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Purification of a Receptor for Formaldehyde-treated Serum Albumin from Rat Liver*

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A membrane-associated receptor involved in a specific uptake of formaldehyde-treated serum albumin (f-Alb) was purified from rat livers by Triton X-100 solubilization of a 105,000 × *g* membrane preparation and affinity chromatography on an f-Alb-Sepharose column. The purified receptor exhibited M_r = 125,000, consisting of two noncovalently linked glycoprotein components with M_r = 53,000 and M_r = 30,000, respectively. Incubation of ^{125}I-receptor with f-Alb, but not with native albumin, resulted in a marked shift of pI value from 5.9 to 5.1, reflecting the presence of a specific ligand-receptor interaction. The receptor incorporated into liposomes displayed a saturable binding to ^{125}I-f-Alb and the binding was effectively replaced by the presence of unlabeled f-Alb, with binding parameters being similar to those obtained from ^{125}I-f-Alb binding to the sinusoidal liver cell membrane (Horiuchi, S., Takata, K., and Morino, Y. (1985) *J. Biol. Chem.* 260, 475–481). Reaction of anti-f-Alb receptor antibody with extracts of sinusoidal cells resulted in a specific precipitation of two proteins whose molecular weights were identical to those for the purified receptor. The anti-receptor IgG fraction effectively blocked ^{125}I-f-Alb binding to the sinusoidal cell membranes. These results indicate that the purified protein represents the membrane-associated receptor which is presumably involved in a specific uptake of this ligand from the circulation.

The phenomenon that, when treated *in vitro* with formaldehyde, serum albumin was rapidly cleared from the bloodstream was discovered in the middle 1960s (1). The specific uptake of this chemically modified albumin was attributed to the membrane associated receptor present on sinusoidal liver cells (2). Physiological function of this membrane-associated receptor remains unknown; it is uncertain whether the same modification could take place *in vivo*, or whether there could be a natural ligand for this receptor in the serum or plasma. However, since this ligand is also effectively taken up by macrophages via a receptor-mediated pathway (3, 4), it is relevant to suggest that the membrane-associated receptor for this ligand belongs to a family of scavenger receptors present on macrophages or macrophage-derived cells. These include the receptors for immune complexes (5, 6), the α_2-macroglobulin-protease complex (7–9), acetylated low-density lipoprotein (10), and glycoproteins with terminal mannose and/or *N*-acetylglucosamine (11–13) or fucose residues (14).

Previously, we have characterized the membrane-associated receptor specific for f-Alb[1] in sinusoidal liver cells, and identified two membrane proteins of M_r = 53,000 and M_r = 30,000, respectively (15). However, it was not known whether the two polypeptide chains represent subunits of a receptor or two distinct binding proteins. In order to obtain a clearer picture of this receptor as well as to facilitate understanding of its physiological role, we have attempted to purify the receptor from the rat livers.

In the current investigation, we describe, first, the purification of the receptor from rat livers, second, the properties of the purified receptor and its interaction with the ligand, and finally, topology of the receptor on sinusoidal liver cell surface by using the specific antibody raised against this receptor.

MATERIALS AND METHODS

Chemicals—BSA (Fraction V) was purchased from Sigma. Radioactive iodine ($Na^{125}I$) and [^{35}S]methionine (1000 mCi/mmol) were obtained from Amersham. Sepharose 4B and Polybuffer (isoelectric focusing kit) were obtained from Pharmacia Fine Chemicals, Sweden. Enzymobeads and Dowex AG 1-X2 (200–400 mesh) were purchased from Bio-Rad (Richmond, California). All reagents used were of analytical grade. f-Alb-coupled Sepharose 4B was prepared as described (15). Concanavalin A was purified and coupled with Sepharose 4B as described previously (16).

Purification of a Receptor from Rat Liver—Livers of Wistar rats were excised after flushing out the blood with 20 ml of ice-cold 10 mM Tris-HCl (pH 7.4), 0.15 M NaCl via the portal vein. Livers from five rats (approximately 50 g) were homogenized in 200 ml of 0.25 M sucrose buffered with 10 mM Tris-HCl to pH 7.4. After removing the nuclear fraction at 500 × *g* for 10 min, homogenates were centrifuged at 7,000 × *g* for 10 min. The resulting supernatant (postmitochondrial fraction) was saved. The pellet was resuspended in 50 ml of the same buffer and recentrifuged at 7,000 × *g* for 10 min. The resulting supernatant was combined with the first postmitochondrial fraction, followed by centrifugation at 105,000 × *g* for 1 h at 2 °C. The supernatant was discarded and the pellet was resuspended in 10 ml of ice-cold buffer A (20 mM Tris-HCl (pH 7.8), 0.15 M NaCl, 0.5 mM $CaCl_2$, and 1 mM phenylmethylsulfonyl fluoride). The membrane suspension was recentrifuged at 105,000 × *g* for 1 h at 2 °C. The resulting pellet (crude membrane fraction) was resuspended in 40 ml of ice-cold buffer A. To this was added 20% of Triton X-100 (v/v) at a final concentration of 2%, followed by incubation for 30 min at 4 °C. Insoluble debris was removed by centrifugation at 105,000 × *g* for 1 h at 2 °C. A solution of solubilized membrane proteins (50 ml, 10.4 mg/ml) was applied on a column (2.6 × 10 cm) of Sepharose 4B coupled with f-Alb which had been equilibrated with buffer A containing 1% Triton X-100. The sample was recycled over the affinity column for 2 h (2–3 cycles). The column was then washed extensively with 2 liters of the same buffer until the effluent fraction (Fraction A) exhibited a protein concentration of less than 1 μg/ml as deter-

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[1] The abbreviations used are: f-Alb, formaldehyde-treated bovine serum albumin; BSA, bovine serum albumin; $NaDodSO_4$, sodium dodecyl sulfate; Staph A, formalin-fixed *Staphylococcus aureus*, Cowan strain A; acetyl-LDL, acetylated low-density lipoprotein.

