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Characterization, size estimation and solubilization of α-macroglobulin complex receptors in liver membranes

Jørgen Gliemann, Ole Davidsen and Søren K. Moestrup

Institute of Physiology, University of Aarhus, Aarhus (Denmark)

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Receptors for α_2-macroglobulin-proteinase complexes have been characterized in rat and human liver membranes. The affinity for binding of ^{125}I-labelled α_2-macroglobulin · trypsin to rat liver membranes was markedly pH-dependent in the physiological range with maximum binding at pH 7.8–9.0. The half-time for association was about 5 min at 37°C in contrast to about 5 h at 4°C. The half-saturation constant was about 100 pM at 4°C and 1 nM at 37°C (pH 7.8). The binding capacity was approx. 300 pmol per g protein for rat liver membranes and about 100 pmol per g for human membranes. Radiation inactivation studies showed a target size of 466 ± 71 kDa (S.D., $n = 7$) for α_2-macroglobulin · trypsin binding activity. Affinity cross-linking to rat and human membranes of ^{125}I-labelled rat α_1-inhibitor-3 · chymotrypsin, a 210 kDa analogue which binds to the α_2-macroglobulin receptors in hepatocytes (Gliemann, J. and Sottrup-Jensen, L. (1987) FEBS Lett. 221, 55–60), followed by SDS-polyacrylamide gel electrophoresis, revealed radioactivity in a band not distinguishable from that of cross-linked α_2-macroglobulin (720 kDa). This radioactivity was absent when membranes with bound ^{125}I-α_1-inhibitor-3 complex were treated with EDTA before cross-linking and when incubation and cross-linking were carried out in the presence of a saturating concentration of unlabelled complex. The saturable binding activity was maintained when membranes were solubilized in the detergent 3-[(3-cholamidopropyl)dimethylammonio]propane sulfonate (CHAPS) and the size of the receptor as estimated by cross-linking experiments was shown to be similar to that determined in the membranes. It is concluded that liver membranes contain high concentrations of an approx. 400–500 kDa α_2-macroglobulin receptor soluble in CHAPS. The soluble preparation should provide a suitable material for purification and further characterization of the receptor.

Introduction

Human α_2-macroglobulin, a 720 kDa plasma protein consisting of four identical subunits, forms stable complexes with a wide variety of proteinases (for review, see Ref. 1). This causes the expression of previously concealed receptor recognition sites in the macroglobulin molecule, followed by rapid elimination of complexes from the circulation with a half-time of about 2 min in mice [2] and rats [3]. It has been demonstrated that this clearance is predominantly caused by receptor-mediated uptake into rodent [3,4] or human [5] hepatocytes.

The receptor binding of α_2-macroglobulin complex, i.e., α_2-macroglobulin · trypsin, has been studied extensively in hepatocytes [6] and other cell-types [7,8] at 4°C. However, binding at physiological temperatures cannot be readily measured separate from uptake in preparations of intact cells.

The purpose of this paper was to characterize α_2-macroglobulin · trypsin binding to rat and human liver membranes and to estimate the size of the receptor by radiation inactivation and by cross-linking the receptor to the 210 kDa ^{125}I-labelled α_1-inhibitor-3 · chymotrypsin. Moreover, since most α_2-macroglobulin receptors in the body are in the liver, hepatic membranes should be a suitable source for preparation and purification of the receptor. As the first step in this direction, we therefore wanted to see whether the receptor could recognize the ligand after solubilization in a suitable detergent.

Materials and Methods

Macroglobulins

Human α_2-macroglobulin was prepared from pooled citrate plasma using Zn^{2+} chelate affinity chromatography as previously described in detail in Ref. 9. Rat α_1-inhibitor-3, an approx. 190 kDa analogue of the α_2-macroglobulin monomer, was prepared from pooled EDTA plasma according to previously published procedures (see Ref. 10). These preparations were gifts from

Correspondence: J. Gliemann, Institute of Physiology, University of Aarhus, Universitetsparken, DK-8000 Aarhus C, Denmark.

