The macrophage mannose receptor mediates phagocytosis of pathogenic microorganisms and endocytosis of potentially harmful soluble glycoproteins by recognition of their defining carbohydrate structures. The mannose receptor is the prototype for a family of receptors each having an extracellular region consisting of 8–10 domains related to C-type carbohydrate recognition domains (CRDs), a fibronectin type II repeat and an N-terminal cysteine-rich domain. Hydrodynamic analysis and proteolysis experiments performed on fragments of the extracellular region of the receptor have been used to investigate its conformation. Size and shape parameters derived from sedimentation and diffusion coefficients indicate that the receptor is a monomeric, elongated and asymmetric molecule. Proteolysis experiments indicate the presence of close contacts between several pairs of domains and exposed linker regions separating CRDs 3 and 6 from their neighboring domains. Hydrodynamic coefficients predicted for modeled receptor conformations are consistent with an extended conformation with close contacts between three pairs of CRDs. The N-terminal cysteine-rich domain and the fibronectin type II repeat appear to increase the rigidity of the molecule. The rigid, extended conformation of the receptor places domains with different functions at distinct positions with respect to the membrane.

The mannose receptor of macrophages and liver endothelial cells is the best characterized member of a family of multidomain cell-surface receptors (1). Each member of the family shares the same overall domain organization, with an extracellular region consisting of an N-terminal cysteine-rich domain followed by a fibronectin type II repeat and 8 or 10 domains related in sequence to the C-type carbohydrate-recognition domains (CRDs)1 of animal lectins. The mannose receptor and one other member of the family, endothelial receptor Endo-180, are true C-type lectins, binding carbohydrates in a Ca2+-dependent manner (2, 3). In contrast, the C-type lectin-like domains of the M-type phospholipase A2 receptor and the dendritic cell receptor DEC-205 do not contain the conserved residues necessary for Ca2+-dependent sugar binding, and these two receptors do not bind carbohydrates (4–6). Each of the receptors mediates endocytosis, but physiological ligands have only been identified for the mannose receptor and the phospholipase A2 receptor.

The mannose receptor acts as a molecular scavenger, binding and internalizing pathogenic microorganisms and potentially harmful glycoproteins (7, 8). The multiple domains in the extracellular region of the receptor allow recognition of a diverse range of glycoconjugate ligands. Several of the eight C-type CRDs are involved in Ca2+-dependent recognition of terminal mannose, N-acetylgalactosamine or fucose residues on the surfaces of microorganisms or on the oligosaccharides of endogenous glycoproteins (9, 10). The receptor plays a role in the immune response against pathogens such as Mycobacterium tuberculosis and Pneumocystis carinii. It also regulates levels of endogenous proteins such as lysosomal enzymes and tissue plasminogen activator released from cells in response to pathological events. In addition, the mannose receptor binds terminal sulfated N-acetylgalactosamine residues on oligosaccharides of the pituitary hormones lutropin and thyrotropin and plays a role in clearing these hormones from the circulation after they have acted on their target cells (11, 12). The cysteine-rich domain of the mannose receptor recognizes sulfated N-acetylgalactosamine by a mechanism distinct from that of the C-type CRDs (13, 14).

Individual C-type CRDs of the mannose receptor show only weak affinity for monosaccharides. High affinity binding to the receptor requires multivalent interactions of oligosaccharides involving several CRDs (9, 10, 15). CRDs 4 and 5 form a protease-resistant ligand-binding core sufficient to bind some ligands but CRDs 4–8 are required for high affinity binding to natural ligands such as yeast mannan. It is likely that the presence of multiple C-type CRDs within the single polypeptide chain of the mannose receptor is important in determining specificity as well as affinity for oligosaccharide ligands, because the spatial arrangement of the CRDs must influence which ligands are able to interact in a multivalent manner. The importance of the spatial arrangement of C-type CRDs in defining ligand selectivity is illustrated by serum mannose-binding protein, in which the orientation of the three CRDs in each trimer is fixed so that mammalian oligosaccharides cannot be recognized and inappropriate complement fixation does not occur (8).