mined by the dye-binding method (see below). The column was then eluted with 300 ml of buffer B (20 mM sodium phosphate buffer (pH 6.0), 0.15 M NaCl, 0.5 mM $CaCl_2$, and 1% Triton X-100) (Fraction B). The final elution was achieved with 200 ml of buffer C (50 mM glycine HCl buffer (pH 3.0), 0.1 M NaCl, 0.5 mM $CaCl_2$, and 1% Triton X-100) (Fraction C). Fraction C was immediately adjusted to pH 7.8 by adding 1 M Tris-HCl buffer (pH 9.0), followed by concentration and dialysis against buffer A containing 0.1% Triton X-100 in an Amicon Diaflow apparatus equipped with a PM-10 membrane. Unless otherwise stated, all the procedures were performed at 4 °C. The concentrated solution of Fraction C (~5 ml) was further applied to the second affinity column of Sepharose 4B-f-Alb (1 × 5 cm) which had been equilibrated with buffer A containing 1% Triton X-100. The sample was recycled over the column for 1 h (3–4 cycles). The column was washed consecutively with 200 ml of buffer A containing 1% Triton X-100 and 100 ml of buffer B. Then, the fractions eluted with 50 ml of buffer C were immediately adjusted to pH 7.8 with 1 M Tris-HCl (pH 9.0) and subjected to concentration and dialysis in an Amicon apparatus as described above. Aliquots of each fraction obtained during the purification were incorporated into liposomes (see below) and determined for binding activity toward ^{125}I-labeled f-Alb.

Iodination—f-Alb was prepared from BSA as described (15) by a modification of the methods reported (1, 2). f-Alb was labeled with ^{125}I to a specific radioactivity of 4–6 × 10^3 cpm/ng by using immobilized Enzymobead lactoperoxidase-glucose oxidase (Bio-Rad) according to the manufacturer's instructions. Free iodine was removed by rapid gel filtration on a mini-column of Sephadex G-25 (Pharmacia Fine Chemicals) as reported (17). Iodination of the purified receptor was performed as follows; 10–20 μg of the purified receptor (Fraction C) dissolved in buffer A containing 0.1% Triton X-100 was iodinated to a specific radioactivity of 2.0 × 10^6 cpm/μg in the same way as described above except that the rapid gel filtration on a mini-column was performed in the presence of 0.1% Triton X-100. The iodinated receptor thus obtained showed more than 99% trichloroacetic acid-precipitable radioactivity. The protein concentration was determined by the method of Lowry *et al.* (18) or by the dye-binding method reported by Bradford (19) with BSA as a standard.

Binding Assay—Liposomes were prepared from egg lecithin (Merck) by a modification of the method employed by Schneider et al. (20). Briefly, egg lecithin (400 mg) was dissolved in 20 ml of ethanol in a 50-ml glass flask. Ethanol was removed under a stream of nitrogen. The dry phospholipid was redissolved in diethyl ether, and a coat of phosphatidyl choline was formed on the inner walls of the flask by evaporating the ether with a stream of nitrogen. A buffer solution (100 ml) containing 50 mM Tris-maleate (pH 6.0) and 2 mM $CaCl_2$ was then added, and a suspension of liposomes was formed by shaking for 5 min on a Vortex mixer. Prior to the binding assay, Triton X-100 was removed from each sample with Bio-Beads SM-2 (Bio-Rad) according to the reported method (21). To the detergent-free samples was added octyl β-D-glucoside to give a final concentration of 40 mM. Incorporation of the solubilized receptor into lipid vesicles was performed by the acetone-precipitation method as reported (20). After vigorous Vortex agitation with cold acetone, liposome precipitates were centrifuged at 20,000 × *g* for 20 min and resuspended in 20 mM Tris-HCl (pH 8.0), 50 mM NaCl, and 1 mM $CaCl_2$ and used for the measurement of ^{125}I-f-Alb binding activity.

A standard binding assay was conducted at pH 8.0 in 100 μl of buffer containing 50 mM Tris-HCl, 0.5 mM $CaCl_2$, 0.1 M NaCl, and 40 mg/ml of BSA in a 1.6-ml polyethylene tube (Eppendorf centrifuge tube). Unless otherwise stated, assay mixtures contained liposome preparations containing graded amounts (0.02–50 μg) of solubilized membrane proteins and 3.75 μg/ml of ^{125}I-f-Alb (4,670 cpm/ng of protein) in the absence or presence of an excess amount of f-Alb (1 mg/ml). After incubation for 1 h at 25 °C, free ^{125}I-f-Alb was separated from the bound ligand by centrifugation at 20,000 × *g* for 20 min at 4 °C. The resulting liposome pellets were washed 3 times with 1.2 ml of ice-cold buffer (50 mM Tris-HCl (pH 8.0), 0.1 M NaCl, and 40 mg/ml BSA). The tube containing pellet was cut off just above the pellet layer by scissors and its radioactivity was determined. Nonspecific binding represented the amount of ^{125}I-f-Alb bound to liposomes in the presence of an excess unlabeled ligand. This was usually less than 15% of the total binding observed in the absence of unlabeled ligand. Specific binding was calculated by subtracting the value for nonspecific binding from that for the total binding. During purification of f-Alb receptor, the binding activity of each fraction was expressed as μg of ^{125}I-f-Alb bound to mg of protein incorporated into liposomes. Experimental conditions for determination of the apparent dissociation constant (K_d) and the effect of unlabeled ligand on the total binding were described in detail for Fig. 4.

Determination of pI Value of ^{125}I-labeled Receptor—The isoelectric point (pI) of the receptor was measured at room temperature by using Polybuffer exchanger (Pharmacia Fine Chemicals) according to the manufacturer's instructions. Briefly, a column (0.8 × 13 cm) packed with a gel of Polybuffer PBE94 was equilibrated with 25 mM imidazol HCl buffer (pH 7.5). The column was first charged with 2 ml of the elution buffer (Polybuffer 74 adjusted to pH 4.0 by HCl), then with 1 ml of same buffer containing ^{125}I-labeled receptor, followed by elution with 120 ml of the same buffer at a flow rate of 0.5 ml/min. Fractions of 2.0 ml were collected and determined both for radioactivity and pH value.