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Dr. L. Sottrup-Jensen, Institute of Molecular Biology, University of Aarhus. The macroglobulins were iodinated using chloramin-T as an oxidizing agent as described in Refs. 6, 11. In brief, 0.4 nmol α_2-macroglobulin (1.6 nmol subunits) or 1.6 nmol α_1-inhibitor-3 in 20 μl 0.2 M sodium phosphate buffer (pH 8.0) was mixed with approx. 0.8 nmol ^{125}I (Amersham, U.K.) followed by the addition of 0.22 nmol chloramin-T in 5 μl phosphate buffer. The reaction was stopped after 1 min by the addition of 34 nmol $Na_2S_2O_5$ in 10 μl. The α_2-macroglobulin complex was formed by reacting for 5 min with a 10-fold excess of trypsin (Boehringer) followed by the addition of soybean trypsin inhibitor. α_1-inhibitor-3 was complexed with a 4-fold excess of chymotrypsin (Worthington, three-times crystallized) followed by the addition of phenylmethanesulfonyl fluoride to 1 mM. The labelled macroglobulin complexes were separated from iodine, decay products, trypsin or chymotrypsin and enzyme inhibitors by gel filtration on Sephacryl S-300 [6].

Protein concentrations were measured according to Bradford [12], using bovine serum albumin as a standard.

Membranes

Rat liver membranes were prepared, essentially as described by Cuatrecasas [13]. In brief, 10 g of rat liver was homogenized (Ultra-Turrax 525 KG-25 GM) for 2×30 s in 250 mM sucrose/10 mM Hepes/5 mM EDTA/0.1 mM phenylmethanesulfonyl fluoride (pH 8.0), followed by centrifugation at $48\,000 \times g$ for 40 min. The pellet (containing all binding activity) was rehomogenized in sucrose buffer and centrifuged at $20\,000 \times g$ for 30 min. The supernatant (containing all binding activity) was centrifuged at $48\,000 \times g$ for 40 min and resuspended in 140 mM NaCl/10 mM Hepes/2 mM $CaCl_2$/1 mM $MgCl_2$/0.1 mM phenylmethanesulfonyl fluoride (pH 8.0). This step was repeated once. All procedures were carried out at 0°C. The $48\,000 \times g$ pellet was frozen at −20°C in small aliquots containing about 4 mg protein/ml. The yield was about 5 mg membrane protein per g rat liver.

Biopsies of human liver were obtained from deceased patients after removal of the kidneys for use in transplantations. The samples were immediately frozen in liquid nitrogen. Specimens of about 5 g were thawed in the sucrose buffer and membranes were prepared as for rat liver with the following modifications: 100 μg/ml leupeptin was added to the sucrose buffer and the $20\,000 \times g$ centrifugation step was changed to $18\,500 \times g$.

Incubations

Digitonin (Sigma), purified as described previously in Ref. 14 was added to the membrane stock solution (0.30 mg digitonin per mg protein) prior to incubation. 60 μg membrane protein were incubated with about 15 pM ^{125}I-labelled complex in 200 μl 140 mM NaCl/10 mM Hepes/2 mM $CaCl_2$/1 mM $MgCl_2$/1% albumin (pH 7.8) at 4°C or 37°C. The incubations were stopped by the addition of 150 μl incubate to 500 μl microfuge tubes with 300 μl 0°C buffer followed by centrifugation for 3 min at about $10\,000 \times g$ and one wash of the pellet with 0°C buffer. Finally, the pellet was cut off and counted in a well-type gamma counter (efficiency 55%, background 7 cpm). The coefficient of variation for replicate values was 5–8%. The results of incubation experiments are presented as mean values of three replicates unless otherwise stated.

Control experiments showed that the activity bound to the membrane preparation – when corrected for radioactivity in trapped buffer – was recovered equally well after microfuge centrifugation with or without washing and after filtration on 0.2 μm Millipore GVWP filters.

Radiation inactivation

Samples of rat liver membranes, frozen in ethanol/solid CO_2 (−72°C) were irradiated using a linear accelerator producing electrons at 10 MeV. 10 mM benzoic acid and 10 mM mannitol were added to the membrane preparations (about 8 mg protein/ml) to minimize secondary damage of membrane proteins due to reactive oxygen species [15]. Control experiments showed that these additions upon dilution to assay conditions did not perturb the ^{125}I-macroglobulin · trypsin binding at 4°C. Doses (10–20 kGy per cycle) were measured by thermocalorimetry. The target size was determined using the equation $M_t = 6.4 \cdot 10^6 \cdot T_i/D_{37}$ [15], where D_{37} is the dose required (in kGy) to reduce binding activity to 37% of the control value at 30°C and T_i is the temperature correction factor of 1.9 for −72°C [16].