The mannose receptor is unusual when compared with other multidomain cell surface receptors in that the domains that seem to be most important for ligand binding, CRDs 4 and 5, are in the middle of the polypeptide rather than at the end. The fifth of the eight C-type lectin-like domains of the M-type phospholipase A2 receptor is also most important for binding of secretory phospholipases (16). One explanation for this phenomenon could be that the extracellular regions of each of these

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1 The abbreviations used are: CRD, carbohydrate recognition domain; MMR, macrophage mannose receptor; MALDI-MS, matrix-assisted laser desorption/ionization time of flight mass spectrometry; MOPS, 2-(N-morpholino)ethanesulfonic acid; Rs, Stokes radius.
receptors adopt a U-shaped conformation rather than extending linearly from the cell surface (Fig. 1) (8). Such an arrangement would put CRDs 4 and 5 furthest from the membrane.

This paper describes hydrodynamic analysis of the mannose receptor combined with analysis of protease resistance. The results indicate that the receptor is likely to adopt an extended conformation with contacts between some domains.

**EXPERIMENTAL PROCEDURES**

**Materials**—Sepharose 6B, α-α-methylmannopyranoside and molecular weight markers for gel filtration were obtained from Sigma. Bis(sulfosuccinimidyl)suberate was obtained from Pierce. Endoglycosidase H and peptide N-glycosidase F were from New England Biolabs. Methotrexate, dialyzed fetal calf serum, and all media were obtained from Life Technologies. Mannose-Sepharose was prepared using the divinyl sulfone method (17).

**Expression and Purification of Mannose Receptor**—Production of Chinese hamster ovary cell lines expressing the extracellular region of the mannose receptor (MMR-S) and a fragment containing only the eight CRDs (MMR1–8) has been described previously (18). Both cell lines were maintained in a minimal essential medium without nucleosides or deoxyribonucleosides, supplemented with 10% dialyzed fetal calf serum and 0.5 mM methotrexate. Following growth to confluence, cells expressing MMR-S were changed into serum-free medium (Chinese hamster ovary-S-SF HIM II) without nucleosides, supplemented with 50 mM Hepes, pH 7.5, 0.11 mM CaCl₂, and 0.5 mM methotrexate. Cells expressing MMR1–8 were kept in serum-containing medium after every 3 days. MMR-S and MMR1–8 were isolated from the medium by affinity chromatography on mannose-Sepharose as described previously (18). MMR1–8 was separated from trace serum contaminants by analytical ultracentrifugation on mannose-Sepharose as described previously (18). MMR1–8 was separated from trace serum contaminants by affinity chromatography on a Mono-S column (Amerham Pharmacia Biotech). Protein was loaded in 20 mM MOPS, pH 6.0, and eluted with a gradient of 0–0.5 mM NaCl in the same buffer. MMR1–8 is isolated from serum-free medium required no further purification. Pure protein fractions were concentrated if necessary using a Centricon-30 micro-concentrator (Millipore).

**Deglycosylation of Mannose Receptor Fragments**—MMR1–8 (0.2 mg) was treated with endoglycosidase H (20,000 units) in 1 ml of 50 mM sodium citrate, pH 5.5, or with peptide N-glycosidase F (10,000 units) in 1 ml of 50 mM sodium phosphate, pH 7.5. Reactions were carried out at 37 °C overnight. Enzymes were separated from MMR1–8 by anion-exchange fast protein liquid chromatography on a Mono-Q column (Amerham Pharmacia Biotech). Protein was loaded in 50 mM Tris-HCl, pH 8.0, and eluted with a gradient of 0–0.5 mM NaCl in the same buffer.

**Matrix-assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-MS)**—Samples of MMR-S and MMR1–8 (100 pmol/μl) were analyzed on a Finnegan MAT Lasermat mass spectrometer, or applied to the target plate in 50% acetonitrile containing 0.1% trifluoroacetic acid as was used in the matrix. Ten spectra were collected for each sample in positive ion mode, each spectrum consisting of signals averaged from 10 to 30 consecutive laser pulses. Dimeric bovine serum albumin ([M + H]²⁺ = 132,860 Da) was used as a calibration standard.

