

# The Human $\alpha_2$ -Macroglobulin Receptor: Identification of A 420-kD Cell Surface Glycoprotein Specific for The Activated Conformation of $\alpha_2$ -Macroglobulin

James D. Ashcom, Steven E. Tiller, Kenneth Dickerson, Janet L. Cravens, W. Scott Argraves, and Dudley K. Strickland

The Biochemistry Department, Holland Laboratory, American Red Cross, Rockville, Maryland 20855

**Abstract.** Ligand affinity chromatography was used to purify a cell surface  $\alpha_2$ -macroglobulin ( $\alpha_2$ M) receptor. Detergent extracts of human placenta were applied to an affinity matrix consisting of  $\alpha_2$ M, previously reacted with methylamine, coupled to Sepharose. Elution with EDTA specifically released polypeptides with apparent molecular masses of 420 and 39 kD. In some preparations, small amounts of a 90-kD polypeptide were observed. The 420- and 39-kD polypeptides appear specific for the forms of  $\alpha_2$ M activated by reaction with proteinases or methylamine and do not bind to an affinity matrix consisting of native  $\alpha_2$ M coupled to Sepharose. Separation of these two polypeptides

was accomplished by anion exchange chromatography, and binding activity was exclusively associated with the 420-kD polypeptide. The purified 420-kD protein binds to the conformationally altered forms of  $\alpha_2$ M that are known to specifically interact with  $\alpha_2$ M receptors and does not bind to native  $\alpha_2$ M. Binding of the 420-kD polypeptide to immobilized wheat germ agglutinin indicates that this polypeptide is a glycoprotein. The cell surface localization of the 420-kD glycoprotein was confirmed by affinity chromatography of extracts from surface radioiodinated fibroblasts. These properties suggest that the 420-kD polypeptide is a cell surface receptor for the activated forms of  $\alpha_2$ M.

$\alpha_2$ -MACROGLOBULIN ( $\alpha_2$ M)<sup>1</sup> is a 718,000-mol wt glycoprotein that is capable of inhibiting enzymes from all four classes of proteinases (2) in a reaction that is essentially irreversible. The reaction of proteinases or methylamine with  $\alpha_2$ M "activates" the molecule by inducing conformational changes in  $\alpha_2$ M (3, 4, 16, 49) that, in the case of the proteinase reaction, reduce the activity of the bound enzyme toward large molecular weight substrates. These conformational changes also generate sites on the inhibitor that result in specific recognition by cell surface receptors (31, 52, 54). It has been possible to prepare monoclonal antibodies that selectively recognize the activated forms of  $\alpha_2$ M (34, 50) by binding to these newly generated regions (25).

In addition to regulating proteinase activity, studies have suggested that  $\alpha_2$ M binds to TGF $\beta$  (41), PDGF (22), and bFGF (7). The association of these molecules with  $\alpha_2$ M results in a reduction of their activity, suggesting that  $\alpha_2$ M may serve not only to modulate proteinase activity but also the activity of these molecules. Interestingly, it appears that several tumor-derived or virus-transformed cell lines are devoid of specific cell surface receptors for  $\alpha_2$ M-proteinase complexes (52).

The process of receptor-mediated endocytosis has been the subject of extensive study, and the mechanisms involved in

endocytosis have been investigated using a variety of labeled ligands (6), including transferrin (20), low density lipoprotein (1), EGF (20), and  $\alpha_2$ M (8, 37, 54, 55). Early studies reported the specific binding of  $\alpha_2$ M to fibroblasts (9, 52), and it was recognized that the binding of  $\alpha_2$ M to cells was specific for the forms of  $\alpha_2$ M activated either by proteolysis or by reaction with methylamine (23, 32, 52). Despite the widespread use of  $\alpha_2$ M in studying the process of endocytosis, little is known about the polypeptide composition and structure of the  $\alpha_2$ M receptor. A more comprehensive understanding of the function of the  $\alpha_2$ M receptor requires a knowledge of the structure of this molecule. The present investigation was initiated to characterize the  $\alpha_2$ M receptor.

## Materials and Methods

### Proteins

$\alpha_2$ M was prepared according to the method of Imber and Pizzo (23) followed by immunoaffinity chromatography (50) to remove trace amounts of the cleaved form when necessary.  $\alpha_2$ M-trypsin complexes were prepared by reacting  $\alpha_2$ M with a 1.8-fold molar excess (active site concentration) of trypsin. Trypsin activity was then inhibited with 20 mM diisopropyl fluorophosphate.  $\alpha_2$ M was iodinated with Enzymobeads (Bio-Rad Laboratories, Richmond, CA). The specific radioactivity of the labeled protein was 1  $\mu$ Ci/ $\mu$ g protein. Upon activation with trypsin or with methylamine, SDS-PAGE followed by autoradiography of this material showed a distribution of products that was essentially identical to that obtained for unlabeled acti-

1. *Abbreviations used in this paper:*  $\alpha_2$ M,  $\alpha_2$ -macroglobulin;  $\alpha_2$ M:Me, methylamine-treated  $\alpha_2$ -macroglobulin; NRK, normal rat kidney.

vated  $\alpha_2M$ . Monoclonal antibody 7H11D6 was prepared as described (25, 50). Mouse laminin was provided by Dr. Albert Chung (Department of Biological Sciences, University of Pittsburgh, Pittsburgh, PA).

### Preparation of Methylamine-treated $\alpha_2M$ ( $\alpha_2M:Me$ )–Sephacrose

Native  $\alpha_2M$  (8.6 mg/ml) was incubated with 200 mM methylamine in 50 mM sodium phosphate, 150 mM NaCl, pH 7.4, for 1 h at room temperature. The  $\alpha_2M:Me$  was then dialyzed extensively against 0.1 M NaHCO<sub>3</sub>, 0.5 M NaCl, pH 8.3. After dialysis, the protein was coupled to CNBr-activated Sepharose 4B (Pharmacia Fine Chemicals, Piscataway, NJ) as recommended by the manufacturer using 10 mg  $\alpha_2M:Me$ /ml resin. The coupling was allowed to proceed for 2 h at room temperature using end-over-end mixing. After coupling, the solution was removed and replaced with 0.1 M Tris, pH 8.0, for an additional 2 h at room temperature. The resin was then washed extensively with alternating buffers of 0.1 M sodium acetate, 0.5 M NaCl, pH 5.0, and 0.1 M sodium bicarbonate, pH 8.3, 0.5 M NaCl.