Cross-linking

The incubations were carried out for 20 h at 4°C in 140 mM NaCl/10 mM sodium phosphate/0.6 mM $CaCl_2$/1% albumin (pH 8.0). The labelled ligand was rat α_1-inhibitor-3 · chymotrypsin which was previously shown to bind to the α_2-macroglobulin receptors [11]. Binding in the phosphate buffer was not different from that obtained in the routine incubation buffer. Cross-linking of the membrane-bound receptor was performed by the addition with vigorous stirring of disuccinimidyl suberate (Pierce, IL, U.S.A.) dissolved to 50 mM in dimethylsulfoxide [18], followed by incubation at 0°C for 10 min. EDTA was added to a final concentration of 4 mM to remove Ca^{2+} and thereby dissociate noncross-linked ligand from the receptor [6]. One vol. of membrane suspension was layered onto 6 vols of 60 mM Tris-HCl (pH 6.8)/5% glycerol followed by centrifugation at $15\,000 \times g$ for 15 min. This provided an efficient wash of the pellet and a replacement of the

incubation buffer with a buffer suitable for electrophoresis. Finally, the pellet was dissolved in 5% SDS in 20 mM Tris-HCl/20% glycerol (pH 6.8), using 100 μl SDS buffer per 0.2 mg membrane protein.

Rat liver membranes (0.2 mg protein) were solubilized in 100 μl phosphate buffer with 3% CHAPS (Aldrich Chemical Co.) and incubated as described for membranes. Cross-linking of the 100000 × *g* supernatant (100 μl centrifuged for 10 min) was performed by incubation with 1.0 mM disuccinimidyl suberate for 5 min at 20 °C followed by the addition of 4 vols of 5% SDS sample buffer.

Electrophoresis was carried out according to Laemmli [19], using 80 mm long and 0.4 mm thick 4% acrylamide/bisacrylamide 30:0.8 slab gels and a sample size of 30 μl.

Results

Table I shows the binding of ^{125}I-labelled α_2-macroglobulin or α_1-inhibitor-3 complexes to rat or human liver membranes at 4 °C, pH 7.8. Digitonin, which in other experiments was found most effective at the displayed concentration of 0.30 mg per mg membrane protein, caused an almost 3-fold increase in binding to both types of membrane probably because permeabilization facilitates the access of ligand to the receptor sites. The presence of a saturating concentration (200 nM) of unlabelled α_2-macroglobulin · trypsin almost abolished the radioactivity associated with the cell pellet. The effect of 200 nM α_1-inhibitor-3 complex (data not shown) was similar. This indicated that the two ligands were bound to the same receptor, in agreement with previous results on rat hepatocytes [11]. Moreover, 15 pM ^{125}I-labelled uncomplexed α_2-macroglobulin or α_1-inhibitor-3 were bound by less than 1% to the membrane preparations in agreement with previous data on rat hepatocytes [6,11]. The residual activity in the presence of 200 nM unlabelled ligand corresponded to the amount of radioactivity in buffer trapped in the membrane pellet, i.e., a blank value. In all subsequent experiments, digitonin was present and binding was corrected for values obtained in the presence of 200 nM α_2-macroglobulin · trypsin.

TABLE I

Binding of ^{125}I-labelled human α_2-macroglobulin and rat α_1-inhibitor-3 complexes to liver membranes

The membrane concentration was 0.3 mg/ml and the labelled ligands were added to concentrations of about 15 pM. The incubation time was 20 h at 4 °C, pH 7.8. Digitonin was present at a concentration of 0.30 mg per mg membrane protein. Excess unlabelled refers to 200 nM α_2-macroglobulin · trypsin. % bound refers to radioactivity in the cell pellet in % total radioactivity added. The values represent mean values of four replicate incubations from a given membrane preparation ± S.D. The values for ^{125}I-macroglobulin · trypsin binding varied from 18 to 32% between rat membrane preparations and from 10 to 15% between human membrane preparations in the presence of digitonin.