**Analytical Ultracentrifugation**—All experiments were carried out at 20 °C in the An50Ti rotor of a Beckman Optima XL-A analytical ultracentrifuge equipped with absorbance optics. Before analysis, protein samples were dialyzed overnight against an appropriate buffer. A calibration standard was run for every centrifuge run. Sedimentation coefficients for MMR-S and MMR1–8 in the absence of Ca²⁺ were determined in 0.1 M Hepes, pH 7.5, and 0.15 mM NaCl. In some experiments, 5 mM CaCl₂ or 5 mM MgCl₂ and 100 mM α-methylmannoside were added. Aliquots were incubated at room temperature for 1 h with different concentrations of bis(sulfosuccinimidyl)suberate before analysis by SDS-gel electrophoresis on 5% polyacrylamide gels.

**Analytical Gel Filtration**—Analytical gel filtration chromatography was performed on a 300 × 7.8-mm BioSep-S30000 column (Phenomenex). The column was eluted with 50 mM Tris acetate, pH 7.5, at a flow rate of 0.5 ml/min. Thyroglobulin (669 kDa), serum albumin (66 kDa), alcohol dehydrogenase (150 kDa), bovine serum albumin (66 kDa), and carbonic anhydrase (29 kDa) were used as standards, with RNase S peptide used as a marker of the included volume of the column. Values for the Stokes radius (Rₛ) of protein standards were calculated from their known diffusion coefficients (20). Values for the Stokes radii of MMR-S and MMR1–8 were determined from their elution positions (21).

**Analysis of Protease Resistance**—MMR1–8 and MMR-S were subjected to limited trypsin digestion after adjustment of Ca⁡²⁺ concentration, following previously described protocols (10). Briefly, MMR1–8 and MMR-S (0.25 mg/ml) in 25 mM Tris-HCl, pH 7.8, 0.15 mM NaCl, 10 mM CaCl₂) were incubated with increasing amounts of trypsin for 30 min at 37 °C. Digestions were stopped by addition of an equal volume of double strength sample buffer and immediate heating to 100 °C for 5 min. Products were analyzed by SDS-PAGE on 15% gels, stained with Coomassie Blue, and scanned using a Molecular Dynamics densitometer. Digestion products separated on 15% mini-gels were subjected to N-terminal sequencing in a Beckman LFS3000 protein sequencer following transfer to polyvinylidene difluoride membranes (22).

**Calculation of Size and Shape Parameters**—Stokes radii determined by analytical gel filtration. Stokes radii were also determined from molar frictional coefficients calculated using sedimentation coefficient values and the molecular masses determined by mass spectrometry (19). Stokes radii obtained from sedimentation coefficient values and from analytical gel filtration were used to calculate frictional ratios (f/f₀) (19). Axial ratios (a/b) were calculated from frictional ratios for prolactin ellipsoid of revolution (24). Hydration of 0.28 g of water per g of protein was assumed (19). Dimensions of MMR-S and MMR1–8 were calculated from their axial ratios using Equations 14–19 of Tanfod (25).

**Hydrodynamic Modeling**—The program HYDRO was used to calculate sedimentation and diffusion coefficients for models of different receptor conformations (26). Models of MMR-S and MMR1–8 were formulated as a series of contiguous spheres. Each domain was represented by a sphere with a volume equal to that calculated from a molecular surface generated with the program GRASP (27). Spheres of 30 Å diameter were used for the CRDs, based on the crystal structures of the C-type CRD of rat mannose-binding protein A and mannose receptor CRD-4 (28, 29). The fibronectin-type II repeat was represented by a sphere of 24 Å, based on the solution structure of the second of these domains from fibronectin (30), while the cysteine-rich domain was modeled as a sphere of 22 Å based on its crystal structure (14). Linker regions between the domains were represented as four spheres of diameter 5 Å. These dimensions were based on the average length and volume calculated for stretches of 10 amino acids taken from six different regions of the rat mannose-binding protein A structure (28).

**RESULTS**

Hydrodynamic and proteolysis experiments were carried out on two purified soluble fragments of the mannose receptor in order to examine the shape, oligomeric state and domain arrangement of the receptor. One fragment (MMR-S) consists of the whole extracellular domain of the receptor, while the other (MMR1–8) consists of just the eight C-type CRDs without the cysteine-rich domain and the fibronectin type II repeat (Fig. 1). MMR1–8 was analyzed in order to determine possible effects of the two N-terminal domains on the conformation of the receptor.