### Purification of the $\alpha_2M$ Receptor

All procedures, unless indicated, were carried out at 4°C. Fresh placenta, obtained from Montgomery General Hospital (Olney, MD) was washed with cold TBS (50 mM Tris, 150 mM NaCl, pH 7.4) and 200 mM sucrose. The fetal membranes and umbilical cord were removed, and the placenta was ground and either used immediately or stored at -20°C until needed. The tissue was suspended in an equal volume of TBS containing 0.005% digitonin, 1 mM each of MgCl<sub>2</sub> and CaCl<sub>2</sub>, and the following proteinase inhibitors: 1 mM PMSF, 0.02 mg/ml leupeptin, and 0.02 mg/ml D-Phe-Pro-Arg-Ch<sub>2</sub>Cl. After stirring for 15 min on ice, the mixture was homogenized in a blender (three times for 30 s each) and then centrifuged at 5,000 g for 20 min. The supernatant was discarded, and the pelleted tissue was suspended in an equal volume of extraction buffer (50 mM octyl- $\beta$ -D-glucopyranoside in TBS containing 1 mM each of MgCl<sub>2</sub> and CaCl<sub>2</sub>, 1 mM PMSF, 0.02 mg/ml leupeptin, 0.02 mg/ml D-Phe-Pro-Arg-Ch<sub>2</sub>Cl for 1 h at 4°C or on ice. The suspension was subjected to centrifugation at 5,000 g for 20 min, and the supernatant from this step was subjected to additional centrifugation at 11,000 g for 20 min. The resultant supernatant was then applied to a 120-ml Sepharose CL-4B column. The unabsorbed material from this column was mixed overnight at 4°C with 40–60 ml of the  $\alpha_2M:Me$ -Sephacrose column. The resin was then washed with eight column volumes of 25 mM octyl- $\beta$ -D-glucopyranoside in TBS containing 1 mM CaCl<sub>2</sub>, 1 mM MgCl<sub>2</sub>, 1 mM PMSF. Elution was achieved using TBS containing 20 mM EDTA, 25 mM octyl- $\beta$ -D-glucopyranoside. Further purification was achieved by applying the eluate from this column to a Mono Q anion exchange column (Pharmacia Fine Chemicals) at room temperature previously equilibrated with 20 mM octyl- $\beta$ -D-glucopyranoside in 50 mM Tris, pH 8.2, at a flow rate of 0.5 ml/min. After washing the column, a linear gradient from 0 to 1 M NaCl was applied over 40 min using a Waters Instruments, Inc. (Rochester, MN) 650E advanced protein purification system. Protein was monitored at 280 nm, and 1-ml fractions were collected. Fractions containing the receptor were stored at 4°C and used for further studies. The concentration of the receptor was estimated by absorbance measurements at 280 nm assuming an  $E_{1\%}^{280\text{ nm}}$  of 10.0.

### SDS-PAGE

A discontinuous pH gel system (Laemmli) was used with 4% polyacrylamide stacking gel and a 5–15% separating gel (1.5 mm  $\times$  16 cm  $\times$  13.5 cm). Proteins were stained with Coomassie blue R-250. Before SDS-PAGE, samples were either reduced in SDS sample buffer with 50 mM DTT at 50°C for 10 min or in SDS sample buffer with 1%  $\beta$ -mercaptoethanol at 100°C for 5 min.

### Surface Labeling of Fibroblasts

Normal rat kidney (NRK) fibroblasts (CRL 1570), obtained from the American Type Culture Collection (Rockville, MD) were grown in a 75-ml flask in DME containing 5% heat-inactivated FCS with 1% penicillin/streptomycin and 1% L-glutamine. Cells ( $2.5 \times 10^7$ ) were removed from the flask with 2 mM EDTA, pH 7.4, counted, washed twice with serum-free DME, and then resuspended in 3–4 ml of 20 mM glucose in Dulbecco's PBS. The cells were concentrated by centrifugation and resuspended in 1 ml of 20 mM glucose/Dulbecco's buffer. Glucose oxidase (0.4 U), lactoperoxidase (200  $\mu$ g), and Na<sup>125</sup>I (1 mCi) were then added and incubated with the cells for

20 min on ice with frequent mixing. After this period, the cells were washed three times (3 ml each wash) with cold PBS, and resuspended in 1 ml of extraction buffer. After stirring for 1 h at 4°C, the supernatant was mixed with 1 ml of  $\alpha_2M:Me$ -Sephacrose overnight. After washing, the column was eluted with a buffer containing 20 mM EDTA.

### Binding of $\alpha_2M$ to the Purified 420-kD Protein

Two assays were used to measure the binding activity of various fractions. The first assay measured the binding of affinity-purified (50) <sup>125</sup>I-labeled  $\alpha_2M:Me$  (or  $\alpha_2M$ ) to microtiter wells coated with either the purified receptor or with various column fractions. Coating was carried out in 50 mM Tris, 150 mM NaCl, 5 mM CaCl<sub>2</sub>, pH 7.4, and was allowed to proceed overnight at 4°C. After washing, the wells were blocked with 3% BSA and incubated with <sup>125</sup>I-labeled  $\alpha_2M:Me$  (5–7 nM) in the presence of increasing concentrations of unlabeled  $\alpha_2M$  or  $\alpha_2M:Me$ . The buffer used was 50 mM Tris, 150 mM NaCl, 5 mM CaCl<sub>2</sub>, 3% BSA, pH 7.4. After a 2-h incubation at 4°C, the wells were washed and counted. Binding data were analyzed as described by Munson and Rodbard (39) using the computer program LIGAND. In some experiments, <sup>125</sup>I-labeled  $\alpha_2M$ -trypsin was used in place of  $\alpha_2M:Me$ . The binding assay was shown to depend upon the concentration of added ligand, upon the mass of protein coated to the microtiter wells, and, further, on the form of  $\alpha_2M$  used. An ELISA (10) was used to measure the specificity of various  $\alpha_2M$  derivatives for the purified 420-kD polypeptide. This assay was performed by coating wells of microtiter plates (Immulon II; Dynatech Laboratories, Inc., Alexandria, VA) with 100  $\mu$ l purified 420-kD polypeptide (5  $\mu$ g/ml) in coating buffer (Dulbecco's PBS plus 1 mM CaCl<sub>2</sub>) for 6 h at room temperature. After washing with PBS, the plate was blocked with 10 mg/ml BSA in coating buffer for 1 h at room temperature. The plates were then washed with PBS containing 0.05% Tween 20, and various amounts of  $\alpha_2M$  or  $\alpha_2M$ -trypsin complexes diluted in PBS containing 0.05% Tween 20, 10 mg/ml BSA, and 1 mM CaCl<sub>2</sub> were added. Incubation was carried out overnight at 4°C. After washing, rabbit anti-human  $\alpha_2M$  (Boehringer Mannheim Biochemicals, Indianapolis, IN) diluted 1:10,000 was added, and detection was accomplished using an anti-rabbit IgG-alkaline phosphatase conjugate. All experiments included control wells that contained no receptor coating. The data were analyzed according to the following relationship (24):

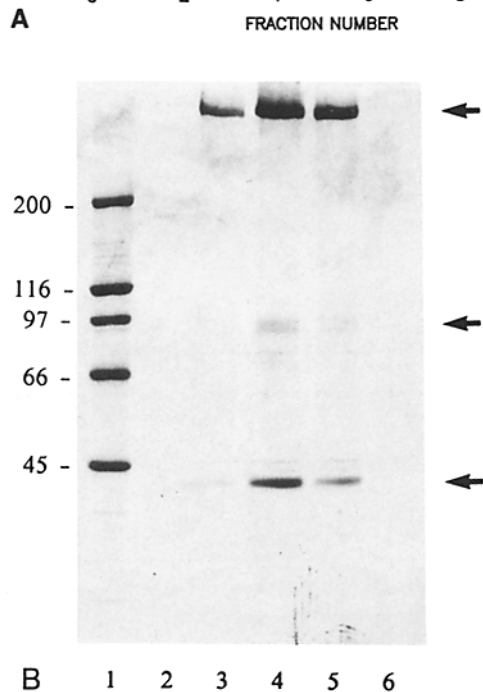
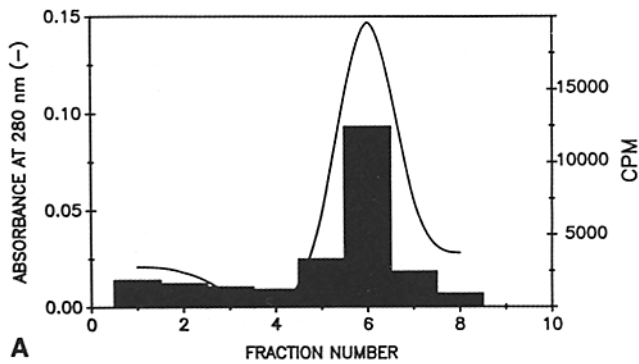
$$A = A_{\max} \left( \frac{[L]}{K_d + [L]} \right),$$

where  $A$  is the absorbance at 410 nm,  $A_{\max}$  is the maximum absorbance at saturation and is treated as a variable parameter,  $[L]$  is the molar concentration of free  $\alpha_2M$  or  $\alpha_2M$ -trypsin, and  $K_d$  is the dissociation constant. Application of this analysis to the present data assumes that the amount of added ligand is greater than the amount of receptor bound to the microtiter wells. This is especially true at higher ligand concentrations. Under these conditions, the concentration of free  $\alpha_2M$  or  $\alpha_2M$ -trypsin is approximately equal to the total concentration of  $\alpha_2M$  or  $\alpha_2M$ -trypsin.