Prior to pI focusing of the receptor-ligand complex, ^{125}I-labeled receptor (1.5 × 10^6 cpm) was incubated for 1 h at 37 °C, in a total volume of 0.1 ml, either with 20 μg of f-Alb or 20 μg of unmodified BSA in 50 mM Tris-HCl (pH 7.4), 0.1 M NaCl, and 0.1% Triton X-100. To this solution was added 0.9 ml of the elution buffer and the solution applied to the column. The elution was conducted in the same way as described above.

Electrophoresis—Polyacrylamide gel electrophoresis of the ^{125}I-labeled receptor preparation was performed as described previously (22) according to Gabriel (23) in a 7.5% disc gel. $NaDodSO_4$-polyacrylamide slab gel electrophoresis was conducted as described previously (24) by using the buffer system of Maizel (25). Details are described in the legends for Figs. 1 and 2.

Preparation of Anti-receptor Antibody—The antiserum against the purified receptor was raised in two rabbits according to a slight modification of the method of Vaitukaitis (26). The purified receptor preparation (30 μg), suspended in 50% complete Freund's adjuvant solution, was injected intradermally into two rabbits at 20–30 skin sites for each rabbit. The rabbits were boostered 10 days later with the same amount of the receptor in 50% complete Freund's adjuvant, followed by two additional booster injections every week thereafter. Each rabbit was finally injected both intradermally and intramuscularly with 20 μg of the antigen suspended in 50% of incomplete Freund's adjuvant. The serum was taken 10 days after the final injection. Preimmune serum was obtained from rabbits prior to their immunization with the purified receptor. IgG fractions both of antiserum and preimmune serum were prepared by precipitation with 33% ammonium sulfate followed by washing the precipitates twice with 40% ammonium sulfate. Following dialysis, the samples were subjected to chromatography on a DEAE-Sephacel column as described (27).

Labeling of Sinusoidal Liver Cells with [^{35}S]Methionine—Sinusoidal liver cells were prepared by the collagenase perfusion method as reported previously (15). To 3 ml of the isolated sinusoidal liver cells suspended in Hanks' balanced solution (3.3 × 10^6 cells/ml) was added 200 μCi of [^{35}S]methionine and the solution incubated for 3 h at 37 °C. After washing with ice-cold 20 mM Tris-HCl (pH 7.4) and 0.15 M NaCl, the cells were resuspended in 2 ml of the same buffer containing 1% Triton X-100 and 1 mM phenylmethylsulfonyl fluoride, followed by vigorous mixing on a Vortex mixer for 5 min at 4 °C. After centrifugation at 12,800 × *g* for 20 min, the supernatant solution was divided into two portions. One-half was incubated with 20 μl of antiserum and the other with preimmune serum on ice for 2 h. To each solution was added a sufficient amount of Staph A suspension to trap all the IgG of the added serum. After incubation on ice for 1 h, Staph A particles were freed from unbound materials by extensive washing. The final pellet was immersed in 80 μl of 0.05 M Tris-HCl (pH 6.8), 1% $NaDodSO_4$, and 1% 2-mercaptoethanol, and boiled for 3 min, followed by centrifugation at 12,800 × *g* for 4 min. A portion (5 μl) of the resulting supernatant was used for measurement of radioactivity and the remaining portion was subjected to $NaDodSO_4$-gel electrophoresis and fluorography as described (28). The film was exposed for 10 days at −75 °C. The total radioactivity of samples applied to gel slots was 3,200 cpm for immunoprecipitates by antiserum and 1,350 cpm for those by preimmune serum. A gel was calibrated with the following M_r standards which were prepared by reductive methylation with [^{3}H]formaldehyde according to the reported method (29): phosphorylase *b*, 94,000; bovine serum albumin, 68,000; ovalbumin, 43,000; carbonic anhydrase, 30,000; soybean trypsin inhibition, 20,000; and α-lactalbumin, 14,500.

RESULTS

Purification of the Receptor—An attempt was made to purify the receptor for f-Alb from Triton X-100 extracts of membrane fractions of rat livers. Table I summarizes the purification of the receptor. Most of the solubilized proteins were not adsorbed to f-Alb-conjugated Sepharose 4B, and appeared in the breakthrough fraction (Fraction A). The ^{125}I-f-Alb binding activity was found exclusively in Fraction C, which was eluted with buffer C (pH 3.0). Rechromatography of this fraction on the affinity column resulted in a further increase in specific activity. Overall purification achieved was almost 1300-fold over solubilized membrane proteins as starting materials. A similar attempt was made to purify a possible BSA-binding protein(s) by using a BSA-coupled Sepharose column. However, the f-Alb binding activity was found only in the flowthrough fraction (data not shown).

Some Molecular Properties of Purified Receptor—A portion of the active fraction (Fraction C) obtained by the second affinity chromatography (Table I) was iodinated with ^{125}I as described under "Materials and Methods." The iodinated sample exhibited almost a single peak on polyacrylamide gel electrophoresis (Fig. 1). As judged from the amount of radioactivity, 92% of the applied sample was recovered in the peak fraction (slices 4–10), indicating a high degree of homogeneity of the active fraction obtained by affinity chromatography. Fig. 2 shows electrophoretic patterns on a $NaDodSO_4$ slab gel of the ^{125}I-labeled preparation of Fraction C. With pretreatment by $NaDodSO_4$ alone, the receptor migrated as a single band slightly slower than the marker protein of M_r = 115,000. The molecular weight of this peak was estimated to be approximately 125,000 when calibrated by standard marker proteins (Fig. 2, *inset*). On the other hand, pretreatment of the labeled receptor both with $NaDodSO_4$ and 2-mercaptoethanol resulted in separation into two peaks whose molecular weights correspond to 53,000 and 30,000, respectively. It is also noted that no band larger than the 53,000 protein was

TABLE I

Summary of purification of f-Alb receptor from Triton X-100 solubilized rat liver membrane fraction

The membrane fractions were isolated from five rat livers (approximately 50 g) as described under "Materials and Methods." The membranes solubilized in 2% Triton X-100 were subjected to an affinity chromatography on a Sepharose 4B-f-Alb column. Fraction C was further purified on the second affinity column of Sepharose-f-Alb. At each step of purification, aliquots of each fraction were determined for ^{125}I-f-Alb binding activity and protein contents. The binding activity was obtained as the amount of ^{125}I-f-Alb bound with liposomes in which proteins from each fraction were incorporated as described under "Materials and Methods."

