I-GST/200-319 (Fig. 2C), or 5 nm 125I-GST/1-319 (Fig. 2D) for 2 h at 4 °C. Following removal of unbound ligand, the cells were transferred to 37 °C for selected intervals and the fate of specifically bound radioactive ligand was followed. As seen in Fig. 2, upon warming at 37 °C, each of the bound radioligands disappeared rapidly from the cell surface. Approximately 95% of specific binding was lost within 5 min (Pronase-sensitive pool). Loss of cell-surface radioligand was associated with the appearance of internalized ligand (Pronase-resistant pool). Maximum intracellular levels for each of the ligands were seen after about 10 min of uptake. Concomitant with the decline of intracellular ligands and after a delay of about 20 min, trichloroacetic acid-soluble radioactivity, representing degraded ligand, appeared in the overlaying medium for each of the ligands. The kinetic pattern of uptake and degradation of a single cohort

![Figure 1](attachment:Figure1.png)

**FIG. 1.** Saturation binding of 125I-GST-39-kDa protein constructs to MHIC1 cells. MHIC1 cells were incubated for 2 h at 4 °C with increasing concentrations of 125I-GST/1-114 (Panel A), 125I-GST/115-319 (Panel B), 125I-GST/200-319 (Panel C), or 125I-GST/1-319 (Panel D) in the absence or presence of 0.5 μM unlabeled ligand. Total (●) and nonspecific (○) ligand binding were determined. Specific binding (▲) was derived as the difference between total and nonspecific binding. Symbols represent the means of duplicate determinations. Insets are Scatchard plots of specific binding. B, bound 125I-labeled ligand; F, bound/free 125I-labeled ligand.

| 125I-Ligand      | Kd (nm) | Binding sites/cell (x10⁶) |
|------------------|---------|--------------------------|
| GST/1-114        | 9.7 ± 3.4 | 553 ± 51                  |
| GST/115–319     | 8.9 ± 2.2 | 2410 ± 300                 |
| GST/200–319     | 8.1 ± 4.8 | 978 ± 250                  |

TABLE I
**Summary of saturation binding experiments with 39-kDa protein constructs to MHIC1 cells**

The average equilibrium dissociation constants (Kd) and average number of binding sites/cell for various 125I-labeled 39-kDa protein constructs are indicated. The number of binding experiments performed are denoted n. Two simultaneous binding experiments were performed with the constructs GST/1-114, GST/115-319, and GST/1-319. The number of independent binding experiments for the constructs GST/1-114, GST/115-319, and GST/1-319 were 3, 2, and 2, respectively. Data are expressed as mean ± S.D.
Amino- and Carboxyl-terminal GST-39-kDa Protein Constructs to Inhibit 125I-GST/115-319 binding to MHICl cells

- GST/l-114, GST/12-107, and GST/18-114 inhibit 125I-GST/1-114 binding. GST alone did not inhibit binding.
- GST/115-319, GST/1200-319, were also able to compete with the binding of 125I-GST/1-319 (Fig. 2).
- The inhibition of 125I-GST/115-319 binding to MHICl cells by various concentrations of amino-terminal GST-39-kDa protein constructs was performed as described above in Panel A. The constructs used were: GST/115-319 (O), GST/200-319 (M), and as a negative control, GST (V). Each symbol represents the average of duplicate determinations.

Of pre-bound amino- and carboxyl-terminal constructs was identical to that observed for the full-length protein, 125I-GST/1-319 (Fig. 2).

Inhibition of 125I-GST/1-114 Binding to MHICl Cells by Amino- and Carboxyl-terminal GST-39-kDa Protein Constructs—We previously demonstrated that the GST-fusion proteins encoding residues 1-114, 12-107, 18-114, 115-319, and 200-319 of the 39-kDa protein each inhibited the binding of 125I-t-PA to LRP/α2MR on MHICl cells (20). To investigate whether amino- and carboxyl-terminal regions of the 39-kDa protein bind to the same site or to distinct sites on LRP/α2MR, competition binding experiments with these constructs were performed. In Fig. 3, the inhibition of 125I-GST/1-114 binding to MHICl cells by amino- and carboxyl-terminal constructs of the 39-kDa protein was examined. As seen in Fig. 3A, GST/1-114, GST/12-107, and GST/18-114 each inhibited the binding of 125I-GST/1-114 (5 nM) to MHICl cells similarly and in dose-dependent manners with apparent K_i values of 15, 20, and 35 nM, respectively. In Fig. 3B, the constructs used were: GST/1-114 (C), GST/12-107 (D), and GST/18-114 (E), and as a negative control, GST (V). Each symbol represents the average of duplicate determinations.

