

Isolation and characterization of a mannose-specific endocytosis receptor from rabbit alveolar macrophages

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Rabbit alveolar macrophages express a plasma-membrane receptor that recognizes glycoprotein ligands bearing terminal mannose, fucose or *N*-acetylglucosamine residues. Macrophage membranes were washed extensively with buffers containing high salt and mannose or EDTA to remove endogenously bound ligand, before Triton X-100 extraction. The extracts were chromatographed on mannose-Sepharose. Elution with mannose, followed by dialysis and a second mannose-Sepharose step with EDTA elution, produced a preparation that migrated as single protein band of M_r 175000 on SDS/polyacrylamide-gel electrophoresis. The purified protein binds mannose-BSA (bovine serum albumin) with a dissociation constant of 1.9×10^{-8} M. Ligand binding is Ca^{2+} and pH-dependent, with maximal binding at neutral pH and low binding below pH 6.0. The binding of ^{125}I -mannose-BSA is inhibited by ligands bearing high-mannose oligosaccharides, such as mannan or β -glucuronidase, as well as the monosaccharides mannose, fucose and *N*-acetylglucosamine. Galactose, galactosylated BSA, glucose and mannose 6-phosphate are non-inhibitory. Amino acid compositional analyses indicate that the receptor contains high concentrations of aspartate/asparagine and glutamate/glutamine, and low amounts of methionine. The carbohydrate composition was studied by lectin overlays of electrophoretically transferred receptor, and the results indicate the presence of *N*-linked complex and *O*-linked sialylated oligosaccharides. A protein of M_r 175000 was immunoprecipitated from radio-iodinated macrophage membranes with an antibody generated against purified rabbit lung mannose receptor.

INTRODUCTION

Macrophages express a cell-surface receptor that binds and internalizes glycoproteins terminating in mannose, *N*-acetylglucosamine and fucose [1]. The mannose receptor is not expressed by circulating monocytes [2], but monocyte-derived macrophages and other primary macrophages are highly positive for receptor activity [3]. Receptor expression is closely regulated in macrophages. Glucocorticoids enhance receptor expression, whereas γ -interferon decreases expression [4]. Glycoprotein ligands for this receptor include the lysosomal hydrolases, as well as a series of non-mammalian glycoproteins terminating in mannose (e.g. ovalbumin, mannans, horseradish peroxidase [5]). In addition to pinocytosis of soluble ligands, the receptor appears to play a significant role in the phagocytosis of organisms such as yeast that have coats rich in mannose or mannan [6].

Previous studies have shown that the mannose receptor recycles constitutively from a pre-lysosomal acidic compartment to the cell surface [7]. Receptor-ligand dissociation occurs under the influence of low pH within endosomal membrane vesicles [8]. A proportion of internalized receptor-ligand complexes fail to dissociate, and are returned to the cell surface intact, suggesting the presence of alternative endocytosis pathways [7,9]. The location and/or function of these alternative pathways remains to be elucidated. In the present paper, we report the isolation and characterization of the mannose

receptor from rabbit macrophages, using affinity chromatography. The protein exhibits both the binding specificity and a requirement for Ca^{2+} and neutral pH that would be expected from studies of the receptor in intact macrophages. The receptor is a glycoprotein containing two classes of oligosaccharides, *N*-linked complex and sialylated *O*-linked.

EXPERIMENTAL

Materials

New Zealand White rabbits and White Leghorn chickens were purchased from Boswell's Bunny Farm, Pacific, MO, U.S.A. Mannose-Sepharose was prepared as previously described [10]. Bovine serum albumin (BSA) was conjugated to mannose, fucose or galactose by the method of Lee *et al.* [11]. Biotinylated concanavalin A (Con A), biotinylated peanut (*Arachis hypogaea*) agglutinin (PNA), neuraminidase and α -mannosidase were purchased from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Endoglycosidase F was obtained from Genzyme (Boston, MA, U.S.A.). Endoglycosidase H was purchased from Miles Scientific (Naperville, IL, U.S.A.). Unless specified, all other reagents were purchased from Sigma. Rabbit anti-chicken IgG was a gift from Dr. Kevin Martin, Washington University, St. Louis, MO, U.S.A.

