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Biochemistry

Identification and quantification of the rat hepatocyte asialoglycoprotein receptor

(monoclonal antibody)

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ABSTRACT **The asialoglycoprotein receptor from rat liver was purified by solubilization and affinity chromatography on asialoorosomucoid-Sepharose. The preparation yielded four distinct polypeptides of M_r 40,000–120,000. We prepared a monoclonal antibody that both immunoprecipitates solubilized receptor activity and blocks the binding of galactose-terminal glycoproteins to immobilized receptor. The monoclonal antibody and a rabbit antireceptor antiserum immunoprecipitated all four polypeptide species. Peptide analysis by two-dimensional chromatography of the individual ^{125}I-labeled species showed nearly identical patterns, which also suggested that the four polypeptides have a similar primary structure. To identify and quantitate the asialoglycoprotein receptor on the hepatocyte cell surface, intact cells were iodinated with lactoperoxidase, and the solubilized membranes were treated with antireceptor antibody. The M_r 55,000 and M_r 65,000 species were the major species found. Our results suggest that the M_r of the surface receptor is at least 55,000 and that it comprises between 1–2% of the iodinated hepatocyte surface protein.**

Once the terminal sialic acid is removed, circulating serum glycoproteins are rapidly and specifically cleared from the circulation by a receptor-mediated process (1). This receptor, localized to the hepatic parenchymal cell, is specific for galactose-terminal carbohydrates of glycoproteins (2). Quantification of asialoglycoprotein receptors on the rat hepatocyte has been limited to functional binding studies, which suggest that this protein is an abundant species (3) with as many as 500,000 surface receptors per cell (4). These receptors appear to represent a homogenous population with a K_d of approximately 10^{-9}M (3, 4). This receptor was purified after solubilization (with detergents) of an acetone powder of rat and rabbit liver and affinity chromatography. The major protein species in this preparation from rat liver has a M_r of ≈45,000 (5). In addition, minor proteins were found with M_rs ≈54,000 and 58,000. The M_r 45,000 species has been presumed to be the receptor polypeptide (5). However, with a specific binding activity of approximately 40 ng of ligand [asialoorosomucoid (ASOR), M_r 40,000] bound per μg of protein, the receptor may represent only a minor amount of this preparation, especially if one receptor molecule binds only one ligand molecule.

We report here one approach to the identification of the receptor protein, independent of functional ligand binding. A monoclonal antibody was raised against the asialoglycoprotein receptor protein. Studies with this antibody reveal that the asialoglycoprotein receptor is a protein of at least M_r 55,000. These results and also peptide mapping studies indicate that the lower M_r species are derivatives of this polypeptide. In addition, with the use of antiserum to the receptor, we have been able to quantitate the number of receptor molecules on the surface of the hepatocyte.

MATERIALS AND METHODS

Purification of Receptor. Asialoglycoprotein receptor was purified essentially as described by Hudgin *et al.* (6) from a Triton X-100 extract of rat liver acetone powder. Affinity chromatography was performed with ASOR-Sepharose (0.3 mg/ml of gel) or asialofetuin-Sepharose (10 mg/ml of gel). Receptor activity was determined after a 30-min incubation at 37°C in 1 M NaCl/40 mM $CaCl_2$/0.1% Triton X-100/100 mM Tris, pH 7.8 containing cytochrome *c* or bovine serum albumin (40 mg/ml) and ^{125}I-labeled ASOR (2 μg/ml), followed by ammonium sulfate precipitation (6). The specific activity varied from 5 to 30 ng of ^{125}I-labeled ASOR bound per μg of protein.

^{125}I-Labeling of Receptor. The receptor preparation was iodinated (^{125}I; Amersham) with immobilized lactoperoxidase and glucose oxidase (Enzymobeads, Bio-Rad), followed by exclusion chromatography on Sephadex G-25. As assayed by $NaDodSO_4$/polyacrylamide gel electrophoresis, the profile of ^{125}I-labeled protein was identical to that of the unlabeled receptor preparation. Specific radioactivities of 10^4–10^5 cpm/ng of protein were obtained. Storage, even at −70°C in the presence of cytochrome *c* (0.5 mg/ml), caused the appearance of bands at M_r ≈20,000 and M_r ≈10,000, presumably due to radiolysis.