	% Bound to liver membrane		
	no addition	plus digitonin	plus digitonin plus excess unlabelled ligand
Rat membranes			
^{125}I-α_2MT	8.4 ± 0.8	23.2 ± 1.3	0.5 ± 0.1
Human membranes			
^{125}I-α_2MT	5.5 ± 0.4 [a]	13.7 ± 1.0	0.4 ± 0.1
Rat membranes			
^{125}I-α_1I_3 complex	7.1 ± 0.4	19.7 ± 1.1	0.8 ± 0.2

[a] Binding measured by filter assay, since human membranes in the absence of digitonin did not pellet quantitatively after centrifugation in the microfuge.

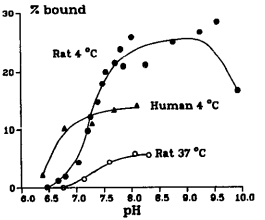

Fig. 1. pH-dependence of ^{125}I-labelled α_2-macroglobulin binding. The incubations were carried out in the presence of digitonin for 20 h at 4 °C (rat, ●; human, ▲) or for 1 h at 37 °C (rat, ○). The data are corrected for radioactivity associated with the cell pellet in the presence of 200 nM unlabelled complex. This amounted to 0.6–0.8% of the added tracer at all pH values.

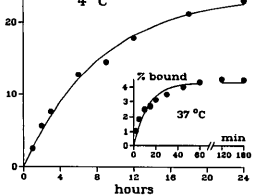

Fig. 2. Time-course of ^{125}I-labelled α_2-macroglobulin binding to rat liver membranes. The ordinate shows the % added tracer bound at 4 °C or 37 °C (inset).

pH-dependence and kinetic characterization

Fig. 1 shows that binding to rat liver membranes at 4°C was markedly dependent on pH with a maximum in the range of 7.8–9.0. The reduced binding at acid pH is probably due to a high dissociation rate constant causing a low affinity, see Fig. 3A. The apparent decrease in binding at pH 9.8 may be due to a partial solubilization of the membranes. Binding to rat membranes at 37°C was much lower than at 4°C, but the pH-dependence was qualitatively similar. The binding to human membranes showed a less steep pH-dependence around physiological pH. The following experiments were carried out at pH 7.8.

Fig. 2 shows a slow binding of ^{125}I-labelled α_2-macroglobulin · trypsin to rat membranes at 4°C with a half-time of about 5 h. The lower steady-state binding at 37°C is obtained much faster, i.e., with a half-time of about 5 min.

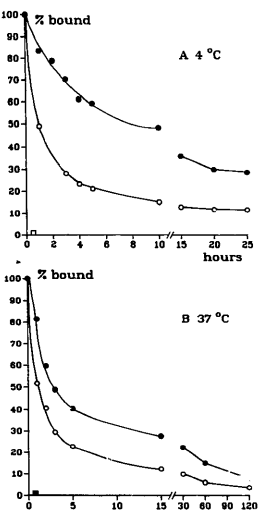

Fig. 3. Time-course of ^{125}I-labelled α_2-macroglobulin dissociation from rat liver membranes. The membranes were incubated for 20 h at 4°C, washed at 4°C to dilute tracer in the buffer more than 200-fold and incubated without (●) or with (○) 200 nM unlabelled complex. The ordinate shows the radioactivity remaining in the cell pellet as a percentage of that at time zero (i.e. immediately after wash) in 4°C (A) or 37°C (B) incubations. The open square shows the effect of pH 6.5 (4°C) and the filled square that of 4 mM EDTA (37°C) in the dissociation medium.

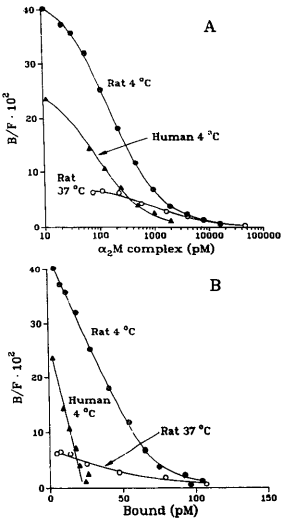

Fig. 4. Concentration-dependence of binding. Panel A shows the competition–inhibition plots for rat (●) or human (▲) liver membranes at 4°C and rat liver membranes at 37°C (○). The abscissa shows the concentration of free ligand (labelled plus unlabelled). The ordinate shows the ratio: membrane-bound/free ligand. Panel B shows the same data plotted in the Scatchard form. The 4°C curve for rat membranes represents the best fit to a two-receptor model with following calculated constants. High-affinity receptors: K_d = 84 pM, receptor concentration = 83 pM; low-affinity receptors: K_d = 1.6 nM, receptor concentration = 65 pM. K_d for binding to human membranes at 4°C (one-receptor model) was calculated as 80 pM and the receptor concentration as 24 pM.