Inhibition of 125I-GST/115-319 binding to MHICl cells by amino-terminal GST-39-kDa protein constructs. The inhibition of 125I-GST/115-319 (5 nM) binding to MHICl cells by various concentrations of amino-terminal GST-39-kDa protein constructs was performed as described above. In Panel A, the constructs used were: GST/115-319 (O), GST/200-319 (M), and as a negative control, GST (V). Each symbol represents the average of duplicate determinations.

The maximum dose of competitor proteins tested, 500 nM, approximately 40% of total binding was inhibited by each of the amino-terminal constructs. GST alone did not inhibit 125I-GST/115-319 binding. Fig. 4B shows that the constructs inhibited 125I-GST/115-319 binding. Each construct inhibited binding to MHICl cells with apparent K_i values of 8, 20, and 50 nM, respectively. These constructs inhibited 80-90% of total 125I-GST/115-319 binding.

Inhibition of 125I-GST/200-319 binding to MHICl cells by amino- and carboxyl-terminal GST-39-kDa protein constructs—To examine whether the pattern of inhibition of 125I-GST-39-kDa protein constructs by amino-terminal con-

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2 K_i is defined as the concentration of competitor which inhibits 50% of radioligand binding as determined by inspection.
structs was similar for different 125I-carboxyl-terminal constructs, the ability of amino- and carboxyl-terminal regions to inhibit 125I-GST/200-319 binding to MH1C1 cells was examined. As seen in Fig. 5A, the amino-terminal constructs GST/1-114, GST/12-107, and GST/18-114 each inhibited, in dose-dependent manners, 125I-GST/200-319 binding to MH1C1 cells. At the maximum dose of competitor added, 500 nM, these constructs inhibited 60% of total 125I-GST/200-319 binding. The patterns of inhibition of 125I-GST/200-319 (Fig. 5A) and 125I-GST/115-319 (Fig. 4A) binding by amino-terminal constructs were similar except whereas approximately 60% of 125I-GST/200-319 binding was inhibited by amino-terminal constructs, only 40% of 125I-GST/115-319 binding was inhibited. Fig. 5B shows that the carboxyl-terminal constructs GST/115-319 and GST/200-319 also inhibited, in dose-dependent manners, 125I-GST/200-319 binding to MH1C1 cells with apparent \( K_d \) values of 8 and 30 nM. Approximately 90% of total 125I-GST/200-319 binding is competed by 500 nM unlabeled GST/115-319 and 500 nM unlabeled GST/200-319.

Saturation Binding of 125I-GST/1-114 in the Absence and Presence of Fixed Concentrations of Unlabeled GST/115-319—
To characterize the nature of the competition of 125I-GST/1-114 binding by carboxyl-terminal constructs, saturation binding experiments with 125I-GST/1-114 were performed in the presence of various fixed concentrations of unlabeled GST/115-319. Fig. 6A shows a plot of femtomoles 125I-GST/1-114 bound per well versus the concentration of 125I-GST/1-114 added in the absence and presence of 3 and 5 nM unlabeled GST/115-319. As seen, addition of GST/115-319 reduced maximum 125I-GST/1-114 binding from approximately 320 fmol bound per well to approximately 200 and 140 fmol bound per well at concentrations of 3 and 5 nM GST/115-319, respectively. The equilibrium dissociation constants (\( K_d \)) were identical in the presence and absence of unlabeled GST/115-319 (see below). Nonspecific binding was determined in the presence of 500 nM GST/1-114 and accounts for approximately 10% of total binding. Fig. 6B shows a double-reciprocal plot of the data in Fig. 6A. All lines, in the presence and absence of inhibitor intersect at the abscissa which corresponds to \(-1/K_d\). The \( K_d \) value in the presence and absence of GST/115-319 is 5.5 nM. Fig. 6B also shows that, with different fixed amounts of GST/115-319, the lines have distinct intercepts on the ordinate. These points correspond to 100% of total femtomoles of 125I-GST/1-114 bound per well and yields values of approximately 320, 200, and 140 fmol in the absence of 3 or 5 nM GST/115-319, respectively.