Receptor Activity. Functional receptor activity was assessed in two ways. Soluble receptor activity was assayed essentially as described by Hudgin *et al.* (6) after binding of ^{125}I-labeled ASOR and ammonium sulfate precipitation. An additional assay (unpublished data) independent of ionic effects and detergent was developed in which soluble receptor was immobilized to polyvinyl chloride plastic in microtiter wells (Dynatech, Alexandria, VA) as described by Klinman *et al.* (7). In general, 0.4 μg of receptor in a 0.1-ml vol of phosphate-buffered saline was applied to each well for 4 hr at 4°C. Nonspecific binding to the plastic was blocked by subsequent treatment with 40 mg of cytochrome *c* or bovine serum albumin per ml for 2 hr. Wells were then washed with distilled water by immersion in a large beaker. ^{125}I-Labeled ASOR was incubated at tracer concentrations (8 ng/ml; specific radioactivity, 25 × 10^3 cpm/ng) or saturating concentrations (2 μg/ml; specific radioactivity, 1500 cpm/ng) in 10 mM Tris, pH 7.8/0.15 M NaCl/4 mg cytochrome *c* per ml/10 mM $CaCl_2$ or 5 mM EDTA. After removal of all unbound radioactivity by rinsing with cold 10 mM Tris, pH 7.8/0.15 M NaCl/10 mM $CaCl_2$ or 5 mM EDTA, ^{125}I bound to the plastic was determined by scintillation counting. Addition of 1000-fold excess of ASOR or asialofetuin reduced the amount of ^{125}I bound to the EDTA blank value.

Rabbit Anti-Receptor Antibody. Rabbits were immunized subcutaneously with 50 μg of soluble receptor in Freund's ad-

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Abbreviation: ASOR, asialoorosomucoid.

juvant. A second injection of 50 μg was given 3 wk later. Serum was collected 1 wk later and assayed by inhibition of binding (see below).

Monoclonal Antibody Isolation. BALB/c mice were immunized by repeated monthly intraperitoneal injections of 20 μg of purified receptor. When the serum demonstrated the ability to inhibit ^{125}I-labeled ASOR binding to immobilized receptor, the splenic lymphocytes were isolated and fused to P3 (P3/X63-AG8) myeloma cells as described (8). After a 14-day growth in selective media, the cells were initially screened for anti-receptor antibody with a fluorescence-activated cell sorter. The 50 most positive cells were regrown and rescreened with ^{125}I-labeled anti-mouse Fab fragment, and positive wells were cloned as single cells in soft agar (8). Individual clones (monoclonals) were rescreened by inhibition of ^{125}I-labeled ASOR binding to immobilized receptor and were grown as ascites in BALB/c mice (8).

Monoclonal Antibody Screening. *Fluorescence activated cell sorter.* Highly fluorescent (green) latex spheres (100 μl) of average diameter 0.77 μm (Polysciences, Warrington, PA) were coupled to 200 μg of affinity-purified receptor in 0.15 M NaCl with 16 mg of 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide·HCl at 4°C. With a coupling efficiency of approximately 30%, this produced 0.15×10^6 receptor molecules per bead. The beads were then incubated with the total population of fused cells, washed, and sorted under sterile conditions in a fluorescence-activated cell sorter (FACS II; Becton Dickinson, Sunnyvale, CA) similar to that described by Parks *et al.* (9).§

^{125}I-Labeled anti-mouse Fab. Aliquots of media from microtiter cloning wells were incubated at 4°C with immobilized receptor in microtiter dishes. After washing with phosphate-buffered saline, 20,000 cpm of ^{125}I-labeled anti-mouse Fab was added, and incubation at 4°C was continued. After again washing with phosphate-buffered saline, the amount of ^{125}I bound was determined by liquid scintillation counting. Negative values were associated with less than 50 cpm, whereas positive clones generally assayed in excess of 2000 cpm bound.