**Hydrodynamic Analysis of the Mannose Receptor**—Comparison of the calculated peptide molecular masses for MMR-S and MMR1–8 with the molecular masses determined by MALDI-MS (Table I) allows estimation of the extent of glycosylation of these mannose receptor fragments. The mass difference is about 20,000 Da for both MMR-S and MMR1–8. There are eight potential sites for N-linked oligosaccharides on...
MMR-S and seven on MMR1–8. Four sites for O-linked oligosaccharides have also been identified based on protein sequencing data (2). The additional mass in each case was attributed to the presence of seven N-linked biantennary complex oligosaccharides and four O-linked disaccharides of GalNAc and GlcNAc. Partial specific volumes of 0.714 cm$^3$/g for MMR-S and 0.717 cm$^3$/g for MMR1–8 were calculated using these estimates of glycosylation (19, 23).

Sedimentation velocity analysis of MMR-S and MMR1–8 by analytical ultracentrifugation was used to determine sedimentation coefficients. Data were analyzed by the $g(s^*)$ method in which the distribution of sedimentation coefficients is calculated. In the absence of Ca$^{2+}$, a narrow symmetrical peak is obtained for each receptor fragment indicating the presence of a single species (Fig. 2). The sedimentation coefficients obtained (Table I) are lower than would be expected for globular proteins of the same mass as the mannose receptor fragments, indicating that the receptor is asymmetric. Equilibrium sedimentation experiments show that the receptor fragments are monomeric in the absence of Ca$^{2+}$. The apparent molecular mass as a function of receptor concentration remains constant across the sample cell (Fig. 3, A and B) indicating that MMR-S and MMR1–8 are monomeric at all protein concentrations. Apparent molecular masses are 173,000 ± 6,700 Da for MMR-S and 165,000 ± 4,400 Da for MMR1–8. These values correspond closely to the values of 174,500 Da and 146,700 Da determined for MMR-S and MMR1–8 by mass spectrometry. Thus, analytical ultracentrifugation indicates that the mannose receptor is monomeric in the absence of Ca$^{2+}$ and sediments at a rate consistent with an asymmetric, extended conformation.

Because at least some of the C-type CRDs of the receptor bind Ca$^{2+}$, sedimentation experiments were also carried out in the presence of 5 mM CaCl$_2$. However, the results indicate that both MMR-S and MMR1–8 aggregate under these conditions. On sedimentation velocity analysis, a broad asymmetric peak is obtained for each receptor fragment, indicating the presence

![Fig. 1. Domain organization of the mannose receptor.](image)

**TABLE I**

Size and shape parameters obtained for mannose receptor fragments by mass spectrometry, sedimentation analysis, and gel filtration

| Parameter                              | MMR-S     | MMR1–8    |
|----------------------------------------|-----------|-----------|
| Calculated peptide molecular mass (Da) | 154,376   | 126,252   |
| Molecular mass determined by MALDI-MS (Da) | 174,500 ± 1400 | 146,700 ± 600 |
| $s_20,w^0 (S)$                          | 7.29 ± 0.08| 7.21 ± 0.08|
| $D_3$ (cm$^2$/s)                       | 3.63 ± 0.02| 4.15 ± 0.02|
| $R_s$ (Å) from sedimentation coefficients | 59.2 ± 0.3 | 52.0 ± 0.7 |
| $g(s^*)$                                | 1.63      | 1.47      |
| a/b                                     | 8.5       | 6.1       |

![Fig. 2. Velocity sedimentation analysis of the mannose receptor.](image)
of multiple species (Fig. 2). Average sedimentation coefficient values of 10.02 ± 0.11 S and 9.01 ± 0.08 S were obtained from three independent sedimentation velocity experiments for MMR-S and MMR1–8, respectively. These average values are considerably higher than those obtained in the absence of Ca\(^{2+}\) (Table I), although overlap in the two curves indicates that a proportion of the protein in Ca\(^{2+}\) is monomeric (Fig. 2). Equilibrium sedimentation analysis confirms that aggregation of MMR-S and MMR1–8 occurs when Ca\(^{2+}\) is present. The apparent molecular mass of each receptor fragment increases as a function of concentration throughout the sample cell (Fig. 3, C and D). The fact that the curves calculated at different loading concentrations do not all overlap indicates that at least some oligomeric forms do not interconvert, consistent with aggregation. Cross-linking experiments confirm the ultracentrifugation data, with monomeric receptor fragments forming at least tetramers on addition of Ca\(^{2+}\) (data not shown). In the absence of Ca\(^{2+}\), cross-linking of monomers does not occur.