	Protein[a]	Activity	Total activity	Purification
	mg			*-fold*
I. Solubilized membrane proteins	720	0.08	57.6 (100)[b]	1
II. First affinity chromatography				
Fraction A	680	ND[c]		
Fraction B	4.5	0.15	0.68	
Fraction C	1.2	25	37.5 (65)	313
III. Second affinity chromatography				
Fraction C	0.21	103	21.6 (38)	1288

[a] Specific activity was expressed as micrograms of ^{125}I-f-Alb bound per protein in which proteins from each fraction were incorporated as described under "Materials and Methods."

[b] Values in parentheses represent the percentage of "total activity" relative to that for solubilized membrane proteins.

[c] ND, not detectable.

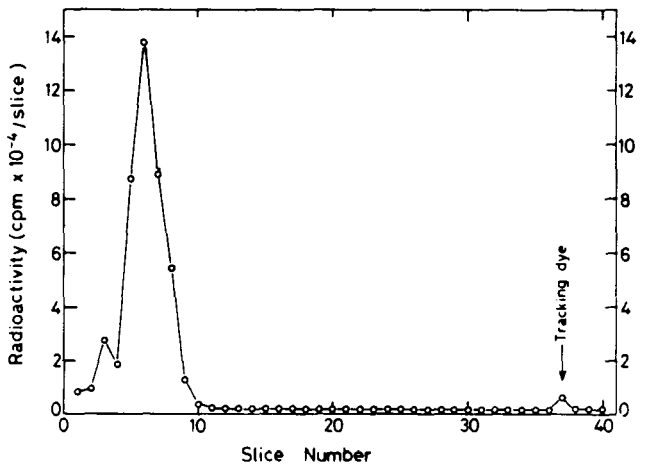

FIG. 1. **Polyacrylamide gel electrophoresis of Fraction C from f-Alb-conjugated Sepharose 4B chromatography of Triton X-100 extracts of liver membranes.** Fraction C obtained by the second affinity chromatography on f-Alb-Sepharose 4B was iodinated to a specific activity of 2.0×10^6 cpm/μg as described under "Materials and Methods." To 30 μl of the radioiodinated sample (5.5×10^5 cpm) was added 10 μl of 0.05 M Tris-HCl buffer (pH 8.2), 30% glycerol, and 0.01% bromphenol blue as a tracking dye, followed by electrophoresis on polyacrylamide disc gel (7.5%). After electrophoresis, the gel was cut into 2-mm thick slices, and radioactivity was determined. Radioactivity in both upper and lower electrophoretic chambers was also determined. The total radioactivity recovered from the upper and lower chambers was 7% and 0.8% of the total radioactivity applied to the gel, respectively.

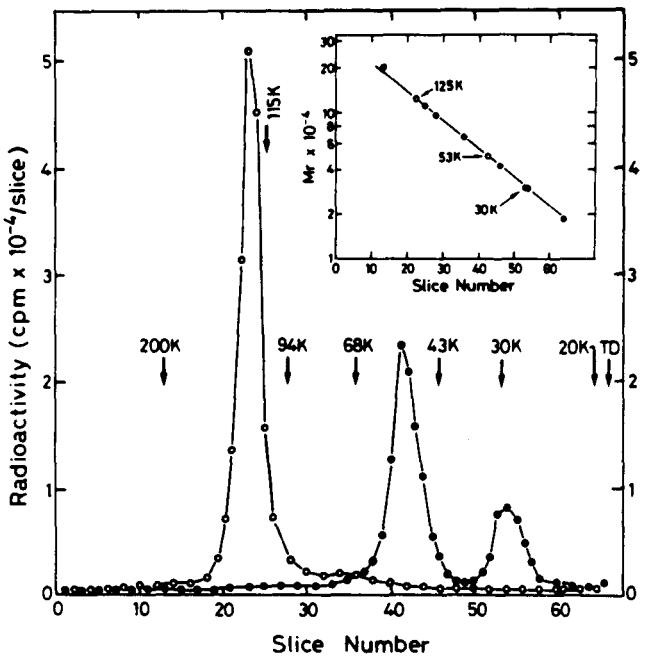

FIG. 2. **Electrophoresis of ^{125}I-labeled receptor on $NaDodSO_4$-polyacrylamide slab gel.** ^{125}I-labeled receptor preparation (2.2×10^5 cpm) was incubated with, in a final volume of 0.1 ml, 50 mM Tris-HCl (pH 6.8), 6% glycerol, and 1% $NaDodSO_4$ for 1 h at 45 °C in the absence (O——O) or presence (●——●) of 1% 2-mercaptoethanol, followed by application to a 6–14% $NaDodSO_4$-polyacrylamide slab gel. The gel was stained with Coomassie Brilliant Blue to locate the following standard proteins; myosin (200,000 (*200K*)), β-galactosidase (115,000), phosphorylase *b* (94,000), bovine serum albumin (68,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (20,000), and α-lactalbumin (14,500). Their locations are shown by *arrows*. Bromphenol blue was used as a tracking dye (*TD*). The corresponding gel slot was cut into 2-mm thick slices and radioactivity for each slice was determined.

detected, suggesting that the minor band (slice 3 in Fig. 1) seen upon polyacrylamide gel electrophoresis might be aggregates. Thus, this result indicates that the receptor was composed of two noncovalently linked polypeptide chains.