Inhibition of radioligand binding. Aliquots of media from microtiter cloning wells were incubated at 4°C with immobilized receptor. After the mixtures were washed with phosphate-buffered saline, 8 ng of high specific activity ^{125}I-labeled ASOR (generally 200,000 cpm) was added to each, and incubation at 4°C was continued. After another wash, bound ^{125}I-labeled radioligand was determined, with results expressed as percentage of control (no antibody present = 100%).

An additional method for measuring inhibition of binding utilized the soluble receptor preparation. After a 6-hr incubation of soluble receptor with culture media, ascites fluid, or antiserum at 4°C in 0.5 ml containing 1 M NaCl, 40 mM $CaCl_2$, 100 mM Tris (pH 7.8), 0.1% Triton X-100, and 20 mg of bovine serum albumin, staphylococcal protein A (IgGsorb; Enzyme Center, Boston) was added, incubated for 1 hr at 4°C, and centrifuged at $12,000 \times g$ for 2 min. The supernatant was assayed for receptor activity by using the ammonium sulphate precipitation assay.

Peptide Analysis by Two-Dimensional Chromatography. Peptide analysis was carried out as described (10). Briefly, 10% (wt/vol) $NaDodSO_4$/polyacrylamide gel electrophoresis was performed on the ^{125}I-labeled receptor. After autoradiography of the fixed, dried gel, appropriate protein bands were excised from the gel and incubated at 37°C with trypsin treated with *N*-tosylphenylalanine chloromethyl ketone (100 μg/ml) or chymotrypsin (100 μg/ml) in 1 ml of 20 mM NH_4HCO_3, pH 8.4/1 mM $CaCl_2$. The supernatants were lyophilized, applied to Whatman 3 MM paper, and subjected to electrophoresis at pH 3.5 at 52 V/cm for 1.5 hr, followed by autoradiography with Kodak SB 5 film at −70°C with Dupont Cronex intensifying screens. Selected peptides from parallel lanes were analyzed by descending liquid chromatography (*n*-butanol/acetic acid/water, 200:30:75, vol/vol) on Whatman 3 MM paper, followed by autoradiography.

^{125}I-Labeling of Hepatocytes. Isolated rat hepatocytes (4) were labeled with ^{125}I by incubating 10^6 cells with 1 mCi (1 Ci = 3.7×10^{10} becquerels) of ^{125}I in the presence of lactoperoxidase and glucose oxidase in Krebs–Ringer bicarbonate buffer containing 10 mM Hepes (pH 7.3), 5 mM $CaCl_2$, 1 mM phenylmethylsulfonyl fluoride, and 0.1 mg of soybean trypsin inhibitor per ml for 15 min at 4°C. The cells were washed twice by centrifugation in this buffer, resuspended in the same buffer, and disrupted by Dounce homogenization. All membranous material was recovered by centrifugation for 5 min at $12,000 \times g$, dissolved at 4°C in 1% Triton X-100 in phosphate-buffered saline containing 1 mM phenylmethylsulfonyl fluoride and 0.1 mg of soybean trypsin inhibitor per ml and stored at −70°C. Immunoprecipitation with washed staphylococcal protein A was performed in phosphate-buffered saline with 1% Triton X-100 and 5 mg of bovine serum albumin per ml. The immunoprecipitated material was analyzed by $NaDodSO_4$/polyacrylamide gel electrophoresis and autoradiography.

RESULTS

The rat liver asialoglycoprotein receptor, purified by affinity chromatography on ASOR-Sepharose or asialofetuin-Sepharose contained at least four discrete polypeptide species (Fig. 1, scan 1). The receptor protein was approximately 60% (Fig. 1, band A) M_r 40,000 species (equivalent to the M_r 45,000 species in ref. 2) and approximately 10–15% each of M_r 55,000, M_r 65,000 and M_r 100,000–120,000 species (bands B, C, and D, respectively). Isolation of the receptor in the presence of phenylmethylsulfonyl fluoride did not alter either the specific activity or the $NaDodSO_4$/polyacrylamide gel electrophoresis pattern. Furthermore, the presence or absence of 2-mercaptoethanol during the electrophoresis gave an identical pattern.