Previous studies on the intact mannose receptor have suggested that the receptor is monomeric (10). Detergent-solubilized receptor sediments on a sucrose gradient as a single band at a position corresponding to a monomer. Furthermore, no evidence of oligomerization was obtained in cross-linking studies in the presence of 10 mM Ca\(^{2+}\) (10). Thus, aggregation seen in the presence of Ca\(^{2+}\) is unlikely to be an indication of the natural state of the receptor. Binding of oligosaccharides on one receptor molecule by the CRDs of another molecule could cause Ca\(^{2+}\)-induced association. For example, high mannose oligosaccharides or hybrid oligosaccharides with terminal mannose or N-acetylglucosamine residues could act as ligands for CRDs 4 and 5. Analytical ultracentrifugation and cross-linking carried out on receptor fragments treated with endoglycosidase H to remove high mannose and hybrid oligosaccharides, or carried out in the presence of α-methylmannoside in an attempt to saturate ligand-binding sites, resulted in a decrease in the amount of aggregation (data not shown). Combination of endoglycosidase H treatment and working in α-methylmannoside decreased aggregation more than either strategy used separately. These results indicate that Ca\(^{2+}\)-induced aggregation is largely due to the presence of terminal sugar residues on one receptor molecule being bound by another receptor molecule, although aggregation could not be completely abolished. Because endoglycosidase H treatment leaves a single GlcNAc residue that could still bind to the mannose receptor, removal of all N-linked oligosaccharides by peptide N glycanses P treatment was attempted. Unfortunately, this treatment resulted in aggregation of receptor fragments under all buffer conditions (data not shown).

Because Ca\(^{2+}\)-induced aggregation could not be completely eradicated, data obtained in the absence of Ca\(^{2+}\) were used for analysis of the conformation of the receptor. Under physiological conditions, Ca\(^{2+}\) would be bound by some of the CRDs of the mannose receptor. Studies with other C-type lectins indicate a local change in the position of the loops forming the sugar-binding site but no overall conformational change in the CRD in the absence of Ca\(^{2+}\) (31). While it is possible that Ca\(^{2+}\) could mediate CRD-CRD interactions within the mannose receptor, there is no evidence for such Ca\(^{2+}\)-dependent interactions in this receptor or in other C-type lectins. The overall conformation of the receptor is therefore not expected to differ in the presence or absence of Ca\(^{2+}\). This conclusion is supported by the data obtained from sedimentation velocity experiments (Fig. 2) which show overlapping peaks in the g(s) analysis, indicating that monomers sediment at similar rates in the presence and absence of Ca\(^{2+}\). Thus the only change occurring on moving from a Ca\(^{2+}\)-free to a Ca\(^{2+}\)-containing buffer is a change in the oligomeric state.

Analytical gel filtration was used to determine the Stokes radii and diffusion coefficients for MMR-S and MMR1–8. Each receptor fragment eluted as a sharp peak indicating the presence of a single species (Fig. 4). Values for Stokes radii and diffusion coefficients obtained from gel filtration for MMR-S and MMR1–8 in the absence of Ca\(^{2+}\) are shown in Table I. Values for Stokes radii were also calculated from the sedimentation coefficients using the molecular masses determined by MALDI-MS. Values obtained by the two different methods are in close agreement, and were thus averaged for calculation of the frictional ratios (f/f_0) (Table I). The frictional ratios obtained for MMR-S and MMR1–8 are considerably greater than 1.00, indicating that the shape of the receptor deviates substantially from that of a sphere. Axial ratios of 8.5:1 and 6:1:1 calculated for MMR-S and MMR1–8 modeled as prolate ellipsoids of revolution indicate that the receptor is elongated. Dimensions calculated from the axial ratios are 306 Å × 36 Å for MMR-S and 232 Å × 38 Å for MMR1–8. Although a prolate ellipsoid is unlikely to be a close representation of the shape of the receptor, these values give a first approximation of its size.