## Results

### Affinity Chromatography of Octyl- $\beta$ -D-glucopyranoside Extracts from Placenta

Binding studies have suggested that relatively large quantities of  $\alpha_2M$  receptor are present in placenta (28). This tissue is an abundant source of receptors for fibronectin (43), vitronectin (43), and transferrin (51). Consequently, studies were initiated to isolate the  $\alpha_2M$  receptor from octyl- $\beta$ -D-glucopyranoside extracts of human placenta. All procedures were carried out in the presence of proteinase inhibitors, and solubilization was performed in a buffer containing 50 mM octyl- $\beta$ -D-glucopyranoside, 1 mM Ca<sup>2+</sup>, and 1 mM Mg<sup>2+</sup>. The extract was first applied to a Sepharose CL-4B column to remove any protein with an affinity for this matrix and then applied to an affinity column prepared by coupling  $\alpha_2M$  previously reacted with methylamine ( $\alpha_2M:Me$ ) to Sepharose CL-4B. After extensive washing, the column was eluted with metal-free buffer containing 20 mM EDTA. The elution



**Figure 1.** Affinity chromatography of placental extract over Sepharose- $\alpha_2$ M:Me. (A) Elution profile after addition of buffer containing 20 mM EDTA (solid line). Individual fractions were diluted 20-fold and coated to microtiter wells, and 5.0 nM  $^{125}$ I- $\alpha_2$ M-trypsin was added. After incubation and washing, the wells were counted (bars). Nonspecific binding was determined by adding a 200-fold molar excess of unlabeled  $\alpha_2$ M-trypsin. (B) Polypeptide composition of fractions eluted from the  $\alpha_2$ M:Me-Sepharose affinity column assessed by SDS-PAGE. Aliquots (20  $\mu$ l) from each fraction were mixed with SDS sample buffer containing 50 mM DTT. After incubation, the samples were subjected to electrophoresis on a 5–15% gradient gel with a 4% stacking gel using the Laemmli buffer system. (Lane 1) Standards; (lanes 2–6) fractions 4–8 from the affinity column.

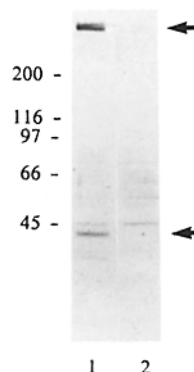
profile from the affinity column is depicted in Fig. 1 A. Dilute aliquots of each fraction were adsorbed onto microtiter wells and tested for their ability to bind  $^{125}$ I-labeled  $\alpha_2$ M-trypsin complexes. Nonspecific binding, determined by including a 200-fold molar excess of unlabeled  $\alpha_2$ M-trypsin with the labeled ligand, was  $\sim$ 9% of total binding. The binding activity measured by this assay correlated with the total protein eluted from the column, and the presence of specific binding in these fractions indicates that it is possible to reconstitute binding activity.

The polypeptide content of each fraction from the affinity

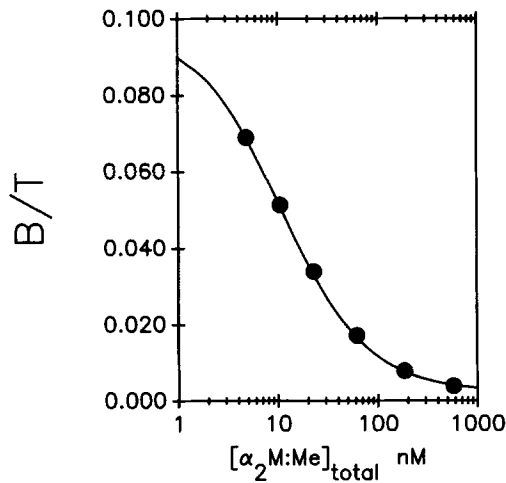
column was assessed by SDS-PAGE. Representative data, shown in Fig. 1 B, reveals that three polypeptides (Fig. 1 B, arrows) are eluted from the affinity column. Polypeptides with apparent molecular mass of 420 and 39 kD are the major proteins eluted from the affinity column. A third polypeptide, with an apparent molecular mass of 90 kD is present in variable amounts in different preparations. In some preparations, this polypeptide can be readily detected by SDS-PAGE (e.g., Fig. 1 B), while in other preparations (e.g., Fig. 2, lane 1) only trace levels are present. The variable amounts of the 90-kD polypeptide in different preparations support the contention that this protein is not exclusively responsible to the binding activity. The data shown in Fig. 1 B represent SDS-PAGE under reducing conditions. Analysis of the samples under nonreducing conditions revealed that none of the polypeptides are covalently linked by disulfide bonds.

Binding studies have documented that cells bind specifically to the activated forms of  $\alpha_2$ M (32, 52). These forms occur after reaction of  $\alpha_2$ M with either methylamine or proteinases. To examine the specificity of the 420- and 39-kD polypeptides for various  $\alpha_2$ M conformers, a portion of a placental extract was applied to an affinity column consisting of native  $\alpha_2$ M coupled to Sepharose. In a control experiment, an equal portion was applied to an  $\alpha_2$ M:Me affinity column. Fig. 2 shows an analysis of the eluted proteins by SDS-PAGE and demonstrates that the 420- and 39-kD polypeptides (Fig. 2, arrows) are specific for the activated forms of  $\alpha_2$ M (Fig. 2, lane 1) and do not bind to native  $\alpha_2$ M (Fig. 2, lane 2). Preincubation of the  $\alpha_2$ M:Me affinity column with stoichiometric amounts of monoclonal antibody 7H11D6 reduces the amount of both the 420- and 39-kD polypeptides recovered from the column (data not shown). This monoclonal antibody binds to the region of  $\alpha_2$ M that is involved in cell recognition (25, 50).

The requirement of metal ions for the binding of this protein to immobilized  $\alpha_2$ M:Me is in agreement with the known requirement of metal ions for the binding of  $\alpha_2$ M:Me and  $\alpha_2$ M-proteinase complexes to cells (11, 13, 18, 32, 40, 42, 52). Many ligands, such as  $\alpha_2$ M-proteinase complexes, that enter the cell by receptor-mediated endocytosis are delivered to lysosomes, while their receptors are recycled back to the cell surface (5). This mechanism requires that the receptor-ligand complex dissociate, which for a number of receptor systems is presumably the result of lowering of the pH. The interaction of activated  $\alpha_2$ M with cells (36) and with solubilized membranes (15) is pH dependent, with maximal binding occurring between pH 7 and 8 and a reduction in affinity



**Figure 2.** Analysis of proteins derived from affinity chromatography of placental extracts on immobilized  $\alpha_2$ M:Me (lane 1) or immobilized native  $\alpha_2$ M (lane 2) by SDS-PAGE. 15-ml extracts were applied to 1-ml columns, incubated overnight at 4°C, and washed. After washing, the columns were eluted in a buffer containing 20 mM EDTA. Equal volumes (65  $\mu$ l) of the eluted fractions were analyzed by SDS-PAGE under reducing conditions on 5–15% gradient gels.



**Figure 3.** Binding of  $^{125}\text{I}$ -labeled  $\alpha_2\text{M:Me}$  to the immobilized receptor preparation obtained from the  $\alpha_2\text{M:Me}$  affinity column. Microtiter wells, coated with receptor, were incubated with 4.9 nM  $^{125}\text{I}$ -labeled  $\alpha_2\text{M:Me}$  in the presence of increasing concentrations of unlabeled  $\alpha_2\text{M:Me}$ . The curve displays the best fit of the data to a single class of sites with a  $K_d$  of 10 nM. Each point represents the average of duplicates.

as the pH decreases below 6.0. Consistent with this is the observation that the 420- and 39-kD polypeptides could be eluted from the affinity resin with a pH 5.0 sodium acetate buffer.