Abbreviations used: Con A, concanavalin A; BSA, bovine serum albumin; Fuc-BSA, bovine serum albumin conjugated to fucose; Gal-BSA, bovine serum albumin conjugated to galactose; Man-BSA, mannosylated bovine serum albumin; HRP, horseradish peroxidase; PNA, peanut agglutinin; PBS, phosphate-buffered saline; TBS, Tris-buffered saline.

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Buffers

Dissociation buffer was 10 mM-Tris/HCl (pH 6.0)/1.25 M-NaCl/15 mM-EDTA; loading buffer, 10 mM-Tris/HCl (pH 7.4)/1.25 M-NaCl/15 mM-CaCl₂; Tris-buffered saline (TBS), 10 mM-Tris/HCl (pH 7.4)/150 mM-NaCl; phosphate-buffered saline (PBS), 10 mM-sodium phosphate (pH 7.4)/150 mM-NaCl.

Preparation of macrophage membranes

Recruitment of macrophages into rabbit lungs and pulmonary lavage for macrophage harvesting were carried out as previously described [1]. Cells were sedimented by centrifugation at 200 g (5 min, 4 °C) and stored at -20 °C before receptor isolation. All procedures were carried out at 4 °C. Membranes were prepared by resuspending macrophages (10¹⁰ cells) in 100 ml of dissociation buffer containing proteinase inhibitors (0.1 mM-phenylmethanesulphonyl fluoride, 1 μg each of leupeptin and chymostatin/ml) and homogenizing for 5 × 30 s at high speed in a Waring blender. Membranes were pelleted (85000 g, 15 min) and subsequently washed by sedimentation, once with 100 ml of loading buffer containing 0.2 M-mannose and the proteinase inhibitors specified above, and twice with loading buffer alone. Mannose receptor was isolated from the pelleted membranes.

Binding assay

The binding assay for determination of the mannose-BSA (Man-BSA) binding constant and for studies on sugar inhibition and pH effects is a modification of the poly(ethylene glycol) method of Gould *et al.* [12]. Total incubation volume was 200 μl. The assay buffer for inhibition studies and *K_D* determination was TBS containing 15 mM-CaCl₂, 0.02% NaN₃ and 1% BSA. Inhibition assays contained ¹²⁵I-Man-BSA. In pH/binding studies, the assay buffer consisted of 30 mM-Mes (pH 5.0, 5.5), acetate (pH 6.0, 6.5) or Hepes (pH 7.0, 7.5, 8.0) containing 150 mM-NaCl, 15 mM-CaCl₂ and 1% BSA. The precipitated receptor-ligand complexes were collected on 0.45 μm Metrical filters (Gelman Sciences, Ann Arbor, MI, U.S.A.), which had been soaked in Hanks' balanced salts solution containing 1% BSA. All other aspects of the assay were unchanged. Non-specific binding was defined as the radioactivity (c.p.m.) precipitated in the presence of excess (1 mg/ml) unlabelled ligand, and amounted to 10–12% of the total radioactivity bound.

Enzyme digests

All enzyme incubations were carried out with 10 μg of receptor in a total incubation volume of 25 μl. All reactions were allowed to proceed to completion (37 °C, 14–17 h), as determined by parallel digests with invertase as the substrate. The conditions for endoglycosidase H and F digestions were those recommended by the manufacturer. Sialic acid residues were removed with neuraminidase (5 units) in 250 mM-acetate, pH 5.5. α-Mannosidase digestions contained α-mannosidase (0.4 unit) in 50 mM-citrate, pH 4.1. Reactions were terminated by boiling for 10 min in SDS/polyacrylamide-gel-electrophoresis sample-preparation buffer containing 6 M-urea.