Saturation Binding of 125I-GST/200-319 in the Absence and Presence of Fixed Concentrations of Unlabeled GST/1-114—
Since amino-terminal constructs inhibited 125I-GST/200-319 binding to a greater extent than 125I-GST/115-319 (Figs. 4A and 5A), we examined the mechanism of inhibition of 125I-GST/200-319 binding by amino-terminal constructs. The mechanism of inhibition of 125I-GST/200-319 binding by unlabeled GST/1-114 was determined by performing saturation binding experiments with 125I-GST/200-319 in the absence and presence of various fixed concentrations of unlabeled GST/1-114. Fig. 7A shows a plot of femtomoles of 125I-GST/200-319 bound per well versus the concentration of 125I-GST/200-319 added in the absence and presence of 5 or 20 nM unlabeled GST/1-114. The addition of GST/1-114 reduced 10 nM 125I-GST/200-319 binding from approximately 330 fmol bound per well to approximately 220 and 120 fmol bound per well at concentrations of 5 and 20 nM GST/1-114, respectively. The apparent \( K_d \) values were identical in the absence and presence of GST/1-114 (see below). Nonspecific binding was determined in the presence of 300 nM unlabeled GST/200-319 and accounts for approximately 10% of total binding. Fig. 7B is a double-reciprocal plot of the data in Fig. 7A and shows that the lines in the absence and presence of inhibitor (GST/1-114) intersect along the abscissa. The intersection point corresponds to a \( K_d \) value of 8 nM. Fig. 7B also shows the lines have distinct intercepts along the ordinate and correspond to values of 330, 220, and 120 fmol of 125I-GST/200-319 bound per well in the absence and presence of 5 and 20 nM unlabeled GST/1-114, respectively.

**DISCUSSION**

Table I summarizes the average \( K_d \) values and number of binding sites/cell from the indicated number of independent and simultaneous saturation binding experiments for various 125I-labeled 39-kDa protein constructs. The \( K_d \) values and the number of binding sites/cell were similar when binding assays...
were performed independently (i.e. GST/1–114 alone, GST/115–319 alone, or simultaneously (i.e. GST/1–114, GST/115–319, and GST/1–319 assayed in separate dishes at the same time) and therefore all results were averaged. The average $K_d$ values for GST/1–114 (9.7 nM) and GST/115–319 (8.9 nM) were similar to the full-length protein, GST/1–319 (8.1 nM). However, the average number of binding sites/cell was different for amino- and carboxyl-terminal regions of the 39-kDa protein with the carboxyl-terminal region having approximately four times as many binding sites/cell as the amino-terminal region (2,410,000 versus 583,000). The average number of binding sites/cell for the full-length protein, GST/1–319, was 978,000. Although amino- and carboxyl-terminal regions each bind independently to LRP/α2MR, it is not clear whether these regions bind to the same site on LRP/α2MR (see below) or whether multiple regions within the full-length 39-kDa protein can simultaneously bind to LRP/α2MR. It is possible that only one region (i.e. amino- or carboxyl-terminal) within the full-length protein binds to LRP/α2MR at any one time and that this binding induces a conformational change in LRP/α2MR such that other regions cannot bind. Therefore the number of binding sites/cell for the full-length protein may reflect a mixed population of different regions of the full-length protein binding to LRP/α2MR.

We previously demonstrated that both amino- and carboxyl-terminal regions of the 39-kDa protein inhibited t-PA binding to LRP/α2MR on MH2C1 cells, whereas only amino-terminal regions inhibited α2M* binding (20). These observations prompted us to examine whether amino- and carboxyl-terminal regions bound to the same site or to separate sites on LRP/α2MR to inhibit t-PA binding. Binding competition experiments demonstrated that amino- and carboxyl-terminal regions of the 39-kDa protein competed with one another for binding to LRP/α2MR. However, the extent of competition varied whereby unlabeled carboxyl-terminal constructs completely inhibited specific 125I-amino-terminal construct binding (Fig. 3B) and unlabeled amino-terminal constructs inhibited only 40–60% of 125I-carboxyl-terminal construct binding (Figs. 4A and 5A). The differences in these extents of inhibition are most likely due to a decrease in the number of binding sites available for binding. Since saturation binding experiments yielded an average of four carboxyl-terminal binding sites to one amino-terminal binding site (Table I), one would predict in competition binding experiments, using a fixed non-saturating concentration of 125I-amino-terminal constructs and saturating concentrations of unlabeled carboxyl-terminal constructs (i.e. 50–500 nM), that all binding sites would be occupied with the carboxyl-terminal constructs and thus no 125I-amino-terminal construct binding would be detected. Indeed, as seen in Fig. 3B, carboxyl-terminal constructs were able to completely inhibit 125I-GST/1–114 binding. Conversely in competition binding experiments with a fixed non-saturating concentration of 125I-carryxyl-terminal constructs and saturating concentrations of unlabeled amino-terminal constructs, approximately 20–25% of the binding sites would be occupied with amino-terminal constructs and therefore the remaining binding sites would be available for 125I-carryxyl-terminal construct binding. Figs. 4A and 5A demonstrate that saturating concentrations of unlabeled amino-terminal constructs maximally inhibited 40–60% of 125I-carboxyl-terminal construct binding.