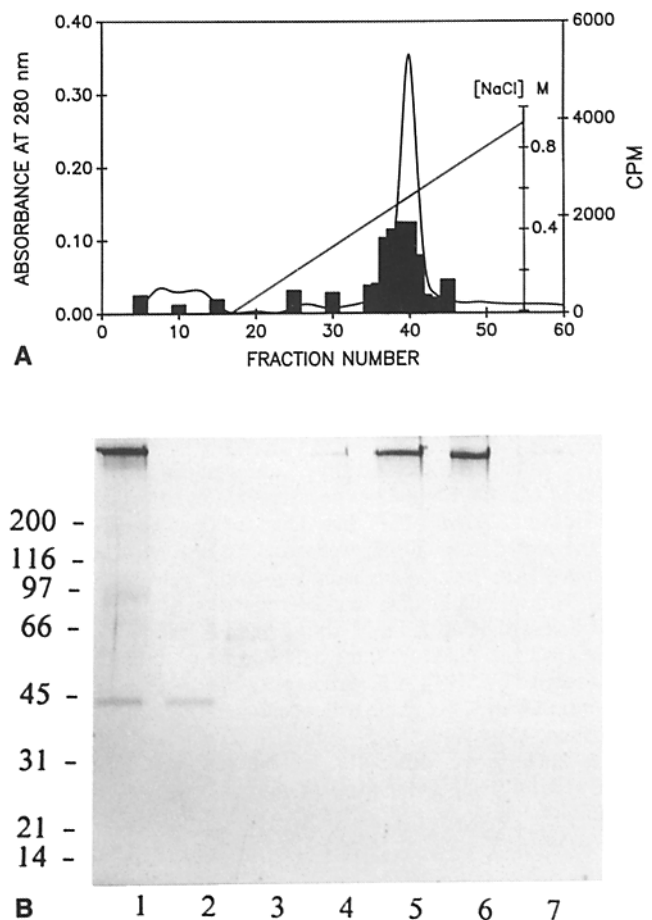
Since it is possible to reconstitute binding activity after elution from the immobilized  $\alpha_2\text{M:Me}$  column, experiments were conducted to measure the affinity of this material for  $\alpha_2\text{M:Me}$ . Fig. 3 demonstrates the results of an experiment in which a constant amount of  $^{125}\text{I}$ -labeled  $\alpha_2\text{M:Me}$ , with increasing amounts of unlabeled  $\alpha_2\text{M:Me}$ , is added to microtiter wells coated with the material eluted from the affinity column. The curve in Fig. 3 represents the best fit of the data to a single class of sites using the procedure described by Munson and Rodbard (39), and a  $K_d$  of 10 nM was derived from the fit. In several different  $\alpha_2\text{M}$  receptor preparations, values of the  $K_d$  ranged from 2 to 14 nM. These values are relatively close to those values of 0.2–10 nM reported for the binding of  $\alpha_2\text{M:Me}$  to various cells (11, 13, 15, 17, 48).

#### **Demonstration That the Binding Activity Is Associated with the 420-kD Polypeptide**

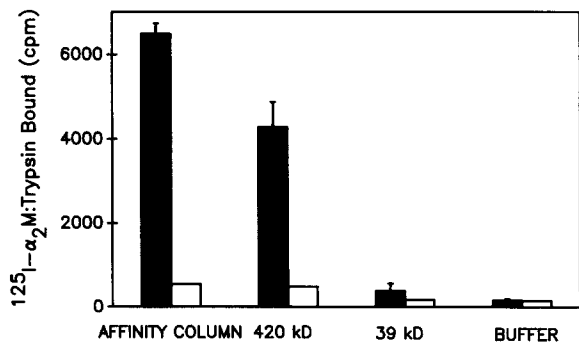
The following experiments were conducted to determine which of the polypeptides eluted from the  $\alpha_2\text{M:Me}$  affinity column is responsible for the binding activity. No binding of  $^{125}\text{I}$ - $\alpha_2\text{M:Me}$  to any polypeptide was detected after SDS-PAGE and transfer to immobililon. An additional chromatography step was used to separate the components eluted from the affinity column. This was accomplished by anion exchange chromatography on a Mono-Q column (Fig. 4 A). The  $\alpha_2\text{M}$ -trypsin binding activity was associated with those fractions that were eluted with 0.5–0.6 M NaCl. Examination of individual fractions by SDS-PAGE (Fig. 4 B) revealed that these fractions contain the 420-kD polypeptide (Fig. 4 B, lanes 4–7). The 39-kD fragment does not bind to the column (Fig. 4, lane 2). Additional experiments revealed that the

majority of the 90-kD polypeptide, when present, does not bind to the Mono Q resin, and is present with the 39-kD polypeptide.

A comparison of the binding of  $^{125}\text{I}$ - $\alpha_2\text{M}$ -trypsin to the various fractions derived from the anion exchange column is shown in Fig. 5. In these experiments, the purified 420-kD polypeptide and a pool of the unabsorbed fractions from the Mono Q column containing the 39-kD polypeptide were coated to microtiter wells. As a control, material recovered from the  $\alpha_2\text{M:Me}$  affinity column was also included.  $^{125}\text{I}$ - $\alpha_2\text{M}$ -trypsin (5 nM) was then added in the absence (Fig. 5, solid bars) or presence (Fig. 5, open bars) of a 200-fold molar excess of unlabeled  $\alpha_2\text{M}$ -trypsin. After incubation and washing, the amount of labeled  $\alpha_2\text{M}$ -trypsin bound to each well was measured. The results confirm that the 420-kD polypeptide specifically binds  $\alpha_2\text{M}$ -trypsin complexes. Very



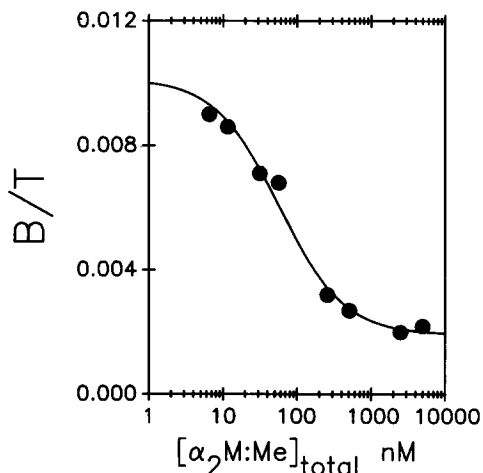
**Figure 4.** (A) Separation of the 420- and 39-kD polypeptides by anion exchange chromatography on a Mono Q column. The flow rate was 0.5 ml/min, and 1-ml fractions were collected. For assay, fractions were diluted 40-fold, coated to microtiter wells, and incubated with 5 nM  $^{125}\text{I}$ - $\alpha_2\text{M}$ -trypsin. After incubation and washing, the wells were counted (bars). Nonspecific binding was determined by adding a 200-fold molar excess of unlabeled  $\alpha_2\text{M}$ -trypsin. (B) Analysis of selected fractions by SDS-PAGE on 5–15% gradient gels. (Lanes 1–7, respectively) Material loaded on the column, pool of fractions 7–14, fraction 35, fraction 37, fraction 39, fraction 41, and fraction 43. In lanes 1 and 2, 40- $\mu\text{l}$  aliquots were applied to the gels, while, in lanes 3–7, 10- $\mu\text{l}$  aliquots were applied.