Lectin blots

Receptor (10 μg) was treated with neuraminidase, endoglycosidase H, endoglycosidase F or α-mannosidase as described above and subjected to SDS/7.5%-polyacrylamide-gel electrophoresis by the method of Laemmli [13]. The resulting gels were equilibrated in transfer buffer (20 mM-Tris, 150 mM-glycine, 20% methanol, pH 8.3) and transferred on to nitrocellulose (45 min, 0.25 A) by using a Hoefer Mini Transphor TE-22 unit (Hoefer Scientific Instruments, San Francisco, CA, U.S.A.) cooled to 4 °C. Transfers were blocked by overnight incubation with periodate-treated BSA and probed with biotinylated lectins bound to avidin-HRP as previously described [14]. The sugars α-methyl glucoside (0.1 M) or galactose (0.5 M) were added to control blots with Con A or PNA respectively, to confirm the binding specificity of the lectins. HRP was detected with chloro-1-naphthol (Bio-Rad, Richmond, CA, U.S.A.).

Generation of chicken antibodies

Antibodies were generated in White Leghorn hens by injection of purified lung receptor [15] into the foot pads. Immunization was initiated with 50 μg of receptor emulsified in Freund's complete adjuvant (ICN Immunobiologicals, Lisle, IL, U.S.A.). The hen was subsequently boosted 21, 42 and 76 days later with 50 μg of antigen in Freund's incomplete adjuvant. The animal was bled at 49 and 84 days, and the serum tested by Ouchterlony double-diffusion analysis [16]. The titre of the antisera was between 1:16 and 1:32. Approx. 3 weeks after the injection of antigen, we began collecting eggs. The yolk immunoglobulins were extracted by the procedure of Polson & von Wechmar [17]. About 1 g of protein (IgY) was recovered from 12 egg yolks. The antiserum and IgY produced identical results in immunoprecipitation experiments.

Iodination of rabbit alveolar macrophages

Macrophages (1 × 10⁷ cells) were surface-labelled by using lactoperoxidase and ¹²⁵I as described previously [18]. Washed cells were homogenized by using one freeze-thaw cycle, followed by ten strokes of a tight-fitting pestle. Nuclei and unbroken cells were pelleted at 200 g (5 min), and the supernatant was solubilized (4 °C, 1 h) in 200 μl of PBS containing 10 mM-iodoacetamide, 1 mM-phenylmethanesulphonyl fluoride, 1 mM-EDTA and 1% digitonin. Debris was removed by high-speed centrifugation (122000 g for 30 min; Beckman Airfuge). The supernatant was pre-cleared by incubation (37 °C, 1 h) with 20 μl of Protein A-Sepharose coated with rabbit anti-chicken IgG. Samples (2 × 10⁶ c.p.m.) were immunoprecipitated with 10 μl of chicken anti-receptor antiserum or normal chicken serum (2 h, room temperature). The immunoprecipitates were immobilized with Protein A-Sepharose coated with rabbit anti-chicken IgG (3 h, 4 °C). The complexes were washed three times with the digitonin solubilization buffer, and the pellet was extracted with 50 μl of SDS sample-preparation buffer. After SDS/polyacrylamide-gel electrophoresis, the gel was subjected to radioautography for observation of the immunoprecipitated bands.