Identification of Protease-resistant CRD Clusters in the Mannose Receptor—Proteolysis experiments were performed to investigate the extent of contact between neighboring CRDs. Examination of the amino acid sequence of the mannose receptor reveals linker regions of about 10–15 amino acids between CRDs (2). Each linker region contains at least one lysine or arginine residue and would be expected to be susceptible to trypsin digestion if the sites were accessible to the enzyme. Because individual C-type CRDs are resistant to proteolysis in the presence of Ca\(^{2+}\), protease digestion of the receptor would be most likely to occur at the linker regions (28). Extensive
contacts between a pair of CRDs protect the linker from proteolysis, whereas linkers that adopt an extended conformation are susceptible to digestion.

The results of digesting MMR1–8 with trypsin in the presence of Ca²⁺ are shown in Fig. 5. Proteolysis yields distinct bands that can be identified based on molecular weights and N-terminal sequences. Western blotting with antibodies specific for CRD-4 and the cysteine-rich domain was also used to confirm the identity of some fragments. At low protease concentrations, MMR1–8 is cleaved to yield two fragments consisting of CRDs 1 to 6 and 7 to 8. Cleavage occurs at an arginine residue at the start of the linker region between CRDs 6 and 7, releasing CRDs 7 to 8 with the linker region still attached. This result suggests that the linker between CRDs 6 and 7 is extended or flexible. At higher trypsin concentrations, the linker attached to CRD-7 is cleaved at a lysine residue close to the start of CRD-7, leaving CRDs 7 and 8 connected. No cleavage occurs between CRDs 7 and 8 even at high concentrations of trypsin, indicating that these domains are in close contact. However, some degradation of CRD 7 is observed, with cleavage of CRD 7 at an internal arginine residue, facilitating further degradation and release of free CRD 8.

Further digestion of the CRD 1 to 6 fragment occurs at higher concentrations of trypsin. Fragments consisting of CRDs 1 to 5, 1 to 3, and 4 to 6 appear transiently, but stable fragments consisting of CRDs 1 to 2 and 4 to 5 are the predominant products of digestion of CRDs 1 to 6. These results suggest that extensive contacts exist between CRDs 1 and 2 and confirm previous findings showing that CRDs 4 and 5 are in close contact (10). In contrast, the linkers between CRDs 2 and 3, between CRDs 3 and 4, and between CRDs 5 and 6 are exposed to the protease.

Digestion of MMR-S with trypsin in the presence of Ca²⁺ shows a very similar pattern to that obtained with MMR1–8 (Fig. 5). Delay is seen in the generation of the CRD 1 to 2 fragment, due to the cysteine-rich domain and the fibronectin type II repeat remaining attached to CRDs 1 and 2. This result suggests that the cysteine-rich domain and the fibronectin type II repeat, along with the fibronectin type II repeat and CRD 1 are in fairly close contact. However, the fragment consisting of CRDs 1 and 2 is seen at higher trypsin concentrations, and some intact cysteine-rich domain is detected, suggesting that proteolysis of the fibronectin type II repeat occurs.

The proteolysis studies show that there are three protease-resistant pairs of CRDs in the mannose receptor: CRDs 1 and 2, CRDs 4 and 5, and CRDs 7 and 8. Close contacts probably exist between the domains in each of these pairs. A conformation in which all the linker regions are extended, separating the domains like beads on a string can therefore be ruled out. However, the data suggest that CRDs 3 and 6 may not be in close contact with their neighboring domains. The linkers on either side of CRD-3 and of CRD-6 could be extended or flexible.

Modeling of Mannose Receptor Conformations—Computer modeling was used to identify conformations of the mannose receptor consistent with the data described here. Using the program HYDRO, hydrodynamic coefficients were predicted for various receptor conformations modeled as arrays of contiguous spheres to represent the domains. Initially, the program was used to predict hydrodynamic coefficients for a simple U-shaped model with CRDs 4 and 5 at the apex and an extended model with the domains arranged linearly (Fig. 6). In each of these two models, neighboring domains are in contact with each other. Comparison of the predicted hydrodynamic coefficients with the values obtained experimentally for MMR-S and MMR1–8 immediately indicates that the U-shaped model can be ruled out. When modeled as a U-shape, the receptor is quite compact, and this compact shape gives predictions for sedimentation and diffusion coefficients that are much higher than the values obtained by experiment. The experimental data fit much more closely to the extended model.