**Figure 5.** Comparison of the binding of  $^{125}\text{I}$ -labeled  $\alpha_2\text{M}$ -trypsin to the purified 420-kD polypeptide and the 39-kD polypeptide. Microtiter wells were coated with 100  $\mu\text{l}$  of 420-kD polypeptide (21 nM) or 100  $\mu\text{l}$  of 39-kD polypeptide (34 nM). After washing, the wells were incubated with 5 nM  $^{125}\text{I}$ - $\alpha_2\text{M}$ -trypsin in the absence (solid bars) or presence of a 1  $\mu\text{M}$  unlabeled  $\alpha_2\text{M}$ -trypsin (open bars). After incubation overnight at 4°C, the wells were washed and counted. As a control, binding to the material derived from the  $\alpha_2\text{M}$ :Me affinity column is also shown.

little binding of these complexes to microtiter wells coated with the 39-kD polypeptide occurs.

The experiments presented in Fig. 5 indicate that the purified 420-kD polypeptide specifically binds  $^{125}\text{I}$ - $\alpha_2\text{M}$ -trypsin complexes whereas the 39-kD polypeptide does not bind these complexes. A comparison of the binding activity of the purified 420-kD receptor with the material eluted from the affinity resin is best accomplished by a comparison of the  $K_d$  values for these two samples. Fig. 6 demonstrates the results of a binding experiment using the purified 420-kD polypeptide. A  $K_d$  of 57 nM was determined from a best fit of the data to a single class of sites. This value is slightly higher than the  $K_d$  measured for the material recovered from the affinity column and most likely reflects the fact that optimal



**Figure 6.** Binding of  $^{125}\text{I}$ -labeled  $\alpha_2\text{M}$ :Me to the purified receptor preparation obtained after anion exchange chromatography. Microtiter wells, coated with receptor, were incubated with 7 nM  $^{125}\text{I}$ -labeled  $\alpha_2\text{M}$ :Me in the presence of increasing concentrations of unlabeled  $\alpha_2\text{M}$ :Me. The curve displays the best fit of the data to a single class of sites with a  $K_d$  of 57 nM. Each point represents the average of duplicates.

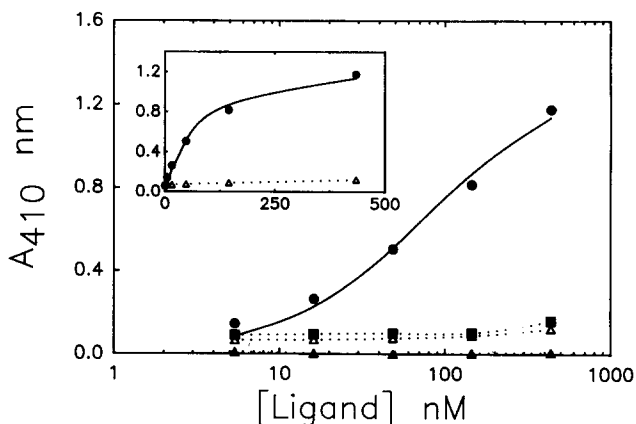
reconstitution conditions have not been achieved. Binding experiments, in which the purified 39- and 420-kD polypeptides were mixed before assay, suggest that the 39-kD polypeptide has little influence on the affinity of the 420-kD polypeptide for  $^{125}\text{I}$ -labeled  $\alpha_2\text{M}$ -trypsin.

### Specificity of the Purified Placental Protein for $\alpha_2\text{M}$ -Proteinase Complexes

An ELISA (10) was used to measure the specificity of the purified 420-kD polypeptide. For these experiments, affinity-purified native  $\alpha_2\text{M}$  (50) was used to ensure no contamination of this form with the proteolyzed form. The results of these experiments (Fig. 7) demonstrate the specific binding of activated  $\alpha_2\text{M}$  to microtiter wells coated with the purified 420-kD polypeptide. Very little binding of native  $\alpha_2\text{M}$  to the 420-kD protein was detected. Furthermore, no binding of any form of  $\alpha_2\text{M}$  to wells coated with BSA or with the purified human fibronectin receptor was observed. Thus, the purified placental protein demonstrates a similar specificity to that documented in studies examining the binding of derivatives of  $\alpha_2\text{M}$  to cells. The data reveal that the solid phase binding approaches saturation (Fig. 7, inset) and when fit to a single class of binding sites (Fig. 7, solid line) yielded an estimate for the  $K_d$  of 70 nM. This value is in good agreement with the  $K_d$  determined from the displacement experiments described earlier.

### Estimate of the Size of the Purified $\alpha_2\text{M}$ Receptor and Demonstration That This Polypeptide Is a Glycoprotein

Fig. 8 A shows an analysis of the purified polypeptide by SDS-PAGE. Comparison of its relative mobility with that of the reduced laminin A chain (~400 kD) confirm that this protein is unusually large. Extrapolation of the molecular



**Figure 7.** Specificity of the purified  $\alpha_2\text{M}$  receptor for activated  $\alpha_2\text{M}$ . The binding of native  $\alpha_2\text{M}$  ( $\Delta$ ) or  $\alpha_2\text{M}$ -trypsin complexes ( $\bullet$ ) to microtiter wells coated with purified 420-kD polypeptide were measured. Control experiments show the binding of  $\alpha_2\text{M}$ -trypsin complexes to microtiter wells coated with BSA ( $\Delta$ ) or with the fibronectin receptor ( $\blacksquare$ ). The binding of native  $\alpha_2\text{M}$  to BSA and the fibronectin receptor was identical to that shown for  $\alpha_2\text{M}$ -trypsin complexes. (Inset) Replot of the data in the form used for the fitting process. Solid line in both graphs represents the best fit of the data to a single class of sites, giving a  $K_d$  of 70 nM and  $A_{\text{max}}$  of 1.33.

mass standard calibration curve suggests that this protein has an apparent molecular mass of 420 kD under reducing conditions. Under nonreducing conditions, the mobility of the  $\alpha_2$ M receptor increases slightly, suggesting that the polypeptide has several internal disulfide bonds. In one experiment (not shown), the 420-kD polypeptide was transferred to immobilon and stained with anti-human  $\alpha_2$ M or anti-human laminin antibodies. These studies revealed that the placental protein is not related to any form of  $\alpha_2$ M or laminin. The presence of carbohydrate on the 420-kD polypeptide was determined by chromatography over immobilized wheat germ agglutinin. The 420-kD polypeptide was bound to this resin and could be eluted with 0.5 M *N*-acetyl-D-glucosamine. All of the  $\alpha_2$ M-proteinase binding ability was eluted with the 420-kD polypeptide.

#### Identification of a 420-kD Polypeptide from Affinity Chromatography of Surface-labeled Proteins from NRK Fibroblasts

Surface labeling experiments were performed to determine if the 420-kD protein is located on the cell surface. NRK fibroblasts were chosen for these experiments since the binding of  $\alpha_2$ M-proteinase complexes to this cell line is well characterized. Cell surface proteins were iodinated using lactoperoxidase. After washing, the cells were solubilized and applied to the  $\alpha_2$ M:Me-Sepharose affinity column. Fig. 8 B demonstrates an analysis of the material eluted from the affinity resin after SDS-PAGE and autoradiography. These results indicate that NRK fibroblasts contain a surface protein with a similar electrophoretic mobility to the human placental protein.

#### Discussion

The general interest in the mechanism of receptor-mediated endocytosis has prompted a number of studies describing the uptake, internalization, and degradation of various derivatives of  $\alpha_2$ M (8, 14, 27, 29, 30, 37, 54, 55). These investigations have yielded considerable insight into mechanisms involved in endocytosis. Despite some insight into the role of the  $\alpha_2$ M receptor in this process, information on its structure is extremely limited. The present studies were initiated with the goal of deriving information on this receptor system. Binding studies have reported the existence of a large number of  $\alpha_2$ M receptors in placental syncytiotrophoblasts (28). This observation, combined with the fact that the placenta has large quantities of receptors for a variety of proteins (43, 46, 51), suggested that this tissue might be an excellent source for the isolation of relatively large quantities of this receptor. Affinity chromatography was used to investigate the  $\alpha_2$ M receptor from this tissue.