To examine whether or not polysaccharides were linked to the receptor, the radioiodinated receptor was passed over a column of Sepharose 4B coupled with concanavalin A, a lectin specific for mannose-type glycoproteins. As shown in Fig. 3, the receptor was effectively adsorbed on to the column and specifically eluted by the buffer containing α-methyl-D-glucoside, a haptenic inhibitor for this lectin, indicating the glycoprotein nature of this receptor. $NaDodSO_4$-polyacrylamide gel electrophoresis of the adsorbed fraction revealed a pattern identical with that observed in Fig. 2 (data not shown).

Each band separated upon electrophoresis of ^{125}I-labeled receptor (Fig. 2) was extracted from the gel slices and $NaDodSO_4$ was removed, followed by binding to Sepharose 4B coupled with concanavalin A. Table II shows that both components displayed a high affinity for this lectin, indicating that both polypeptide chains were of a glycoprotein nature.

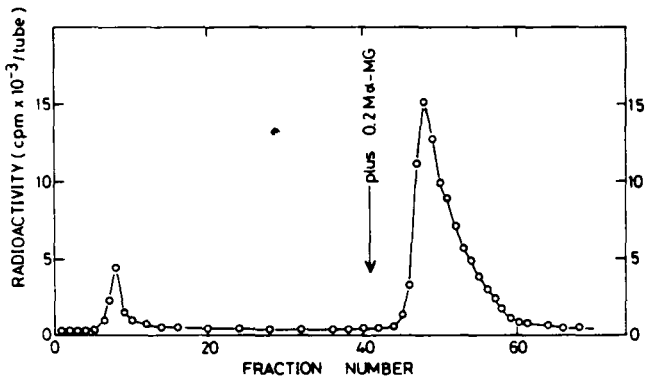

FIG. 3. **Affinity chromatography of ^{125}I-labeled receptor on a Sepharose 4B-concanavalin A column.** Twenty microliters of ^{125}I-labeled receptor preparation (4.3×10^5 cpm) in buffer A was diluted to 600 μl with buffer (50 mM Tris-HCl (pH 8.0), 0.1 M NaCl, 1 mM $CaCl_2$, 1 mM $MnCl_2$, and 0.1% Triton X-100). It was then charged on a column of Sepharose 4B-concanavalin A column which had been equilibrated with the above buffer. The column was washed with the same buffer, followed by elution with buffer containing 0.2 M α-methyl-D-glucoside (*α-MG*). Each fraction contained 1.2 ml. Further washing with the buffer containing 0.2 M α-methyl-D-glucoside and 1% Triton X-100 did not elute appreciable radioactivity from the column. The overall recovery of radioactivity in the effluents from the column was around 85% of the total radioactivity applied.

TABLE II

Affinity of 53,000 and 30,000 protein for concanavalin A

After ^{125}I-labeled receptor preparation was electrophoresed on $NaDodSO_4$-polyacrylamide gel, the gel was cut into slices (2-mm thickness) and radioactivity was determined. The gel slices corresponding to the bands for 53,000 and 30,000 proteins were separately extracted by incubating with 1 ml of 50 mM sodium phosphate buffer (pH 7.2) and 0.1% $NaDodSO_4$ for 6 h at 37 °C. After removing gel slices by brief centrifugation, the supernatant was passed over a column of Dowex AG 1-X2 (1 × 5 cm) to remove $NaDodSO_4$. The radioactive fractions were collected, combined, and concentrated with a Diaflow apparatus equipped with PM-10 membrane (Amicon). To the concentrate was added 2 ml of concanavalin A-Sepharose 4B gel equilibrated with 20 mM Tris-HCl, (pH 7.5), containing 0.15 M NaCl, 1 mM $MnCl_2$, and 1 mM $CaCl_2$. The mixture was incubated for 30 min at room temperature and the gel slurry was transferred to a mini-column. The column was washed with 40 ml of the same buffer (nonabsorbed fraction), followed by elution with 40 ml of the above buffer containing 0.1 M α-methyl-D-glucoside (adsorbed fraction).

Protein	Radioactivity applied	Nonadsorbed	Adsorbed
		cpm/system	
53,000 protein	43,500	2,600	37,800
30,000 protein	32,400	3,500	26,200

Binding of Ligand to Purified Receptor—Fig. 4 shows the binding of ^{125}I-labeled f-Alb to the purified receptor preparation incorporated into liposomes. The binding of the radiolabeled ligand to the liposome preparation was inhibited in a dose-dependent fashion by the presence of unlabeled ligand (Fig. 4*B*). The presence of an excess amount of unlabeled f-Alb replaced 80–85% of the total binding, and the amount of unlabeled ligand required to inhibit the total binding by 50% was approximately 16 μmg/ml, a value similar to that obtained from the binding-kinetics study reported previously (8). Under the conditions, no significant binding of ^{125}I-f-Alb was observed with the liposomes into which the receptor was not incorporated (data not shown). ^{125}I-f-Alb binding to the purified receptor showed typical saturation kinetics (Fig. 4*A*) with an apparent K_d of about 6.2 μg/ml and maximum binding of 285 μg/mg of purified receptor, which were obtained from the Scatchard plot (30) shown in the inset of Fig. 4*A*. Thus, the binding affinity of receptor purified from rat liver membrane fraction is in good agreement with that of sinusoidal cell membranes (15).

Ligand-receptor interaction was also demonstrated even in the soluble state of the receptor (Fig. 5). Isoelectric focusing of ^{125}I-labeled receptor revealed a single peak centered at pH 5.9. Preincubation of the labeled receptor with native serum albumin (BSA) did not affect the pI value of the receptor. By contrast, incubation of the labeled receptor with its ligand (f-Alb) resulted in a marked decrease in pI value down to 5.1, presumably under the influence of interacting with f-Alb (pI, 4.6). This indicates the presence of a specific interaction between the ligand and its solubilized receptor molecules.