Values for sedimentation and diffusion coefficient predicted for the simple extended model are still higher than the values derived experimentally. Addition of linker regions between the domains extends the model further and lowers the predicted sedimentation and diffusion coefficients. For MMR-S, inclusion of linkers on either side of CRDs 3 and 6 gives predicted values of sedimentation and diffusion coefficients that are almost identical to those obtained by experiment (Fig. 6). The very close agreement between the predicted hydrodynamic parameters and those derived from the experiment, combined with the fact that the proteolysis experiments suggest that only four linkers are likely to be extended, provides good evidence that this extended model approximates the actual conformation of the mannose receptor. Adding together the diameters of the spheres in this model gives an estimate of 380 Å for the length of the receptor. Inclusion of four linker regions in the U-shaped model still gives predicted values of sedimentation and diffusion coefficients that are substantially higher than the values derived from the experimental data (Fig. 6). Other U-shaped models, in which the molecule is bent at different places, also yield predicted hydrodynamic coefficients that are much higher than the experimental values (data not shown).

For MMR1–8, inclusion of the four linkers in the extended model gives predicted sedimentation and diffusion coefficients that are slightly lower than those determined by experiment (Fig. 6). This result suggests that in the absence of the cysteine-rich domain and the fibronectin type II repeat, the eight CRD fragment may be somewhat flexible, so that it sediments faster than predicted for a rigid molecule. However, predicted coefficients for MMR1–8 in the U-shaped model with four linkers are still inconsistent with the experimental values, indicating that the portion of the polypeptide containing the eight CRDs must be extended.

As well as indicating that the receptor is extended with

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2 C. E. Napper and M. E. Taylor unpublished observations.
contacts between some domains, the modeling studies also show that the receptor must be fairly rigid. Flexibility allowing some bending in the receptor would lead to a more compact molecule that would sediment and diffuse faster than a rigid linear molecule. If the receptor were flexible, it would have to be much longer for diffusion and sedimentation coefficients to be as low as those determined. Such an increase in length would require all the domains to be separated without contacts between them, and the proteolysis studies indicate that this is not the case.

**Fig. 5.** Protease resistance of the mannose receptor. Concentrations of trypsin used are indicated above each lane. Bands identified by mobilities and N-terminal sequences are labeled on the right. Positions of molecular weight markers are shown on the left.

**Fig. 6.** Hydrodynamic modeling of mannose receptor conformations. Sedimentation coefficients ($s_{20,w}^n$) and diffusion coefficients ($D$) calculated using HYDRO for various conformations of MMR-S (A) and MMR1–8 (B). Experimental values are shown at the top for comparison.
A combination of hydrodynamic analysis and proteolysis experiments provides good evidence that the mannose receptor adopts a rigid, extended conformation with close contacts between some pairs of neighboring domains. A U-shaped conformation can be ruled out, as can a conformation in which the domains are spread out like beads on a string. The approximate dimensions calculated from the modeling studies suggest that the mannose receptor may project about 380 Å from the cell surface. These findings have a number of implications for the function of members of the mannose receptor family in endocytosis and cell adhesion.

With the mannose receptor in an extended conformation, the two C-type CRDs most important for binding glycoproteins, CRDs 4 and 5, are not outermost from the membrane. However, when the receptor is localized in fluid-filled clathrin-coated pits, it will be surrounded by lipid, allowing binding to CRDs 4 and 5 from the side of the molecule. An extended conformation of the receptor results in the cysteine-rich domain being projected furthest away from the membrane. The cysteine-rich domain would not need to be in this exposed position to allow binding of glycoprotein hormones in coated pits, but such an arrangement would be important if binding of cell-surface sulfated ligands by the cysteine-rich domain plays a role in directing macrophages to germinal centers during the immune response (32, 33). For comparison, CD8, E-selectin, and sialoadhesin, three proteins involved in cell-cell interactions, are predicted to extend about 200, 270, and 500 Å from the cell surface (34–36). Projection of the cysteine-rich domain 380 Å from the membrane would be sufficient to allow trans interactions between the mannose receptor and sulfated ligands on other cells.