This approach identified two polypeptides, with apparent molecular masses of 420 and 39 kD, that are specifically bound to an affinity column prepared by reacting native  $\alpha_2$ M with methylamine. The specificity of these two components for the conformationally altered form of  $\alpha_2$ M was documented by demonstrating that these polypeptides did not bind to a column prepared by coupling native  $\alpha_2$ M to Sepharose. Additional chromatography steps were used to separate these two components, and the results of these experiments indicated that the binding activity was associated

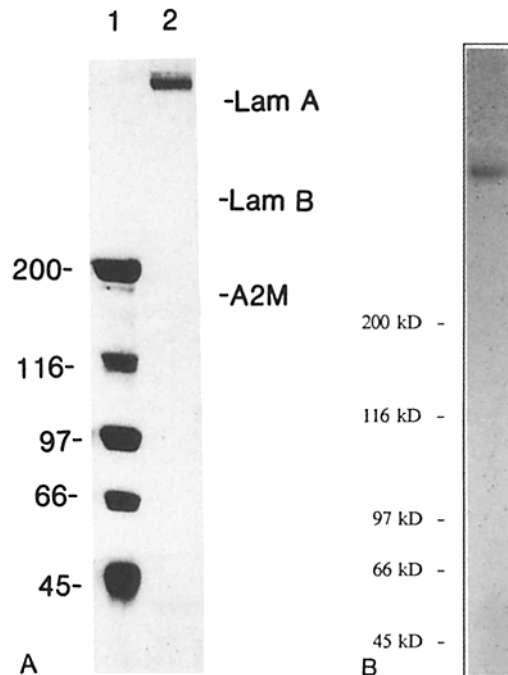


Figure 8. (A) Estimate of the size of the purified receptor by SDS-PAGE under reducing conditions on a 5% gel. (Lane 1) Standards, which include myosin,  $\beta$ -galactosidase, phosphorylase b, BSA, and ovalbumin; (lane 2) purified  $\alpha_2$ M receptor. The migration position of the  $\alpha_2$ M subunit and the A and B chains of laminin are also shown. (B) SDS-PAGE analysis of the material eluted from  $\alpha_2$ M:Me affinity chromatography after surface labeling of proteins from NRK fibroblasts. The migration position of standard proteins are shown.

with the 420-kD polypeptide. Considerable evidence has been obtained that indicates that this polypeptide has properties of the  $\alpha_2$ M receptor. Most important, the purified glycoprotein exhibits an identical specificity and a similar high affinity for the activated form of  $\alpha_2$ M that is displayed by the binding of  $\alpha_2$ M derivatives to cells. Very little, if any, binding of the purified protein to the native molecule was observed in the present study. Second, the purified protein demonstrates a similar requirement for metal ions, and a similar pH dependence for binding, that is found for the binding of proteolyzed  $\alpha_2$ M to cells. Third, surface labeling experiments indicate that the 420-kD polypeptide is located on the surface of fibroblasts. All of these data provide compelling evidence that the 420-kD glycoprotein is an  $\alpha_2$ M receptor.

At this time, the relationship between the 420- and 39-kD polypeptides is not known. The 39-kD polypeptide, like the 420-kD polypeptide, does not bind to native  $\alpha_2$ M immobilized on Sepharose and thus is specific for the affinity column containing activated  $\alpha_2$ M. Interestingly, this polypeptide does not appear to bind directly to  $^{125}$ I-labeled  $\alpha_2$ M-trypsin or  $\alpha_2$ M:Me. These results suggest that the 39-kD polypeptide is associated with the 420-kD polypeptide and could represent either a subunit of the  $\alpha_2$ M receptor or, possibly, a cytosolic protein that binds to the  $\alpha_2$ M receptor. Further experiments are required to determine the potential interaction between these two components and the significance of this interaction.

Most studies examining the binding of  $\alpha_2$ M-proteinase complexes (or the methylamine-reacted form) to cells have resulted in nonlinear Scatchard plots. The data have been interpreted to represent two classes of binding sites. At this time it is not clear if these two binding sites represent distinct molecules or, as suggested (18), whether they represent ligand binding to aggregated receptor molecules with high affinity and to single receptor molecules with a lower affinity. The binding affinity for the purified receptor recovered from the affinity column is similar to that measured for the binding of  $^{125}$ I-labeled  $\alpha_2$ M-trypsin complexes to solubilized membranes (15, 18). Further purification of the  $\alpha_2$ M receptor by anion exchange chromatography results in a molecule with a reduced binding affinity. It is quite likely that the methods used in the present study, in which microtiter wells are coated with purified receptor, are not optimal for receptor-ligand binding. This is particularly true if aggregation of the receptor is required for high affinity binding. Further, reconstitution of the receptor into appropriate phospholipid vesicles would likely result in an increased affinity of the 420-kD polypeptide for ligand. Studies are currently in progress to examine these and other possibilities. The fact that  $\alpha_2$ M contains four identical subunits and the observation that  $\alpha_2$ M appears to be a multivalent ligand for the receptor (53) further complicates interpretation of the binding interaction.

The large size of the  $\alpha_2$ M receptor in the present study differs from some previous reports. Three reports in the literature have described the partial purification of the  $\alpha_2$ M receptor from membranes of spontaneously transformed fibroblasts (NIH-3T3 cells) (17) and from human diploid fibroblasts (12, 35). There does not appear to be any general consensus as to the identity of this molecule from these reports. Hanover et al. (17, 19) identified two polypeptides (90 and 180 kD) as potential candidates for the  $\alpha_2$ M receptor. A second study (12) identified a single 125-kD component that appeared to bind to  $^{125}$ I- $\alpha_2$ M-trypsin complex after transfer of the polypeptide to nitrocellulose. Using affinity chromatography over immobilized  $\alpha_2$ M:Me, Marynen et al. (34) identified three components from  $^{35}$ S-labeled normal human fibroblasts with molecular masses of 360, 130, and 83 kD as the putative  $\alpha_2$ M receptor. The largest of these polypeptides has a molecular mass close to that obtained in the present study. The relationship between these proteins and the large single chain molecule identified in the present study remains to be determined. It is likely that some or all of these polypeptides may represent degradation products of the larger polypeptide, and it does not appear likely that fibroblasts contain a different and much smaller molecular mass receptor than that isolated in the present study since studies with surface-labeled fibroblasts identified a large molecular mass component as the major protein eluted from an  $\alpha_2$ M:Me affinity column. An 85-kD polypeptide was noted in some of the receptor preparations in the present studies. The presence of only trace amounts of this polypeptide in most preparations suggest that it is not responsible for the binding activity. It is possible that this polypeptide arises as a proteolytic product of the larger 420-kD polypeptide. This proposal is supported by Jensen and Pizzo (26) who noted an accumulation of an 80–90-kD polypeptide upon storage of their receptor preparation for several days. Further, incubation of the purified 420-kD polypeptide with elastase

(Cravens, J. L., and D. K. Strickland, unpublished observation) in the present study yielded several polypeptides with apparent molecular masses in the 66–90-kD range in addition to other distinct polypeptides. These results indicate that it is possible to generate fragments with similar molecular masses by proteolysis of the purified 420-kD polypeptide.

In excellent agreement with the present work is a report (15) examining the functional molecular mass of the  $\alpha_2$ M receptor by radiation inactivation. This approach, in conjunction with cross-linking studies, concluded that the  $\alpha_2$ M receptor is a large polypeptide with a molecular mass ranging from 420 to 500 kD. During the preparation of the present manuscript, two studies appeared in the literature describing large molecular mass polypeptides as components of the  $\alpha_2$ M receptor. The size of these polypeptides, isolated from human, baboon, rat, and mouse liver (26) and from rat liver membranes (38), agree with the size of the  $\alpha_2$ M receptor in the present investigation.