Although the binding competition experiments demonstrated that amino- and carboxyl-terminal constructs competed with one another for binding, these data did not demonstrate whether the constructs bound to the same site or to distinct sites on LRP/α2MR. To characterize the nature of competition (i.e. competitive inhibition versus noncompetitive inhibition), saturation binding experiments were performed with 125I-amino- and 125I-carboxyl-terminal constructs in the presence of various fixed concentrations of unlabeled competitor protein. The amino- and carboxyl-terminal regions of the 39-kDa protein contain no sequence identity and, therefore, would be predicted to bind to different sites on LRP/α2MR. Double-reciprocal plots of the data using 125I-GST/1–114 in the absence and presence of unlabeled GST/115–319 showed that all lines intersected at the abscissa and had distinct intercepts on the ordinate (Fig. 6B). Thus the $K_d$ value in the presence and absence of GST/115–319 was 5.5 nM, whereas the maximum femtomoles bound per well was reduced in the presence of inhibitor. Similarly, double-reciprocal plots of the data using 125I-GST/200–319 in the absence and presence of unlabeled GST/1–114 showed that all lines intersected at the abscissa (corresponding to a $K_d$ value of 8 nM) and had distinct intercepts on the ordinate (Fig. 7B). These double-reciprocal patterns are consistent with a mechanism of noncompetitive inhibition (24), whereby the inhibitor and substrate bind to
different sites on LRP/α2MR.

A model is depicted in Fig. 8 to summarize t-PA and α2M* binding to LRP/α2MR and the regulatory role of the 39-kDa protein on ligand binding. As seen, t-PA and α2M* bind to separate sites on LRP/α2MR (arrow 1) because these ligands do not cross-compete for binding (10). We previously demonstrated that several amino-terminal regions of the 39-kDa protein, generated as fusion proteins with GST (GST/1-114 and GST/12-107), and several carboxyl-terminal GST-fusion proteins (GST/115-319, GST/151-319, GST/200-319, and GST/187-311) bound directly to LRP/α2MR immobilized on nitrocellulose. Based on our observations that carboxyl-terminal constructs may function as noncompetitive inhibitors for 125I-amino-terminal construct binding to MH1C1 cells (Fig. 6) and amino-terminal constructs may function as noncompetitive inhibitors of 125I-carboxyl-terminal construct binding (Fig. 7), we propose that the amino- and carboxyl-terminal regions of the 39-kDa protein bind to different sites on LRP/α2MR (arrow 2). We have previously shown that the GST-39-kDa protein inhibits both t-PA and α2M* binding to MH1C1 cells whereas t-PA and α2M* inhibit 125I-GST-39-kDa protein binding only slightly (10). These results may suggest that the GST-39-kDa protein inhibits ligand binding indirectly, for example by steric hindrance. Since the amino-terminal region of the 39-kDa protein inhibited both t-PA and α2M* binding to MH1C1 cells, whereas the carboxyl-terminal region only inhibited t-PA binding, it is possible that the amino-terminal region overlaps both the t-PA and α2M* binding sites on LRP/α2MR to sterically hinder t-PA and α2M* binding (arrow 3), whereas the carboxyl-terminal region overlaps only the t-PA binding site and thus inhibits t-PA binding but not α2M* binding (arrow 4). Arrows 5 and 6 demonstrate a possible model for the noncompetitive inhibition by amino- and carboxyl-terminal regions of the 39-kDa protein. Binding of the amino-terminal region to LRP/α2MR may induce a conformational change in LRP/α2MR such that the carboxyl-terminal region of the 39-kDa protein is unable to bind (arrow 5). Similarly, binding of the carboxyl-terminal region to LRP/α2MR may induce a conformational change in LRP/α2MR such that the amino-terminal region of the 39-kDa protein cannot bind (arrow 6).

In summary, we have demonstrated that amino- and carboxyl-terminal regions of the 39-kDa protein each bind specifically to LRP/α2MR on rat hepatoma MH1C1 cells with similar affinities as the full-length protein. Following binding, these regions are rapidly endocytosed and degraded with kinetics identical to the full-length protein. In addition, the amino- and carboxyl-terminal regions bind to separate sites on LRP/α2MR, and this implies that these regions may differentially regulate ligand binding to LRP/α2MR.

Acknowledgments—We thank Carl Frieden for helpful discussions and comments. We also thank Jonathan Gitlin, Dave Wilson, and Aaron Ciechanover for critical reading of the manuscript.

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