Fig. 3 shows that the dissociation of prebound labelled complex is faster at 37°C (B) than at 4°C (A). Excess unlabelled complex accelerated the dissociation at both temperatures, although less markedly at 37°C. Dissociation at pH 6.5 (A) or in the presence of excess EDTA (B) rapidly abolished the binding.

Fig. 4 shows the concentration-dependencies as competition–inhibition plots (A) or in the Scatchard form (B) in rat and human membranes. Binding was mainly accounted for by receptors with an apparent dissociation constant (K_d) of about 100 pM at 4°C, although some sites with lower affinity were also present in the

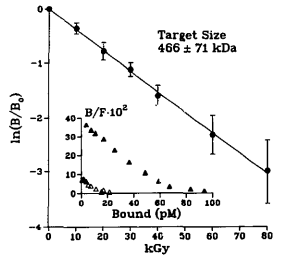

Fig. 5. Radiation inactivation of binding activity. The ordinate shows the natural logarithm to binding in membranes irradiated with a given dose divided by binding in nonirradiated membranes. The points are the mean values from seven experiments ± 1 S.D. The target size was calculated from the 7 regression lines as 466 ± 71 kDa (1 S.D.). The inset shows Scatchard plots of membranes irradiated with 40 kGy as compared with nonirradiated membranes.

rat membranes in agreement with previous results on rat hepatocytes [6]. An increase in the temperature to 37 °C caused a marked decrease in the receptor affinity.

Size estimation

Fig. 5 shows the effect of radioactive dose on α_2-macroglobulin · trypsin binding at 4 °C, pH 7.8. The data represent radioactivity in the membrane pellet after incubation with tracer in the absence of unlabelled complex minus radioactivity in the presence of 200 nM complex. It is important to note that the subtracted value remained unchanged at any dose. The binding activity declines exponentially and the target size is calculated as 466 ± 71 kDa (S.D., $n = 7$). Glucose-6-phosphate dehydrogenase was irradiated in the same series as an internal control and the target size was estimated as 97 kDa compared to 104 kDa determined by conventional biochemical methods [20].

TABLE II

Efficiency of cross-linking

Rat membranes were incubated with 100 pM ^{125}I-labelled α_2-macroglobulin · trypsin followed by the addition of disuccinimidyl suberate. After 10 min at 4 °C 1 vol. of 16 mM EDTA in 62 mM Tris (pH 6.8) was added to 3 vol. of membrane suspension followed by incubation at 37 °C for 10 min. The membranes were layered over 62 mM Tris/5% glycerol, then pelleted and dissolved in 5% SDS. The values refer to activity in the pelleted membranes after EDTA or in the 100000 × *g* supernatant after solubilization, both in % activity bound to membranes not treated with EDTA.

	Activity (% of control)						
Disuccinimidyl suberate (mM)	1.0	0.5	0.25	0.125	0.06	0.03	0
% bound after EDTA	98	104	92	79	42	17	0
% soluble in 5% SDS	42	72	86	77	43	19	0