A monoclonal antibody was raised against the asialoglycoprotein receptor preparation. After screening with the fluorescence-activated cell sorter and ^{125}I-labeled anti-mouse Fab fragments, positive cloned cell lines were raised as ascites. Fig. 2*A* shows the ability of one such monoclonal ascites (C11) to bind detergent-solubilized receptor. Here the solubilized receptor preparation was mixed with antibody. Antigen–antibody complexes were removed by using staphylococcal protein A, and residual receptor activity in the supernatant was quantified. There was no effect of either control ascites containing a monoclonal antibody against a different antigen [azophenylarsonate coupled to bovine serum albumin (8)] or of staphylococcal protein A alone. An independent assessment of the potency and specificity of the ascites serum was the inhibition of binding ^{125}I-labeled ASOR to immobilized receptor (Fig. 2*B*). Immobilized receptor was incubated with antibody. After removing unbound antibody, saturating concentrations of ^{125}I-labeled ASOR were added to determine residual receptor activity. C11 ascites completely inhibited ^{125}I-labeled binding to immobilized receptor, whereas control monoclonal ascites had no significant effect.

§ Preliminary experiments were carried out by using a parent myeloma and a monoclonal cell line directed against the hapten azophenylarsonate coupled to a bovine serum albumin carrier. There was a perfect correlation between the coupling of appropriate fluorescent beads (azophenylarsonate-bovine serum albumin vs. serum alone) to the monoclonal cell line (producing anti-azophenylarsonate antibodies) when mixed with the parent myeloma.

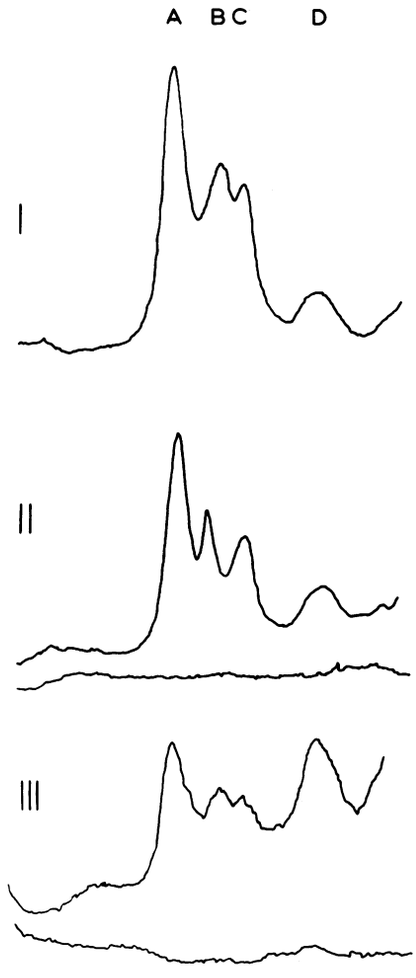

FIG. 1. Densitometric scans of $NaDodSO_4$/10% (wt/vol) polyacrylamide gel electrophoresis separation of immunoprecipitated ^{125}I-labeled receptor. ^{125}I-Labeled receptor was incubated with the appropriate serum for 12 hr at 4°C in buffer containing 0.5% Triton X-100, 0.25% deoxycholate, 0.5% $NaDodSO_4$, and 2.5 mg of bovine serum albumin per ml of phosphate-buffered saline. After precipitation with staphylococcal protein A, samples were separated by electrophoresis. Autoradiograms were scanned with a Joyce–Loebl microdensitometer with a wedge, so that the full-scale pen deflection represented 1.4 OD units, a value within the linear range of the film and the densitometer. The four distinct polypeptide species (A–D) correspond to apparent M_rs 40,000, 55,000, 65,000, and 100,000–120,000, respectively. Scans: I, ^{125}I-labeled receptor alone; II, rabbit anti-receptor antiserum (*Upper*) and preimmune serum (*Lower*); III, monoclonal ascites C11 (*Upper*) and control monoclonal ascites (*Lower*).