Anti-receptor Antibody—In order to confirm that the receptor purified from the whole rat liver was indeed derived from sinusoidal liver cells, the following experiments were carried out. The isolated sinusoidal liver cells were metabolically labeled with [^{35}S]methionine. The cells thus labeled were solubilized with 1% Triton X-100. The solubilized proteins were reacted with the antiserum raised against the purified f-Alb receptor, and immune complexes thus formed were trapped by Staph A and subjected to $NaDodSO_4$-polyacrylamide gel electrophoresis, followed by fluorography. As shown in Fig. 6, preimmune serum (*Lane b*) appeared to precipitate only a faint protein band at the position for $M_r = 43{,}000$. This band was similarly observed in the precipitates obtained by immune serum. In addition to this band, the precipitates with immune serum gave two distinct bands; a major band of $M_r = 53{,}000$ and a minor band of $M_r = 30{,}000$. Their mobilities were indistinguishable from those for the purified receptor used as an antigen. Thus, it is likely that the f-Alb binding protein purified from the whole liver was identical to the receptor occurring on sinusoidal liver cells.

Fig. 7 shows the effect of anti-receptor antibody on the ^{125}I-f-Alb binding to the sinusoidal liver cell membranes. Pretreatment of the membrane fractions with IgG obtained from the antiserum effectively inhibited the specific binding of the radioactive ligand to the membranes. IgG from the preimmune serum, on the other hand, did not affect the binding process. This indicates that the antibody not only recognizes the membrane-associated receptor on sinusoidal liver cells, but also has a capacity to block the ligand binding to its receptor. These results obtained by using anti-receptor antibody strongly suggest that the membrane protein purified in the present study would represent the membrane-bound receptor involved in a specific uptake of f-Alb from the bloodstream.

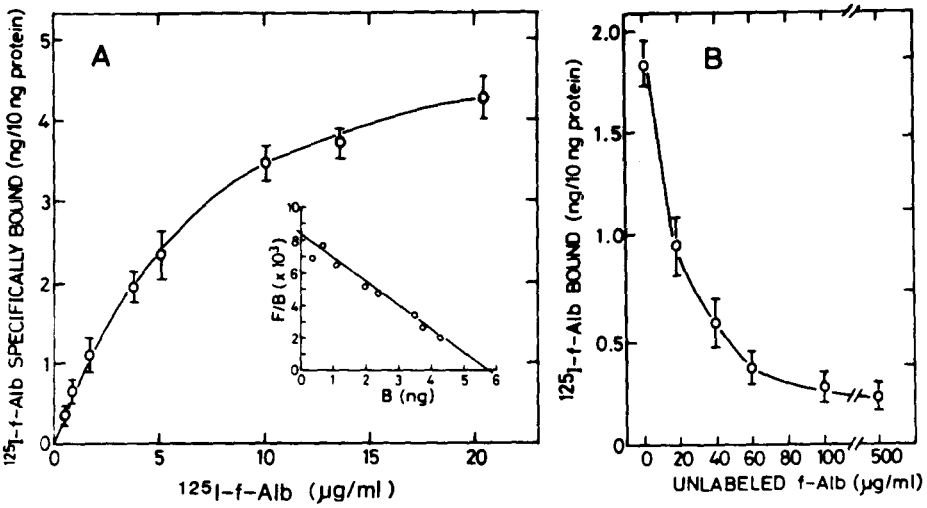

FIG. 4. **^{125}I-f-Alb binding to purified f-Alb receptor.** *A*, aliquots containing 20 ng of pure f-Alb receptor (Fraction C from the second chromatography on f-Alb-Sepharose) were incubated with appropriate concentrations of ^{125}I-f-Alb for 1 h at 25 °C as described under "Materials and Methods." Nonspecific binding was determined by parallel incubations in the presence of 4 mg/ml of unlabeled f-Alb (less than 15% of the total binding). Assays were run in duplicate. Binding data were plotted after correction for nonspecific binding. *Open circles* denote the mean value and the *bars* indicate the range. The *inset* shows the Scatchard plot: *F*, free ^{125}I-f-Alb; *B*, bound ^{125}I-f-Alb. *B*, effect of unlabeled f-Alb on ^{125}I-f-Alb binding to the purified receptor. Aliquots containing 20 ng of the purified receptor were incubated, in a total volume of 0.1 ml, with 3.75 μg/ml of ^{125}I-f-Alb (4,670 cpm/ng) in the absence or presence of appropriate concentrations of unlabeled f-Alb for 1 h at 25 °C. Amounts of ^{125}I-f-Alb to the receptor preparation were determined as described under "Materials and Methods." Assays were run in duplicate. The range was shown by *vertical bars*.