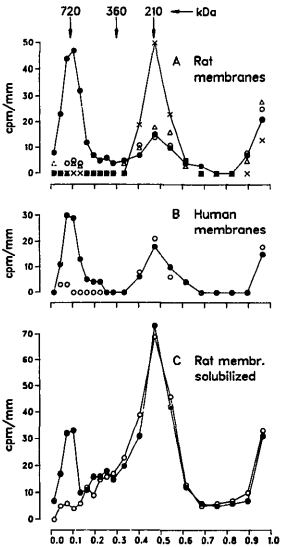

Fig. 6. Size of the cross-linked receptor. Panel A (rat) and B (human) shows the electrophoretic migration of α_1-inhibitor-3 cross-linked to membranes and solubilized in SDS. The membranes were incubated with approx. 200 pM labelled complex at 4 °C and cross-linked with 0.1–0.2 mM disuccinimidyl suberate, see legend to Table II. The membranes were solubilized in 5% SDS sample buffer and 30 µl applied to a 4% polyacrylamide gel. The dried gel was cut in 2–5 mm slices and counted. The marker proteins were α_1-inhibitor-3 · chymotrypsin (210 kDa), α_2-macroglobulin (360 kDa) and cross-linked α_2-macroglobulin (720 kDa). The symbols represent: cross-linked with tracer only (●), cross-linked in the presence of 80 nM unlabelled α_1-inhibitor-3 (○), 4 mM EDTA added before cross-linking (△). ^{125}I-labelled α_1-inhibitor-3 from the incubation medium, i.e. in the presence of cross-linker and membranes, is also shown (×). The results are the mean values of six (A) or four (B) replicate cross-linking experiments run on the same batch of gels. Panel C shows the pattern obtained when 150 µg membrane protein was solubilized in 100 µl phosphate buffer with 3% detergent (CHAPS) and incubated with ^{125}I-labelled α_1-inhibitor-3 in the absence (●) or presence (○) of 80 nM unlabelled complex. The results are the mean values of six experiments.

The estimation of target size relies on the assumption that increasing radiation dose causes a progressive disappearance of binding sites rather than a decrease in affinity. The inset of Fig. 5 shows that this is the case within the limits of interpretation of nonlinear Scatchard plots.

Cross-linking to the receptors was carried out using the 210 kDa α_1-inhibitor-3 complex, which has about the same affinity as α_2-macroglobulin complex for the receptor (Ref. 11, Table I). When using a cross-linking reagent, it is essential to aim at conditions which irreversibly link the ligand to the receptor with a minimum of attachment to other membrane components. Table II shows that disuccinimidyl suberate at concentrations higher than 0.5 mM causes complete irreversible cross-linking. The labelled protein is then poorly dissolved in 5% SDS. In addition, part of the solubilized radioactivity remained in the stacking gel after electrophoresis (data not shown). We used 0.1–0.2 mM disuccinimidyl suberate with 70–80% of the ^{125}I-labelled α_1-inhibitor-3 cross-linked and complete solubilization of the radioactivity in the 5% SDS sample buffer.

Fig. 6, panel A, shows the electrophoretic pattern of α_1-inhibitor-3 · chymotrypsin cross-linked to rat membranes. As markers in the Coomassie-blue-stained gels we used cross-linked α_1-inhibitor-3 · chymotrypsin (210 kDa), α_2-macroglobulin (360 kDa) and α_2-macroglobulin cross-linked to maintain the tetrameric form in the presence of SDS (720 kDa). ^{125}I-labelled α_1-inhibitor-3 · chymotrypsin in the incubation medium was cross-linked in the presence of membranes and used as a control. It migrated according to its molecular size (210 kDa). The migration of ^{125}I-labelled α_1-inhibitor-3 · chymotrypsin cross-linked to membranes was not distinguishable from the 720 kDa band. Radioactivity was absent in this band when membranes were cross-linked after incubation with tracer plus a saturating concentration of α_1-inhibitor-3, when EDTA was added after incubation but before cross-linking and when the procedure was carried out in the absence of disuccinimidyl suberate. Thus, the ligand was affinity cross-linked to a 400–500 kDa membrane protein. Panel B shows the same pattern for human membranes.

Panel C shows the pattern for rat membranes first solubilized, incubated with tracer and then cross-linked. A peak is seen in the 720 kDa region similar to that observed with cross-linked membranes. This peak is absent when cross-linking is carried out in the presence of excess unlabelled ligand. Thus, saturable binding can be detected after solubilization and the size of the putative receptors remains the same as that estimated in the membranes.

Discussion

The results obtained with liver membranes at 4°C in terms of kinetics and concentration-dependence are similar to those obtained previously with isolated hepatocytes at that temperature [5,6], except that the pH-dependence of binding has not been evaluated before. The α_2-macroglobulin complex receptors therefore retain their basic characteristics in the membrane preparation. The amount of high-affinity receptor present in membranes from one rat liver (7 g, approx. 40 mg membrane protein) is about 5 pmol, see legend to Fig. 4B. Previous morphometric results [3] have shown that a 7 g rat liver contains about $6 \cdot 10^8$ hepatocytes and these contain about 10 pmol high-affinity receptors as estimated from incubations at 4°C (Ref. 6, Fig. 5B, legend). This calculation, which should be taken with much caution, suggests that the membrane preparation contains about half of the α_2-macroglobulin complex receptors expressed on the surface of the hepatocytes.