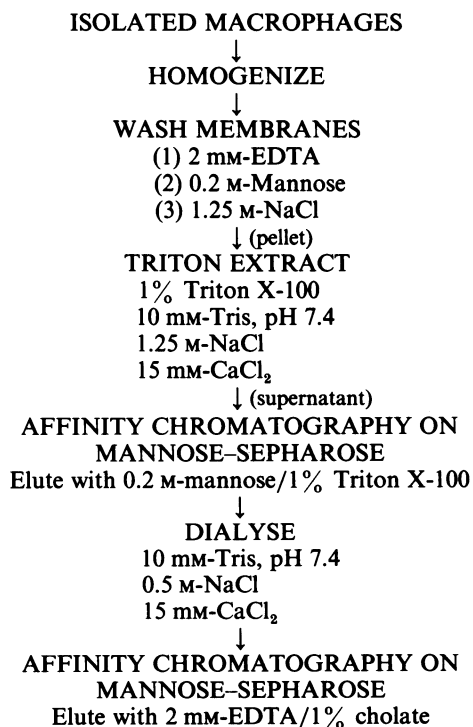
Amino acid compositional analysis

Amino acid composition data were collected from samples which had been subjected to acid hydrolysis (6 M-HCl, evacuated sealed tubes, 24 h, 110 °C). Cysteine was determined after conversion into cysteic acid by performic acid oxidation [19] before standard hydrolysis. All analyses were performed after post-column modification of the hydrolysates with ninhydrin. Amino acid determinations were done with a Beckman auto-analyser (model 6300).

RESULTS

Isolation of the mannose receptor

Macrophage membranes were extracted (4 °C, overnight) with 1% Triton X-100 in 100 ml of loading buffer containing the proteinase inhibitors used for the isolation of the membranes (see the Experimental section), and the unextracted debris was removed by centrifugation (100 000 g, 30 min). The mannose receptor was isolated from the supernatant by the protocol summarized in Scheme 1. This procedure is a modification of that published for the mannose-binding protein from rabbit lung [15]. The modifications include the substitution of a second mannose-Sepharose chromatography step for the *N*-acetylglucosamine-agarose column and elution of the second affinity column with EDTA buffer containing 1% cholate. The eluted fractions were assayed for protein by the method of Bradford [20]; the protein peak was pooled and dialysed against TBS. This procedure yielded a purified preparation of mannose receptor which exhibited an M_r of 175 000 on SDS/polyacrylamide-gel electrophoresis (Fig. 1). Receptor used for binding assays was dialysed again against TBS containing 15 mM-CaCl₂ and 0.02% NaN₃.



Scheme 1. Purification scheme for isolation of mannose receptor from rabbit alveolar macrophages

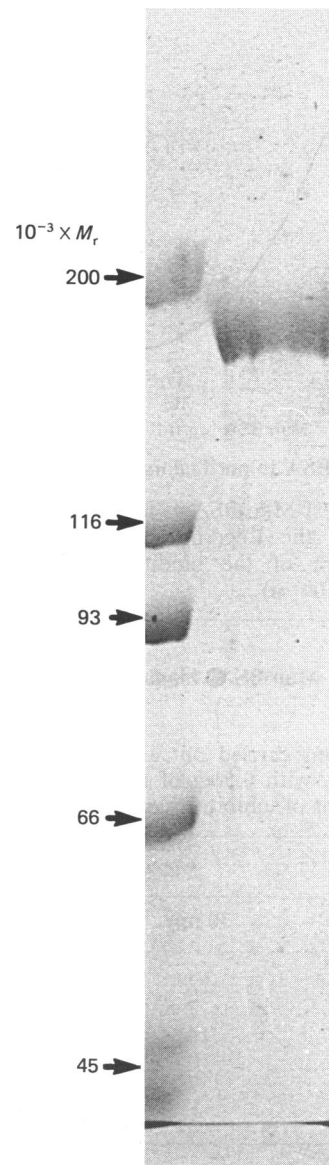


Fig. 1. Polyacrylamide-gel electrophoresis of macrophage membrane protein eluted from mannose-Sepharose with 2 mM-EDTA

Purified mannose receptor (2 µg) was subjected to 7.5%-polyacrylamide-gel electrophoresis in SDS under reducing conditions (right lane). Proteins were stained with Coomassie Blue. Standard protein markers (left lane) were myosin heavy chain (M_r 200 000), β -galactosidase (116 000), phosphorylase *b* (93 000), BSA (66 000) and ovalbumin (45 000).

and stored at 4 °C. All other material was dialysed against TBS and simultaneously concentrated with a Micro-ProDiCon Concentrator Dialyzer (Bio-Molecular Dynamics, Beaverton, OR, U.S.A.). The concentrated receptor (1 mg/ml) was stored at -80 °C. Although the yield of receptor varied, 100–350 µg of receptor protein was recovered from 1×10^{10} cells.