Few receptors have subunits as large as the  $\alpha_2$ M receptor identified in the present study. It is considerably larger than other well characterized receptors involved in receptor-mediated endocytosis. For example, the transferrin receptor is a dimer of 90 kD (44), while the low density lipoprotein receptor appears to be a single chain of 164 kD (45). The CRI (C3b/C4b) receptor, a molecule that binds to proteins closely related to  $\alpha_2$ M (47), is a polymorphic polypeptide (33) with a relatively large molecular mass (ranging from 160 to 250 kD). This receptor was found to contain 30 domains containing the short consensus repeat units also found in C4b/C3b binding protein (33). Herz et al. (21) have identified a large 500-kD liver membrane protein, closely related to low density lipoprotein receptor, whose function remains undefined. It is quite possible that a family of large molecular mass receptors exists on a variety of cells. The availability of large amounts of homogenous  $\alpha_2$ M receptor will facilitate structural determination of this molecule which will be of great value in establishing the role and function of this receptor.

This work was supported by U. S. Public Health Service grants HL30200 and GM42581 and by Research Career Development award HL-02113 to D. K. Strickland.

Received for publication 10 July 1989 and in revised form 13 November 1989.

#### References

1. Barak, L. S., and W. W. Webb. 1981. Fluorescent low density lipoprotein for observation of dynamics of individual receptor complexes on cultured human fibroblasts. *J. Cell Biol.* 90:595–604.
2. Barrett, A. J. 1981.  $\alpha_2$ -macroglobulin. *Methods Enzymol.* 80:737–754.
3. Barrett, A. J., M. A. Brown, and C. A. Sayers. 1979. The electrophoretically "slow" and "fast" forms of the  $\alpha_2$ -macroglobulin molecule. *Biochem J.* 181:401–418.
4. Bjork, I., and W. W. Fish. 1982. Evidence for similar conformational changes in  $\alpha_2$ -macroglobulin on reaction with primary amines or proteolytic enzymes. *Biochem J.* 207:347–356.
5. Brown, M. S., R. G. W. Anderson, and J. L. Goldstein. 1983. Recycling receptors: the round trip itinerary of migrant membrane proteins. *Cell.* 32:663–667.
6. Cheng, S. Y., F. R. Maxfield, J. Robbins, M. C. Willingham, and I. H. Pastan. 1980. Receptor-mediated uptake of 3,3',5-triiodo-L-thyronine by cultured fibroblasts. *Proc. Natl. Acad. Sci. USA.* 77:3425–3426.
7. Dennis, P. A., O. Saksela, P. Harpel, and D. B. Rifkin. 1989.  $\alpha_2$ -macroglobulin is a binding protein for basic fibroblast growth factor. *J. Biol. Chem.* 264:7210–7216.
8. Dickson, R. B., M. C. Willingham, and I. Pastan. 1981.  $\alpha_2$ -macroglobulin adsorbed to colloidal gold: a new probe in the study of receptor-