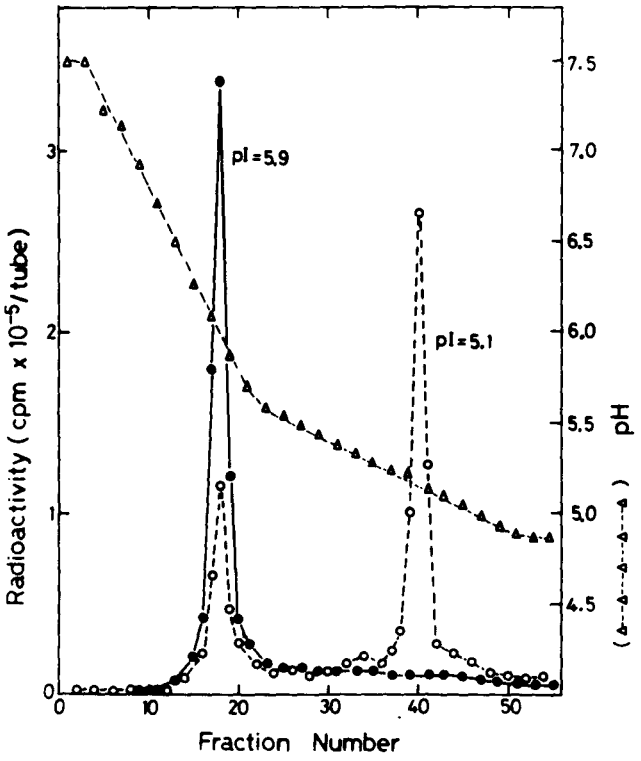

FIG. 5. **Isoelectric point of ^{125}I-labeled receptor and its shift upon interaction with f-Alb.** ^{125}I-labeled receptor (1.5 × 10^6 cpm) was incubated for 1 h at 37 °C either with 20 μg of f-Alb (○——○) or 20 μg of BSA (●——●). The samples thus treated were then applied to Polybuffer exchanger columns, followed by elution with Polybuffer as described under "Materials and Methods." Each fraction was determined both for the radioactivity and pH (△——△). ^{125}I-labeled receptor alone focused at a position identical with that preincubated with native albumin (data not shown).

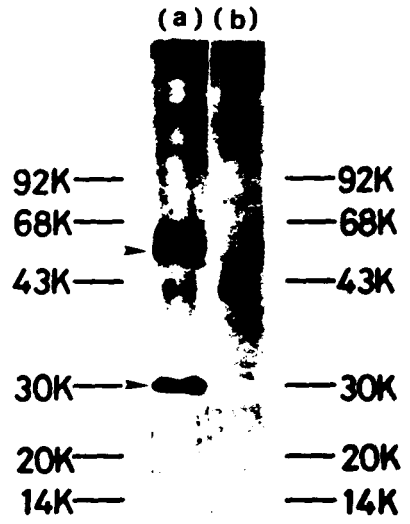

FIG. 6. **Identification of proteins reactive with anti-f-Alb receptor antibody in [^{35}S]methionine-labeled sinusoidal liver cell membranes.** Sinusoidal liver cells (1 × 10^7 cells) suspended in 3 ml of Hanks' balanced solution were labeled with 200 μCi of [^{35}S] methionine by incubating for 3 h at 37 °C. The labeled cells were lysed in a buffer solution containing phenylmethylsulfonyl fluoride and Triton X-100, followed by centrifugation. To portions of the resulting supernatant were added either antiserum to f-Alb receptor or preimmune serum. After incubation for 2 h on ice, the immunoprecipitates were trapped by Staph A, followed by washing with the same buffer to remove unbound materials. The washed precipitates were boiled for 3 min in 1% NaDodSO$_4$ and 2-mercaptoethanol and analyzed on a 10% NaDodSO$_4$-polyacrylamide slab gel. See "Materials and Methods" for further details. M_r standards indicated on both sides of the gel slots were the same as those described for Fig. 2. *Lane a*, immunoprecipitates with antiserum; *Lane b*, immunoprecipitates by preimmune serum. *Arrow heads* indicate the two protein bands specifically detected by antiserum.

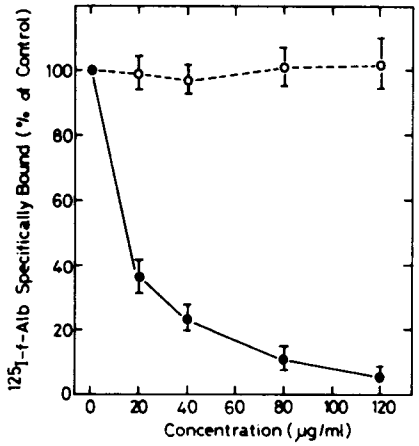

FIG. 7. **Effect of anti-receptor antibody (IgG) and preimmune IgG on ^{125}I-f-Alb binding to sinusoidal liver cell membranes.** Each tube contained, in a final volume of 90 μl, 1.65 μg of proteins from membranes prepared (15) from sinusoidal liver cells, indicated concentrations of either anti-receptor IgG (●——●) or preimmune IgG (○——○), 50 mM Tris-HCl (pH 7.5), 0.1 M NaCl, 0.5 mM $CaCl_2$, and 2 mg of BSA. Incubation was performed for 20 min at 0 °C. ^{125}I-f-Alb binding to the membrane fractions were initiated by adding 10 μl of 3.75 μg/ml of ^{125}I-f-Alb (4,670 cpm/ng) and incubating for 1 h at 0 °C. The unbound ^{125}I-f-Alb was removed as described by washing with the same buffer solution using centrifugation at 35,000 × *g* for 1 h. The specific binding was determined by subtracting nonspecific binding from the total binding. Nonspecific binding was determined by parallel incubations in the presence of 4 mg/ml of the unlabeled f-Alb. Binding data were plotted after correction for nonspecific binding.

DISCUSSION

The present study has clearly shown that the membrane-associated receptor presumably responsible for the uptake of intravenously injected f-Alb from the bloodstream is a glycoprotein of M_r = 125,000, comprised of two noncovalently associated polypeptide chains of M_r = 53,000 and 30,000. However, no definite data are available about the subunit composition of the receptor molecule. Further study is needed to understand its molecular architecture.

The purified protein incorporated into liposomes exhibited binding kinetics similar to that obtained for the binding of ^{125}I-f-Alb to sinusoidal liver cell membranes (15). Even if the purified receptor was present as a complex with Triton X-100, the receptor-ligand interaction was demonstrated by a marked shift in pI value of the receptor upon preincubation with f-Alb. The presence of native albumin, a parent molecule of f-Alb, did not shift the pI value (Fig. 5). These results are consistent with the previous finding that receptor-ligand complexes were detected when the ^{125}I-labeled solubilized membrane proteins were reacted with f-Alb but not with native albumin (15). Although, in the present study, the f-Alb receptor was purified from membrane fractions of whole liver, it was derived from sinusoidal cells as was clearly shown by the experiment using ^{35}S-labeled sinusoidal liver cells and the antibody raised against the purified receptor protein. Furthermore, the inhibitory effect of the antibody on the binding of f-Alb to sinusoidal cells is consistent with the topology of the receptor protein on the surface membrane of cells.