Ligand specificity

The binding of Man-BSA was studied by a poly-(ethylene glycol) precipitation assay. The assay mixture contained 2 µg of receptor, 1 pM-¹²⁵I-ligand and

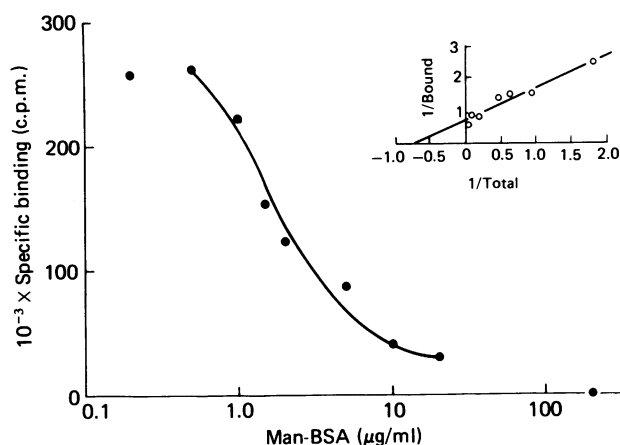


Fig. 2. Binding of Man-BSA to purified mannose receptor

Binding assays with ^{125}I -Man-BSA as ligand were carried out as described in the Experimental section. Inset: double-reciprocal plot of the binding data, for K_D determination ($1.9 \times 10^{-8} \text{ M}$).

Table 1. Inhibition of Man-BSA binding to the mannose receptor

The binding assay was carried out as described in the Experimental section, with $0.5 \mu\text{g}$ of receptor per assay and the concentrations of inhibitor indicated.

Inhibitor	(% Inhibition)	
	30 mM	100 mM
Mannose	22	47
<i>N</i> -Acetylglucosamine	7	22
Fucose	14	36
Galactose	0	0
Mannose 6-phosphate	7	10
10 mM		
EDTA	30 $\mu\text{g}/\text{ml}$	100
		100 $\mu\text{g}/\text{ml}$
Mannan	71%	66%
β -Glucuronidase	23%	42%
Man-BSA (37)*	93%	93%
Gal-BSA (32)*	0	0
Fuc-BSA (52)*	86%	97%

*The numbers in parentheses indicate mol of sugar residues per mol of BSA.

0.02 – $40 \mu\text{g}$ of unlabelled ligand in a total volume of $200 \mu\text{l}$. A binding constant of $1.9 \times 10^{-8} \text{ M}$ was estimated from a double-reciprocal plot (inset, Fig. 2). Inhibition assays contained ^{125}I -Man-BSA ($1.5 \mu\text{g}/\text{ml}$; $2 \times 10^5 \text{ c.p.m.}/\mu\text{g}$) as ligand with various concentrations of inhibitor. The concentration of ligand chosen was sufficient to saturate half of the available binding sites, as determined by the binding constant for Man-BSA. Gal-BSA (at $100 \mu\text{g}/\text{ml}$) has no apparent affinity for the receptor (Table 1). Monosaccharides are poor ligands for the receptor, requiring higher concentrations than the polyvalent mannan, β -glucuronidase and fucose-BSA to inhibit Man-BSA binding ([21]; Table 1). Mannose,

fructose and, to a lesser extent, *N*-acetylglucosamine inhibit binding in the millimolar range, whereas glucose, galactose and mannose 6-phosphate inhibit minimally even at 100 mM concentrations.

Ion effects on ligand binding

Previous work in whole cells demonstrated that the macrophage mannose receptor binds ligands in a pH- and Ca^{2+} -dependent manner [7,22]. To determine if the purified receptor retained these properties, the effect of pH and removal of bivalent cations on binding was investigated. Binding is maximal in the physiological range, but is lost below pH 6.0 (Fig. 3). Binding can be inhibited by 42% on addition of 5 mM -EDTA to the assay (Table 1).