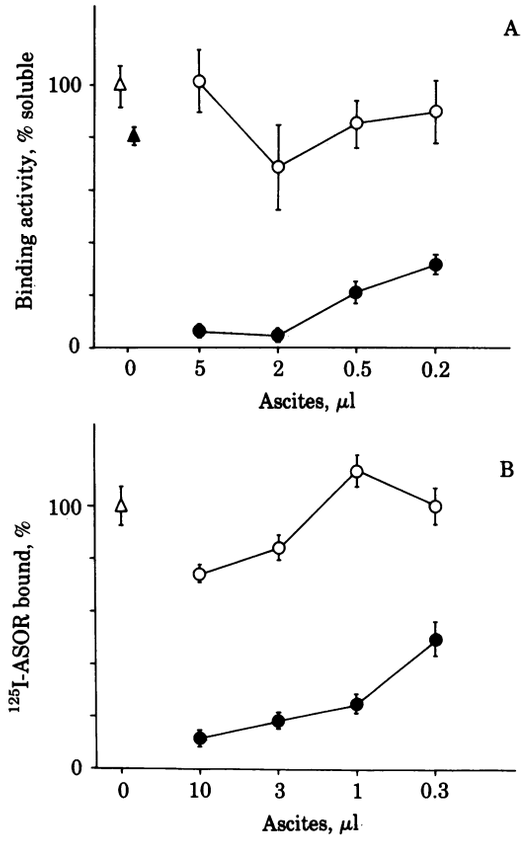

FIG. 2. (*A*) Precipitation of the solubilized receptor by monoclonal ascites C11. Solubilized receptor (1 μg) was incubated with either ascites C11 or a control monoclonal ascites. Antigen–antibody complexes were precipitated with staphylococcal protein A, and the supernatants were assayed for receptor activity. The 100% value represents 19×10^3 cpm. All values were corrected for nonspecific binding activity in the absence of calcium (0.9×10^3 cpm). Each figure represents the mean ± SEM for four determinations on two preparations. ▲, No ascites, no staphylococcal protein A; △, staphylococcal protein A alone; ●, monoclonal ascites C11 and staphylococcal protein A; ○, control monoclonal ascites and staphylococcal protein A. (*B*) Inhibition of the activity of immobilized receptor by monoclonal ascites C11. Immobilized receptor (0.2 μg) was preincubated with monoclonal ascites. After washing, a second incubation with 8 ng (200,000 cpm) of ^{125}I-labeled ASOR (^{125}I-ASOR) was performed. Bound radioactivity was determined, and the results are presented as mean ± SEM of four determinations. The 100% value represents 14×10^3 cpm. All values were corrected for binding in the absence of calcium (2×10^3 cpm). △, No ascites; ●, monoclonal ascites C11; ○, control monoclonal ascites.

Taken together, these data suggest that the monoclonal C11 is either directed against the ligand binding site on the receptor or against another site on the receptor protein, binding to which alters the configuration necessary for ligand binding.

All four polypeptides present in the preparation of ^{125}I-labeled receptor were immunoprecipitated by the monoclonal serum (Fig. 1). Each protein species was precipitated to nearly the same relative extent. Essentially no immunoprecipitation of ^{125}I-labeled receptor was found with control monoclonal ascites or rabbit preimmune serum, whereas rabbit antiserum prepared against the receptor preparation also immunoprecipitated all four polypeptides (Fig. 1). Therefore, either the four protein species are closely related (i.e., share the same monoclonal determinants) or are very tightly associated, despite the use of potent detergents, in immunoprecipitation mixtures.

Peptide mapping of the ^{125}I-labeled polypeptides revealed that all four proteins have a similar primary structure (Fig. 3). The trypsin and chymotrypsin digest of the polypeptides were resolved by paper electrophoresis at pH 3.5. Subsequent chromatographic analysis of individual peptides from parallel lanes revealed that the peptides that comigrated on electrophoresis indeed were identical (data not shown).

Analysis of the four individual protein species also was carried out by simultaneous partial protease digestion with staphylococcal V8 protease or chymotrypsin according to Cleveland *et al.* (11) All proteolytic fragments generated from the four protein species exhibited the same relative mobility in $NaDodSO_4$/polyacrylamide gel electrophoresis (data not presented).