One advantage of using isolated membranes is that binding can be determined independent of uptake. We find that the α_2-macroglobulin complex-receptor affinity is much lower at 37°C and that binding at low concentrations of the complex (far below the K_d) occurs with a half-time of about 5 min. Uptake of a low concentration of labelled complex in isolated hepatocytes at 37°C occurs with a half-time of about 60 min [21]. The rate-limiting step for uptake into hepatocytes is therefore the rate of endocytosis of receptor-bound complex rather than the rate of binding of complex.

We used radiation inactivation and affinity cross-linking of membrane-bound α_1-inhibitor-3 complex to determine the size of the receptor. Both methods estimate a functional size rather than the size of a putative binding subunit. Thus, the target size estimated from radiation inactivation studies has in several cases been shown to represent oligomers of membrane-associated proteins [22,23]. Likewise, cross-linking of the labelled ligand may occur not only to a single receptor in the membrane, but also to other proteins associated with high affinity to the receptor, including neighboring receptors.

The two methods gave similar estimates, i.e., 400–500 kDa. Even though it should be noted that this estimate is not very accurate with the techniques available, it is fair to conclude that the receptor is unusually large. It is possible, therefore, that the receptor consists of two or more subunits. If so, the subunits must remain closely associated in the detergent, since the size of the cross-linked solubilized receptor was not distinguishable from that of the cross-linked membrane-bound receptor. One possibility is that the receptor is a disulfide-linked oligomer. Unfortunately, it was not possible to test this hypothesis, since reduction caused fragmentation of α_1-inhibitor-3 [17].

The size of the α_2-macroglobulin receptor has previously been estimated in human fibroblasts [24,25] and fibroblast-like cells [26–28]. Hanover et al. [26] have solubilized membranes in octyl β-D-glucoside and mea-

sured the binding activity in the protein precipitate after removal of the detergent by dialysis. Gel filtration showed elution of most of the binding activity in a broad peak corresponding to approx. 160 kDa. Hanover et al. [27] subsequently radioiodinated the precipitate containing binding activity followed by solubilization in Triton X-100. A small fraction of the activity was retained on an affinity column and subsequently eluted in EDTA buffer. A major part of the radioactivity in the eluate (which did not contain binding activity) was in a 85 kDa band on SDS-polyacrylamide gels and this was tentatively identified as a component of the receptor. In a subsequent study, Hanover et al. [28] suggested that a 180 kDa protein, which readily fragments to a 90 kDa form, is a component of the receptor.

Frey and Afting [24] have lysed fibroblasts with receptors occupied by a saturating concentration of α_2-macroglobulin complex in Nonidet P-40 followed by affinity chromatography on immobilized protein A preloaded with antiserum against α_2-macroglobulin. Elution was performed with SDS followed by electrophoresis and transfer to nitrocellulose sheets. After renaturation, a 125 kDa band was able to bind α_2-macroglobulin complex as detected by incubations with anti-α_2-macroglobulin and labelled protein A. A potential problem with this technique is that a 125 kDa fragment of α_2-macroglobulin might be bound to the sheets and could be detected by the anti-α_2-macroglobulin.

Finally, Marynen et al. [25] have solubilized membranes from metabolically labelled fibroblasts in Triton X-100. Binding activity was detected using precipitation with poly(ethylene glycol) and was eluted from columns with immobilized α_2-macroglobulin complex together with two major (360 and 83 kDa) and one minor (130 kDa) labelled band identified by SDS-polyacrylamide electrophoresis.

No clear picture emerges from these previously published results. One possibility is that the receptor in fibroblasts is a 360 kDa protein and that the 130 kDa and 83–90 kDa components are proteolytic breakdown products of the receptor. If so, the size of the receptor in fibroblasts may be similar to that in hepatocytes, since our size estimates are compatible with a 400 kDa receptor. Affinity cross-linking of receptor preparations should be a valuable tool for comparing the receptor size in various cell types. Moreover, the solubilized hepatic receptor preparation with preserved binding activity should be suitable for purification of the quantitatively dominating type of α_2-macroglobulin complex receptors.

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