Carbohydrate constituents of the receptor

Lectin-blotting techniques were used to characterize the oligosaccharides associated with the receptor. Concanavalin A conjugated to HRP (Con A-HRP) was used to detect *N*-linked oligosaccharides. Endoglycosidases H and F were used in conjunction with Con A-HRP to identify the classes of *N*-linked sugars present on the protein. Endoglycosidase H cleaves specifically high-mannose and hybrid oligosaccharides [23]; endoglycosidase F removes all *N*-linked sugar chains, i.e. high-mannose, hybrid and complex types [24]. The results presented in Fig. 4 demonstrate that endoglycosidase H treatment has no effect on Con A-HRP binding, whereas incubation with endoglycosidase F removes the Con A-binding oligosaccharides. The specificity of the endoglycosidases, coupled with the

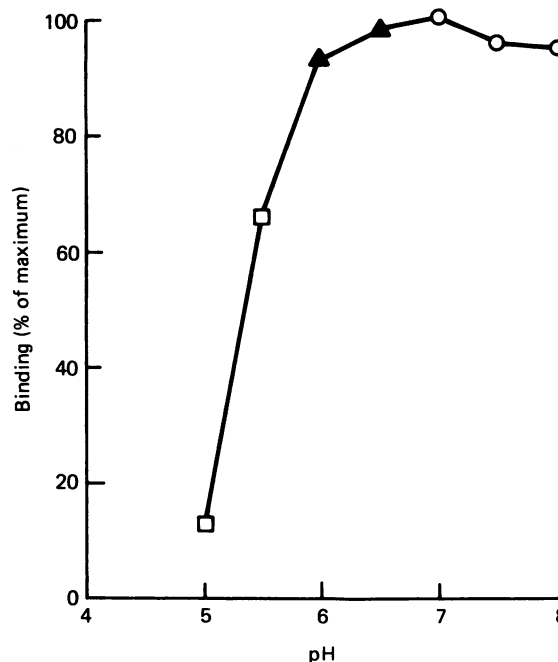


Fig. 3. Effect of pH on binding of Man-BSA to the mannose receptor

Receptor ($2 \mu\text{g}$) and ligand ($1.5 \mu\text{g}/\text{ml}$, $2 \times 10^5 \text{ c.p.m.}/\mu\text{g}$) were incubated in buffers containing 30 mM -acetate (\square), -Mes (\blacktriangle) or -Hepes (\circ) for 60 min as described in the Experimental section. Non-specific binding was determined for each pH by the addition of $100 \mu\text{g}$ of Man-BSA/ml to companion assays at each pH.

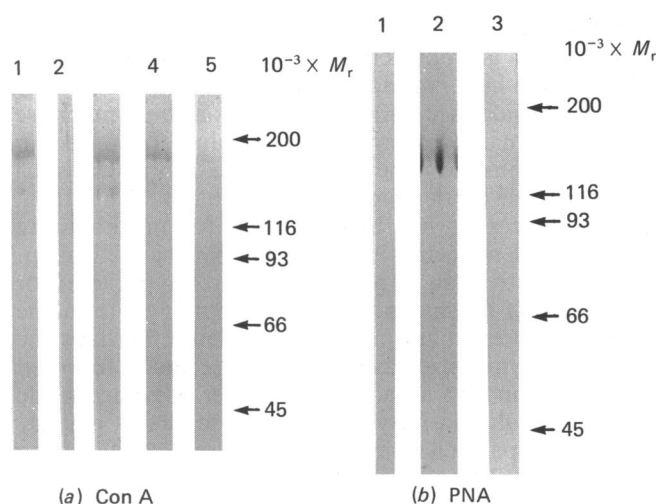


Fig. 4. Effect of sugar modification on the binding of (a) concanavalin A and (b) peanut agglutinin to the mannose receptor

Receptor ($10 \mu\text{g}$) was subjected to glycosidase treatment as described in the text. The modified receptor and untreated controls were electrophoresed, transferred to nitrocellulose and probed with Con A (a) or PNA (b). (a): lane 1, native receptor; lane 2, native receptor probed with Con A in the presence of the competing sugar, α -methyl glucoside; lanes 3, 4 and 5, receptor modified with α -mannosidase, endoglycosidase H and endoglycosidase F respectively. (b): lane 1, native receptor; lane 2, neuraminidase-treated receptor; lane 3, neuraminidase-treated receptor probed with PNA in the presence of the competing sugar galactose. The positions of M_r markers are indicated on the right.

observation that α -methyl glucoside inhibits binding of Con A to complex oligosaccharides [25], suggests that the Con A-binding oligosaccharides on the mannose receptor belong to the *N*-linked complex class.