Which of these polypeptides are present on the surface of hepatocytes and what fraction do they represent of total surface protein? To answer these questions, the surface of intact hepatocytes was labeled with ^{125}I. Membranes were solubilized and immunoprecipitated with preimmune serum or immune

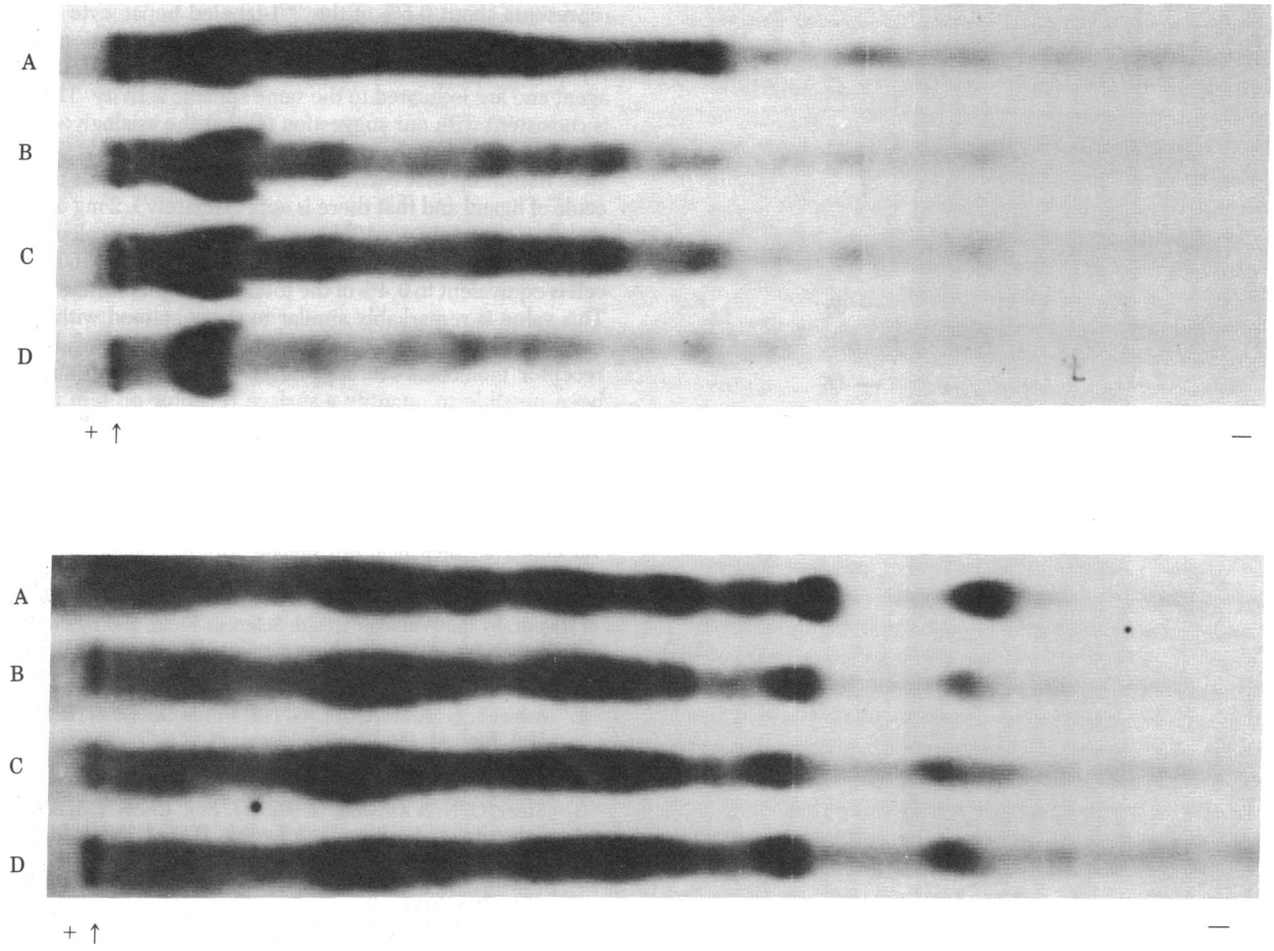

FIG. 3. High-voltage electrophoresis separation of ^{125}I-labeled peptides after protease digestion of isolated ^{125}I-labeled receptor polypeptides. Individual polypeptides of the ^{125}I-labeled receptor were isolated by NaDodSO$_4$/polyacrylamide gel electrophoresis and digested with either trypsin treated with *N*-tosylphenylalanine chloromethyl ketone (*Upper*) or chymotrypsin (*Lower*) *in situ*. ^{125}I-Labeled peptides were separated by paper electrophoresis and visualized by autoradiography. The individual polypeptides are lettered as in Fig. 1.

rabbit antiserum. Immunoprecipitation with anti-receptor antibody of ^{125}I-labeled hepatocyte surface proteins revealed two major bands of M_r ≈55,000 and M_r ≈65,000 (Fig. 4). Neither of these bands were present when preimmune or control serum were used, and immunoprecipitation of both of these species was specifically inhibited by the presence of 1 μg of purified receptor in the immunoprecipitation mixture. These species represent approximately 90% of the immunoprecipitated material. Of note is the small amount (5%) of radioactivity at M_r 40,000. Identical results were obtained with the monoclonal antibody C11 and control monoclonal antibody.

DISCUSSION

The asialoglycoprotein receptor of the rat hepatocyte was purified by affinity chromatography and characterized with an anti-receptor monoclonal antibody and peptide analysis by two-dimensional chromatography. The receptor preparation is composed of four polypeptide species of M_r 40,000, 55,000, 65,000 and 100,000–120,000, all of which may contain the ligand binding site. Peptide