It was reported that radiolabeled f-Alb was readily digested inside the cells by lysosomal protease(s) subsequent to internalization of the ligand (2, 31). Hence, it is possible that the f-Alb-binding protein obtained from whole liver in the present study may represent one of the lysosomal proteases which is involved in degradation of this particular ligand. Incubation of the purified protein with ^{125}I-labeled f-Alb, however, resulted in no significant degradation of the radiolabeled ligand as shown by the absence of any increase in trichloroacetic acid-soluble radioactivity (data not shown). Thus, this possibility seems unlikely.

The glycoprotein nature of this receptor is in good agreement with the prevailing view that the membrane and secretory proteins were synthesized on membrane-bound polysomes and glycosylated during delivery from endoplasmic reticulum to the surface membrane (32–35). The presence of mannose-rich oligosaccharide in the receptor molecules was also suggested from the previous finding that the binding of ^{125}I-f-Alb to the membranes of the sinusoidal liver cells was significantly inhibited by the presence of concanavalin A and its effect was prevented by α-methyl-D-glucoside, a haptenic inhibitor for this lectin (15). Whether or not the oligosaccharide moiety of the receptor molecule is involved in the specific recognition of the ligand is currently under investigation.

Previous studies on several membrane receptors such as asialoglycoprotein (36) and low-density lipoprotein receptor (37) revealed the presence of binding sites other than the surface membrane (*i.e.* intracellular receptor). It is uncertain whether or not the intracellular pool of the receptor for f-Alb exists, or whether the surface receptors are recycled back to the plasma membrane after receptor-ligand complexes are internalized in the cell interior. The specific antibody for this receptor may serve as a probe to solve this interesting question.

Sinusoidal liver cells represent a heterogeneous cell population comprised of endothelial cells and Kupffer cells (38). It has not precisely been clarified yet which type of cells is responsible for the uptake of f-Alb. The finding that rat peritoneal macrophages showed a similar uptake capacity for ^{125}I-labeled f-Alb (3, 4) supports a preferential involvement of Kupffer cells, a main intravascular macrophage population *in vivo*. The same corollary applies to other receptors for high-mannose glycoproteins, immune complexes, α_2-macroglobulin-protease complex, and acetyl-LDL in that (i) these ligands were specifically taken up by sinusoidal liver cells upon intravenous injection and (ii) peritoneal or alveolar macrophages exhibited a specific affinity for these ligands. Thus, it may be reasonable to assume that the f-Alb receptor is specifically, although not exclusively, expressed in cells of the monocyte-phagocyte family as a member of the scavenger receptors of macrophages. Of particular interest in this respect is the recent finding by Goldstein *et al.* (39), Mahley *et al.* (40), and Henriksen *et al.* (41) that the receptor for acetyl-LDL present on macrophages plays a pivotal role in the pathogenesis of foam cells, cells characteristic of the atheroma lesion in atherosclerosis (10). Since the f-Alb receptor shares common features with this receptor ((*a*) they are localized on macrophages or macrophage-derived cells, (*b*) the chemical modification unique to these ligands is unlikely to occur *in vivo*, (*c*) a possible physiological ligand for each receptor has not yet been identified, and (*d*) the binding is divalent cation independent), the answer to the question of whether these two receptors expressed on macrophages are distinct from each other would be a clue to elucidate the scavenger function of macrophages at a molecular level. In this connection, Via *et al.* (42) described that the highly purified membrane receptor for acetyl-LDL from the mouse macrophage cell line P388D_1 exhibited a molecular weight of 283,000 as a detergent-receptor complex with a pI value of 5.9.

In our recent studies, the binding of ^{125}I-f-Alb to sinusoidal cells was effectively inhibited by unlabeled acetyl-LDL. By contrast, the binding of ^{125}I-acetyl-LDL to the cells was found

to be inhibited neither by unlabeled f-Alb nor by the anti-f-Alb receptor antibody. Since the f-Alb receptor was sensitive to polyanions such as dextran sulfate and poly(L-glutamic acid), it was suggested that the scavenger receptors for acetyl-LDL and f-Alb might be distinct from each other (43).

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Purification of a Receptor for Formaldehyde-treated Serum Albumin from Rat Liver.
The Journal of Biological Chemistry. 1985;260(1):482-488.
FORMALDEHYDE /ALBUMIN /WOW
HEART /FOAM CELL /MACROPHAGE
When treated in vitro with formaldehyde, serum albumen is rapidly cleared from the bloodstream by binding to scavenger receptors on the membranes os sinusoidal liver cells. The receptor is specific to this chemically modified albumin. This ligand is also effectively taken up by macrophages via a receptor-mediated pathway. These authors suggest that the membrane-associated receptor for this ligand belongs to a family of scavenger receptors present on macrophages or macrophage-derived cells. It is proposed that the Kupffer cells are the cells responsible for the uptake of formaldehyde treated albumen. It is also suggested that the formaldehyde modified albumen receptor is specifically, although not exclusively, expressed in cells of the monocyte-phagocyte family as a member of the scavenger receptors of macrophages.
Of particular interest in this respect is the finding by (1003) that the receptor for acetyl-LDL present on macrophages plays a pivotal role in the pathogenesis of foam cells, cells characteritic of the atheroma lesion in arthrosclerosis (1004). it is suggested that the receptors for acetyl-LDL and f-Alb might be distinct from each other. The authors think it unlikely that the chemical modification unique to these ligands is unlilely to occur in vivo."Physiological function of this membrane-associated receptor remains unknown;it is uncertain whether the same modification could take place in vivo, or whether there could be a latural ligand for this receptor in the serum or plasma". Macrophagestake up this f-Alb via a receptor-mediated pathway(1005).
see 315