The presence of *O*-linked sugars was demonstrated by using PNA conjugated to HRP (PNA-HRP). PNA recognizes the galactose-(β 1,3)-*N*-acetylgalactosamine linkage unique to *O*-linked sugar chains. The galactose is usually penultimate to a sialic acid residue, a linkage which masks the PNA-HRP recognition marker. Therefore, neuraminidase pretreatment is necessary to detect PNA-HRP-binding oligosaccharides. Fig. 4 shows that PNA-HRP binds to neuraminidase-treated receptor, but does not recognize the native receptor. The inhibition of PNA-HRP binding in the presence of galactose (Fig. 4b, lane 3) demonstrates the specificity of the PNA for the galactose linkage. These results indicate that the mannose receptor contains *O*-linked oligosaccharides, many of which bear terminal sialic acid residues which mask the PNA recognition marker.

Immunoprecipitation

A chicken antibody to the rabbit lung receptor was prepared as described in the Experimental section. This antibody was used to probe for the presence of immunologically related proteins on the macrophage cell surface. Immunoprecipitation of surface-iodinated macrophages reveals a single labelled band on SDS/10% polyacrylamide-gel electrophoresis (Fig. 5). The

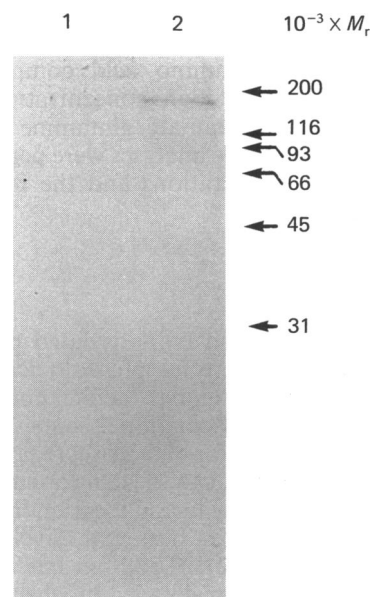


Fig. 5. Immunoprecipitation of radio-iodinated macrophage membranes by antibody to lung mannose receptor

Rabbit alveolar macrophages (1×10^7 cells) were radio-iodinated and immunoprecipitated as described in the Experimental section. Solubilized membranes (3×10^6 c.p.m.) were immunoprecipitated with normal chicken serum (lane 1) or antibody to lung mannose receptor (lane 2) and analysed by SDS/7.5% polyacrylamide-gel electrophoresis, followed by autoradiography. The positions of M_r markers are indicated on the right.

Table 2. Amino acid composition of macrophage mannose receptor

Receptor ($100 \mu\text{g}$) was subjected to amino acid compositional analysis as described in the Experimental section. The data represent the results of assays performed on two preparations of receptor.

Amino acid	Content (mol/mol)
Asx	191
Thr	93
Ser	133
Glx	169
Pro	71
Gly	134
Ala	88
Cys	41
Val	67
Met	19
Ile	60
Leu	115
Tyr	60
Phe	75
His	31
Lys	109
Arg	68

absence of detergent during iodination results in cell-surface labelling, indicating that the antibody recognizes a single cell-surface protein of M_r 175000.

Amino acid composition

The results of amino acid compositional analysis (Table 2) indicate high concentrations of aspartate/asparagine and glutamate/glutamine and low amounts of methionine. Three analyses were performed on each of two receptor preparations and the results averaged to produce Table 2.