The finding that six CRDs form pairs with close contacts between the two CRDs in each pair, while CRDs 3 and 6 appear to be separated from their neighboring domains by extended linker regions, is likely to be important in explaining how the mannose receptor can interact with both exogenous and endogenous oligosaccharides. While CRDs 4 and 5 could be close enough together to recognize mannose residues on a mammalian high mannose oligosaccharide, the extended linkers on either side of CRD 3 and CRD 6 would provide wider spacing between CRDs 4 and 5 and the accessory domains. Such an arrangement might be suitable for matching the more widely spaced arrays of sugars on the surfaces of pathogens. In serum mannose-binding protein, the sugar-binding sites in adjacent CRDs are 53 Å apart (37). The linker regions in the mannose receptor could enable a comparable separation of some of its CRDs. A large amount of flexibility between the CRDs would be energetically unfavorable for binding to oligosaccharides. However, having a limited amount of flexibility in the linker regions between some domains, while the position of other CRDs are fixed relative to one another by close contact, might be important in allowing the mannose receptor to recognize a diverse range of ligands.

Carbohydrate-mediated aggregation of soluble receptor fragments observed in the presence of Ca$^{2+}$ probably results from interactions that would be less likely to take place with the receptor in the membrane. The soluble extracellular domain of the receptor adopts a rigid extended conformation, but without the constraints imposed by a membrane anchor, a high mannose oligosaccharide on any part of one receptor molecule is free to interact with CRDs 4 or 5 on another. However, under some circumstances, a high mannose or hybrid oligosaccharide might occur on some receptor molecules at the correct distance from the membrane to allow limited oligomerization to occur. In this way, post-translational modifications could alter the ligand-binding properties of the receptor. Cross-binding of receptor molecules is consistent with the proposal that some cell types might express some dimeric receptor able to bind glycoprotein hormones, but unable to bind mannose-terminated glycoproteins (12, 38).

It is likely that other members of the mannose receptor family will also adopt an extended conformation, which might be important for roles of these receptors in cell adhesion. Like the mannose receptor, the phospholipase A$_2$ receptor binds its ligands, phospholipases A$_2$ predominantly through domains in the middle of the polypeptide (16). As with the mannose receptor, sequestering of the phospholipase A$_2$ receptor into clathrin-coated pits would allow phospholipases A$_2$ to bind from the side. A role in cell adhesion has also been proposed for the phospholipase A$_2$ receptor as it has been shown to mediate cell spreading on collagens type I and IV through collagen binding by the fibronectin type II repeat (39). Examination of the sequence of Endo-180, the other member of the family that binds carbohydrate ligands in a Ca$^{2+}$-dependent manner, suggests that CRDs 1 and 2 are responsible for this activity (3). Once again, although Endo-180 is an endocytic receptor, it binds strongly to collagen V, suggesting that it might also have a role in cell adhesion and making an extended conformation more important (40). It is interesting that the presence of the N-terminal cysteine-rich domain and the fibronectin type II repeat seem to confer extra rigidity on the mannose receptor, since these are the domains predicted to be involved in cell adhesion functions.

Conservation of the linker regions between domains is seen in the members of the mannose receptor family (3–6). Therefore it is likely that the same pattern of close contacts between some neighboring domains with extended linkers on either side of CRDs 3 and 6 will be found in each protein. As in the mannose receptor, such spacing of the domains might be important for allowing DEC-205 to interact with a diverse range of ligands. Although no ligands have yet been identified for DEC-205, there is a considerable amount of evidence to suggest that it is involved in enhancing antigen processing and presentation (6, 41). In such a role, it is probable that DEC-205 would need to interact with multiple different ligands, rather than being specific for a single ligand.

In all of the receptors in the mannose receptor family, a rigid, extended conformation positions different domains at distinct distances from the membrane. It is likely that the constraints imposed by this conformation influences which ligands each receptor can bind.

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