analysis after digestion with trypsin, chymotrypsin, or staphylococcal V8 protease has shown that all four species have a similar, if not identical, primary sequence. This suggests that the lower M_r proteins are proteolytic fragments of the native protein, although alterations in the carbohydrate or lipid content, or both, of certain species might alter their apparent M_rs. Consistent with this notion is our finding that the M_r 55,000 and M_r 65,000 polypeptides are the major species labeled on the surface of intact hepatocytes with ^{125}I and lactoperoxidase. This suggests that proteolytic processing of the liver receptor occurs during the solubilization or affinity chromatography steps of purification. It is presently not clear whether the receptor is a molecule of M_r 55,000, M_r 65,000, or M_r 100,000–120,000. However, preliminary observations have demonstrated that association of isolated M_r 55,000 or M_r 65,000 molecules will take place to form a species of apparent M_r of about 110,000 or 120,000 when it is boiled in NaDodSO$_4$ solutions preparatory to electrophoresis (data not shown). The absence of additional ^{125}I-labeled peptides in the M_r 100,000–120,000 species compared to the M_r 55,000 or M_r 65,000 species is consistent with the notion that the M_r 100,000–120,000 species are dimers of the lower M_r species. Consistent with this notion is the recent high-resolution NaDodSO$_4$/polyacrylamide gel electrophoresis (C. N. Steer and G. Ashwell, personal communication) which demonstrates three distinct bands in the M_r 100,000–120,000 region. Thus, it appears most likely that the asialoglycoprotein receptor of rat hepatocytes is a molecule of at least M_r 55,000 and may represent two nonidentical subunits. The rabbit liver asialoglycoprotein receptor appears to be composed of two nonidentical subunits (M_r 40,000 and M_r 48,000) (12). The identity of the ligand binding subunit is not presently known.