DISCUSSION

Macrophages bind mannosylated proteins with high specificity and affinity at physiological pH and deliver them to an intracellular acidic compartment where receptor-ligand dissociation occurs [8]. This event is necessary for receptor-ligand segregation, for return of the mannose receptor to the macrophage cell surface and for the delivery of the ligand to secondary lysosomes. The mannose receptor has been extensively characterized in intact cells [6-8,11,21,22,26], but has not been available in a purified form for detailed biochemical analysis. The present paper reports the isolation of microgram quantities of macrophage receptor and demonstrates that the purified protein retains the binding characteristics of the cellular receptor. The development of a rapid, reproducible and quantitative poly(ethylene glycol) binding assay has allowed the binding specificity of the receptor to be studied in detail. The binding assay is linear with increasing concentrations of receptor (2-20 $\mu\text{g/ml}$) and saturable with increasing concentrations of ligand. It can also be readily modified to examine the effects of putative inhibitors and pH on binding of receptor to ligand.

Using the poly(ethylene glycol) assay, we were able to measure a dissociation constant of 1.9×10^{-8} M for ^{125}I -Man-BSA binding to purified receptor (Fig. 2). This is identical with that reported for intact macrophages [7] and demonstrates that the receptor loses little binding activity during isolation. The results of the inhibition assays show that mannose receptor binds neoglycoproteins prepared by using mannose (Fig. 2) or fucose, but not galactose (Table 1). Monosaccharides are poorer ligands for the receptor than are the polyvalent neoglycoproteins, an observation which is in agreement with the work of Hoppe & Lee [21]. The potency of inhibition of simple sugars (mannose > fucose > *N*-acetylglucosamine; Table 1) mimics that reported by Shepherd *et al.* [1] for neoglycoproteins bearing these monosaccharides. Studies of the effect of pH on ligand binding (Fig. 3) indicate that the receptor binds ligand at neutral pH and releases it when the pH falls below 6.0, an observation which is consistent with assimilation of ligand into macrophages and its release within an acidic endocytic vesicle [8].

The availability of large amounts of receptor provided the opportunity to elucidate some of the chemical properties of the protein. Compositional analysis reveal relatively high concentrations of serine and glycine, suggesting that these amino acids may be useful for studies involving biosynthetic labelling of receptor.

Lectin-blot analyses revealed the presence of both *N*- and *O*-linked oligosaccharides on the protein. The presence of oligosaccharides is a characteristic of many membrane proteins. However, the presence of *N*-linked oligosaccharides on the mannose receptor raises an interesting issue with respect to the binding specificity of

the protein. The receptor is known to bind to mammalian glycoproteins, such as β -glucuronidase, which bear high-mannose chains [1]. Since *N*-linked oligosaccharides are added to proteins as high-mannose precursors in the endoplasmic reticulum (reviewed in [27]), there must be some period of time during which the receptor itself carries high-mannose chains. Why, then, does the receptor not bind to itself (or neighbouring glycoprotein molecules) in the endoplasmic reticulum, causing inactivation of receptor? Several hypotheses for protection of the mannose-receptor binding site in the endoplasmic reticulum may be considered. Perhaps the receptor is synthesized as an inactive precursor, followed by conversion into an active form after oligosaccharide processing; alternatively, high-mannose chains may be physically separated from the receptor's active site during biosynthesis. The availability of the binding assay and antibodies to the receptor now make it feasible to address these possibilities.

Recent papers report the isolation of a mannose receptor from rabbit lung [15] and a fucose-binding lectin from rat lung [28,29]. Both proteins exhibit an M_r of 175000 and bind mannosylated ligands. Although we are not able to comment on the origin of the rat lectin, several lines of investigation provide strong evidence that the rabbit lung protein is the macrophage receptor. Antibody raised against the purified M_r -175000 lung protein [15] immunoprecipitates the macrophage receptor (Fig. 4), the dissociation constant for Man-BSA is identical for both proteins (Fig. 2), both receptors contain the same amino acid composition (Table 2) and *N*-terminal sequence analysis reveals identical *N*-termini (results not shown).

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