- mediated endocytosis. *J. Cell Biol.* 89:29-34.
9. Dickson, R. B., M. C. Willingham, and I. Pastan. 1981. Binding and internalization of  $^{125}\text{I}$ - $\alpha_2$ -macroglobulin by cultured fibroblasts. *J. Biol. Chem.* 256:3454-3459.
  10. Engvall, E., and P. Pearlman. 1972. Enzyme-linked immunosorbent assay, ELISA. III. Quantification of specific antibodies by enzyme labeled anti-immunoglobulin in antigen-coated tubes. *J. Immunol.* 109:129-135.
  11. Feldman, S. R., M. R. Rosenberg, K. A. Ney, G. Michalopoulos, and S. V. Pizzo. 1985. Binding of  $\alpha_2$ -macroglobulin to hepatocytes: mechanism of *in vivo* clearance. *Biochem. Biophys. Res. Commun.* 128:795-802.
  12. Frey, J., and E. G. Afting. 1983. Isolation and renaturation of  $\alpha_2$ -macroglobulin receptor from diploid human fibroblasts. *Biochem. J.* 214:629-631.
  13. Gliemann, J., and O. Davidsen. 1986. Characterization of receptors for  $\alpha_2$ -macroglobulin-trypsin complexes in rat hepatocytes. *Biochem. Biophys. Acta.* 855:49-57.
  14. Gliemann, J., and O. Sonne. 1985. Uptake and degradation of insulin and  $\alpha_2$ -macroglobulin-trypsin complex in rat adipocytes: evidence for different pathways. *Biochem. Biophys. Acta.* 845:124-130.
  15. Gliemann, J., O. Davidsen, and S. K. Moestrup. 1989. Characterization, size estimation and solubilization of alpha-macroglobulin complex receptors in liver membranes. *Biochem. Biophys. Acta.* 980:326-332.
  16. Gonias, S. L., J. A. Reynolds, and S. V. Pizzo. 1982. Physical properties of human  $\alpha_2$ -macroglobulin following reaction with methylamine and trypsin. *Biochem. Biophys. Acta.* 705:306-314.
  17. Hanover, J. A., J. E. Budick, M. C. Willingham, and I. Pastan. 1983.  $\alpha_2$ -macroglobulin binding to cultured fibroblasts: identification by affinity chromatography of high-affinity binding sites. *Arch. Biochem. Biophys.* 227:570-579.
  18. Hanover, J. A., S. Cheng, M. C. Willingham, and I. H. Pastan. 1983.  $\alpha_2$ -macroglobulin binding to cultured fibroblasts: solubilization and partial purification of binding sites. *J. Biol. Chem.* 258:370-377.
  19. Hanover, J. A., P. O'Souza, T. August, I. Pastan, and M. C. Willingham. 1986. Monoclonal antibodies against a glycoprotein localized in coated pits and endocytic vesicles inhibit  $\alpha_2$ -macroglobulin binding and uptake. *J. Biol. Chem.* 261:16732-16737.
  20. Hanover, J. A., M. C. Willingham, and I. Pastan. 1984. Kinetics of transit of transferrin and epidermal growth factor through clathrin-coated membranes. *Cell.* 39:283-293.
  21. Herz, J., U. Hamann, S. Rogne, O. Myklebost, H. Gausepohl, and K. K. Stanley. 1989. Surface location and high affinity for calcium of a 500-kDa liver membrane protein closely related to the LDL-receptor suggest a physiological role as lipoprotein receptor. *EMBO (Eur. Mol. Biol. Organ.) J.* 7:4119-4127.
  22. Huang, J. S., S. S. Huang, and T. F. Deuel. 1984. Specific covalent binding of platelet-derived growth factor to human plasma  $\alpha_2$ -macroglobulin. *Proc. Natl. Acad. Sci. USA.* 81:342-346.
  23. Imber, M. J., and S. V. Pizzo. 1981. Clearance and binding of two electrophoretic "fast" forms of human  $\alpha_2$ -macroglobulin. *J. Biol. Chem.* 256:8134-8139.
  24. Ingham, K. C., S. A. Brew, and B. S. Isaacs. 1988. Interaction of fibronectin and its gelatin-binding domains with fluorescently-labeled chains of type I collagen. *J. Biol. Chem.* 263:4624-4628.
  25. Isaacs, I. J., J. P. Steiner, P. A. Roche, S. V. Pizzo, and D. K. Strickland. 1988. Use of anti-idiotypic antibodies to establish that monoclonal antibody 7H11D6 binds to the  $\alpha_2$ -macroglobulin receptor recognition site. *J. Biol. Chem.* 263:6709-6714.
  26. Jensen, P. E. H., and S. V. Pizzo. 1989. Comparison of  $\alpha_2$ -macroglobulin receptors from human, baboon, rat, and mouse liver. *Biochem. Arch.* 5:171-176.
  27. Jensen, P. H., O. Davidsen, J. Gliemann, and C. M. Petersen. 1988. Cell association and degradation of pregnancy zone protein-chymotrypsin complex in cultured human monocytes. *Scand. J. Clin. Lab. Invest.* 48:165-176.
  28. Jensen, P. H., S. K. Moestrup, L. Sottrup-Jensen, C. M. Petersen, and J. Gliemann. 1988. Receptors for  $\alpha_2$ -macroglobulin- and pregnancy zone protein-proteinase complexes in the human placental syncytiotrophoblast. *Placenta.* 9:463-477.
  29. Kaplan, J., and E. A. Keogh. 1981. Analysis of the effect of amines on inhibition of receptor-mediated and fluid-phase pinocytosis in rabbit alveolar macrophages. *Cell.* 24:925-932.
  30. Kaplan, J., and E. A. Keogh. 1983. Studies on the physiology of macrophage receptors for  $\alpha_2$ -macroglobulin-protease complexes. *Ann. NY Acad. Sci.* 421:442-456.
  31. Kaplan, J., and M. L. Nielsen. 1979. Analysis of macrophage surface receptors. I. Binding of  $\alpha_2$ -macroglobulin-protease complexes to rabbit alveolar macrophages. *J. Biol. Chem.* 254:7323-7328.
  32. Kaplan, J., F. A. Ray, and E. A. Keogh. 1981. Recognition of nucleophile-treated  $\alpha_2$ -macroglobulin by the alveolar macrophage  $\alpha_2$ -macroglobulin-protease complex receptor. *J. Biol. Chem.* 256:7705-7707.
  33. Klickstein, L. B., W. W. Wong, J. A. Smith, J. H. Weis, J. G. Wilson, and D. T. Fearon. 1987. Human C3b/C4b receptor (CR1): demonstration of long homologous repeating domains that are composed of the short consensus repeats characteristic of C3/C4 binding proteins. *J. Exp. Med.* 165:1095-1112.
  34. Marynen, P., F. Van Leuven, J. J. Cassiman, and H. Van den Berghe. 1981. A monoclonal antibody to a neo-antigen on  $\alpha_2$ -macroglobulin complexes inhibits receptor-mediated endocytosis. *J. Immunol.* 127:1782-1786.
  35. Marynen, P., F. Van Leuven, J.-J. Cassiman, and H. Van den Berghe. 1984. Solubilization and affinity purification of the  $\alpha_2$ -macroglobulin receptor from human fibroblasts. *J. Biol. Chem.* 259:7075-7079.
  36. Maxfield, F. R. 1982. Weak bases and ionophores rapidly and reversibly raise the pH of endocytic vesicles in cultured mouse fibroblasts. *J. Cell Biol.* 95:676-681.
  37. Maxfield, F. R., M. C. Willingham, H. T. Haigler, P. Dragsten, and I. H. Pastan. 1981. Binding, surface mobility, internalization, and degradation of rhodamine-labeled  $\alpha_2$ -macroglobulin. *Biochemistry.* 20:5353-5358.
  38. Moestrup, S. K., and J. Gliemann. 1989. Purification of the rat hepatic  $\alpha_2$ -macroglobulin receptor as an approximately 440 kDa single chain protein. *J. Biol. Chem.* 264:15574-15577.
  39. Munson, P. J., and D. Rodbard. 1980. LIGAND: a versatile computerized approach for the characterization of ligand binding systems. *Anal. Biochem.* 107:220-239.
  40. Ney, K. A., S. Gidwitz, and S. V. Pizzo. 1984. Changes in the binding of "fast"-form  $\alpha_2$ -macroglobulin to 3T3-L1 cells after differentiation to adipocytes. *Biochemistry.* 23:3395-3403.
  41. O'Connor-McCourt, M. D., and L. M. Wakefield. 1987. Latent transforming growth factor  $\beta$  in serum: a specific complex with  $\alpha_2$ -macroglobulin. *J. Biol. Chem.* 262:14090-14099.
  42. Petersen, C. M., B. S. Christiansen, P. H. Jensen, S. K. Moestrup, J. Gliemann, L. Sottrup-Jensen, and J. Ingerslev. 1988. Human hepatocytes exhibit receptors for  $\alpha_2$ -macroglobulin and pregnancy zone protein-proteinase complexes. *Eur. J. Clin. Invest.* 18:184-190.
  43. Pytela, R., M. D. Pierschbacher, W. S. Argraves, S. Suzuki, and E. Ruoslahti. 1987. Arginine-glycine-aspartate adhesion receptors. *Methods Enzymol.* 144:475-489.
  44. Schneider, C., R. Sutherland, R. Newman, and M. Greaves. 1982. Structural features of the cell surface receptor for transferrin that is recognized by the monoclonal antibody OKT9. *J. Biol. Chem.* 257:8516-8522.
  45. Schneider, W. J., U. Beisiegel, J. L. Goldstein, and M. S. Brown. 1982. Purification of the low density lipoprotein receptor, an acidic glycoprotein of 164,000 molecular weight. *J. Biol. Chem.* 257:2664-2673.
  46. Siegel, T. W., S. Ganguly, S. Jacobs, O. M. Rosen, and C. S. Rubin. 1981. Purification and properties of the human placental insulin receptor. *J. Biol. Chem.* 256:9266-9273.
  47. Sottrup-Jensen, L., T. M. Stepanik, T. Kristensen, P. B. Lonblad, C. M. Jones, D. M. Wierzbicki, S. Magnusson, H. Domdey, R. A. Wetsel, A. Lundwall, B. F. Tack, and G. H. Fey. 1985. Common evolutionary origin of  $\alpha_2$ -macroglobulin and complement components C3 and C4. *Proc. Natl. Acad. Sci. USA.* 82:9-13.
  48. Straight, D. L., L. Jakoi, P. A. McKee, and R. Snyderman. 1988. Binding of  $\alpha_2$ -macroglobulin-thrombin complexes and methylamine-treated  $\alpha_2$ -macroglobulin to human blood monocytes. *Biochemistry.* 27:2885-2890.
  49. Strickland, D. K., and P. Bhattacharya. 1984. Kinetics of the conformational alterations associated with nucleophilic modification of  $\alpha_2$ -macroglobulin. *Biochemistry.* 23:3115.
  50. Strickland, D. K., J. P. Steiner, M. Migliorini, and F. D. Battey. 1988. Identification of a monoclonal antibody specific for a neoantigenic determinant on  $\alpha_2$ -macroglobulin: use for the purification and characterization of binary proteinase-inhibitor complexes. *Biochemistry.* 27:1458-1466.
  51. Turkewitz, A. P., J. F. Amatruda, D. Borhani, S. C. Harrison, and A. L. Schwartz. 1988. A high yield purification of the human transferrin receptor and properties of its major extracellular fragment. *J. Biol. Chem.* 263:8318-8325.
  52. Van Leuven, F., J. J. Cassiman, and H. Van den Berghe. 1979. Demonstration of an  $\alpha_2$ -macroglobulin receptor in human fibroblasts, absent in tumor-derived cell lines. *J. Biol. Chem.* 254:5155-5160.
  53. Van Leuven, F., P. Marynen, L. Sottrup-Jensen, J.-J. Cassiman, and H. Van Den Berghe. 1986. The receptor-binding domain of human  $\alpha_2$ -macroglobulin: isolation after limited proteolysis with a bacterial proteinase. *J. Biol. Chem.* 261:11369-11373.
  54. Willingham, M. C., F. R. Maxfield, and I. H. Pastan. 1979.  $\alpha_2$ -macroglobulin binding to the plasma membrane of cultured fibroblasts: diffuse binding followed by clustering in coated regions. *J. Cell Biol.* 82:614-625.
  55. Willingham, M. C., F. R. Maxfield, and I. Pastan. 1980. Receptor-mediated endocytosis of  $\alpha_2$ -macroglobulin in cultured fibroblasts. *J. Histochem. Cytochem.* 28:818-823.