The amount of receptor on the hepatocyte cell surface has

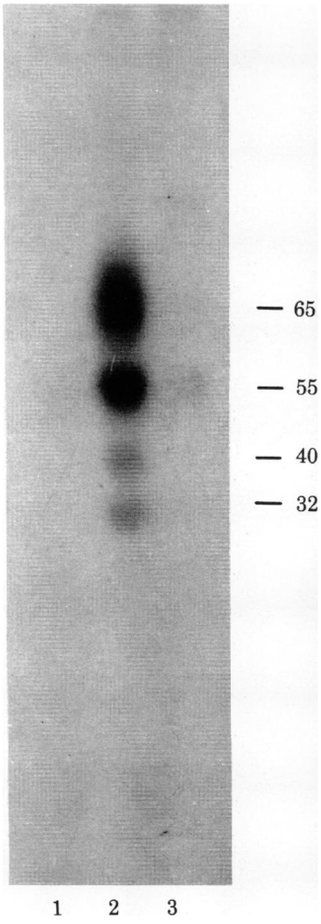

FIG. 4. Autoradiogram of immunoprecipitation of labeled asialoglycoprotein receptor from ^{125}I-surface-labeled hepatocytes. Isolated hepatocytes were surface-labeled with ^{125}I, and membranes were prepared and solubilized with detergent. Immunoprecipitation was with preimmune serum (lane 1), anti-receptor antiserum (lane 2), or anti-receptor antiserum in the presence of 1 μg of unlabeled receptor (lane 3). M_rs are $\times 10^{-3}$.

been quantitated by immunoprecipitation with anti-receptor antibody after ^{125}I labeling of the hepatocyte. From densitometric scans of the data in Fig. 4 and the percentage of immunoprecipitated radioactivity, it can be calculated that each of the M_r 55,000 and M_r 65,000 asialoglycoprotein receptors represents about 0.5% of the ^{125}I-labeled hepatocyte cell-surface proteins. This calculation carries the assumption that all surface proteins have residues susceptible to the iodination reagent and are iodinated to the same specific activity. This value is consistent with our suggestion (4) that the asialoglycoprotein receptor is an abundant species on the rat hepatocyte cell surface. Assuming that each molecule of receptor binds one molecule of ligand and that there is approximately 1.2 mg of plasma membrane protein per 10^8 hepatocytes (2, 13), we calculate that 500,000 functional surface receptor molecules of M_r 60,000 per cell is equivalent to 0.4% of the total plasma membrane protein. This value is remarkably similar to that obtained with anti-receptor antibody. Thus, it appears that the majority of all surface receptor molecules are capable of ligand binding, and it has been possible to quantify a surface receptor protein independent of ligand binding. In addition, our anti-receptor monoclonal antibody will now permit detailed studies of receptor topology and biosynthesis.

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1. Ashwell, G. & Morell, A. G. (1974) *Adv. Enzymol. Relat. Areas Mol. Biol.* **41**, 99–128.
2. Pricer, W. E. & Ashwell, G. (1971) *J. Biol. Chem.* **246**, 4825–4833.
3. Steer, C. N. & Ashwell, G. (1980) *J. Biol. Chem.* **255**, 3008–3013.
4. Schwartz, A. L., Rup, D. & Lodish, H. F. (1980) *J. Biol. Chem.* **255**, 9033–9036.
5. Tanabe, T., Pricer, W. E. & Ashwell, G. (1979) *J. Biol. Chem.* **254**, 1038–1043.
6. Hudgin, R. L., Pricer, W. E., Ashwell, G., Stockert, R. & Morell, A. G. (1974) *J. Biol. Chem.* **249**, 5536–5543.
7. Klinman, N. R., Pickard, A. R., Sigal, N. H., Gearhart, P. J., Metcalf, E. S. & Pierce, S. K. (1976) *Ann. Immunol. (Paris)* **127C**, 489–502.
8. Marshak-Rothstein, A., Fink, P., Gridley, T., Raulet, D. H., Bevan, M. J. & Gefter, M. L. (1979) *J. Immunol.* **122**, 2491–2497.
9. Parks, D. R., Bryan, V. M., Boi, V. T. & Hertzenberg, L. A. (1979) *Proc. Natl. Acad. Sci. USA* **76**, 1962–1966.
10. Morrison, T. G. & Lodish, H. F. (1974) *J. Biol. Chem.* **249**, 5860–5866.
11. Cleveland, D. W., Fischer, S. G., Kirschner, M. W. & Laemmli, U. K. (1977) *J. Biol. Chem.* **252**, 1102–1106.
12. Kawasaki, T. & Ashwell, G. (1976) *J. Biol. Chem.* **251**, 1296–1302.
13. Seglen, P. O. (1975) *Methods Cell Biol.* **13**, 29–83.