Structural Model for the Mannose Receptor Family Uncovered by Electron Microscopy of Endo180 and the Mannose Receptor*

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The mannose receptor family comprises four members in mammals, Endo180 (CD280), DEC-205 (CD205), phospholipase A2 receptor (PLA2R) and the mannose receptor (MR, CD206), whose extracellular portion contains a similar domain arrangement: an N-terminal cysteine-rich domain (CysR) followed by a single fibronectin type II domain (FNII) and 8–10 C-type lectin-like domains (CTLDs). These proteins mediate diverse functions ranging from extracellular matrix turnover through collagen uptake to homeostasis and immunity based on sugar recognition. Endo180 and the MR are multivalent transmembrane receptors capable of interacting with multiple ligands; in both receptors FNII recognizes collagens, and a single CTLD retains lectin activity (CTLD2 in Endo180 and CTLD4 in MR). It is expected that the overall conformation of these multivalent molecules would deeply influence their function as the availability of their binding sites could be altered under different conditions. However, conflicting reports have been published on the three-dimensional arrangement of these receptors. Here, we have used single particle electron microscopy to elucidate the three-dimensional organization of the MR and Endo180. Strikingly, we have found that both receptors display distinct three-dimensional structures, which are, however, conceptually very similar: a bent and compact conformation built upon interactions of the CysR domain and the lone functional CTLD. Biochemical and electron microscopy experiments indicate that, under a low pH mimicking the endosomal environment, both MR and Endo180 experience large conformational changes. We propose a structural model for the mannose receptor family where at least two conformations exist that may serve to regulate differences in ligand selectivity.

The mannose receptor (MR4 and CD206), together with Endo180 (also known as the urokinase-type plasminogen activator receptor-associated protein (uPARAP) and CD280), the dendritic cell receptor DEC-205 (CD205) and the M-type phospholipase A2 receptor (PLA2R) encompass the mannose receptor family of endocytic receptors (1, 2). The members of this family are unique within the superfamily of C-type lectins as these alone contain multiple C-type lectin-like domains (CTLDs) within a single polypeptide backbone. All these receptors are recycled between the plasma membrane and the endosomal apparatus and mediate the uptake of extracellular ligands for intracellular delivery (2). Recently, an avian structural homologue of the mammalian mannose receptor family members, FcRγ, has been discovered to function as a yolk sac IgY receptor (3).

The MR was originally described as a transmembrane receptor that recognized saccharide chains terminating in mannose and designated as the macrophage mannose receptor. Since its expression was found not to be restricted to macrophages, it is now known as MR (2). MR executes important roles in the immune system by functioning as a pattern recognition receptor toward a wide range of pathogens. Other members of the family were found subsequently as proteins with a significant homology with the human MR. Together, they represent an exceptional family of multidomain multifunction proteins where each member has evolved distinct ligand binding properties (2). These include calcium-dependent sugar binding to specific CTLDs (MR and Endo180), binding of sulfated sugars to the cysteine-rich domain (MR), calcium-independent binding of proteins to CTLDs (PLA2R), and the binding of collagen (MR, PLA2R, and Endo180).

Members of the mannose receptor family have very diverse functions. The MR acts as a molecular scavenger that binds and internalizes potentially harmful glycoproteins with terminal mannose, fucosyl, or N-acetylglucosamine (4, 5). The MR may also mediate phagocytosis of a wide variety of microbes including fungi, protozoa, yeast, and bacteria, all of them displaying a high density of mannose at their cell surface (6). Endo180 is involved in the remodelling of extracellular matrix through its interaction with collagen and the uPA-uPAR complex (7–10), and in vivo studies have implicated Endo180 as a major pathway of collagen turnover during malignancy (11). PLA2R can internalize soluble lipo-

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The abbreviations used are: MR, mannose receptor; PLA2R, phospholipase A2 receptor; CTLD, C-type lectin-like domain; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; CysR, cysteine-rich domain; FNII, fibronectin type II domain; CRD, carbohydrate recognition domain; EM, electron microscopy; HA, hemagglutinin; sMR-HA, HA-tagged soluble form of MR; PAA, polyclamydite; PDB, Protein Data Bank; mAb, monoclonal antibody.
lytic enzymes (PLA₂) (12), whereas DEC-205 is involved in the internalization of antigens for presentation to T lymphocytes (13). Last, the avian structural homologue FcRY is a protein responsible for pH-dependent binding of IgY in the chicken yolk sac membrane (3).

Despite their diverse functions the members of the mannose receptor family present a similar structure. Their extracellular portion contains an N-terminal cysteine-rich domain (CysR), a single fibronectin type II domain (FNII), and eight (10 in the case of DEC-205) CTLDs (Fig. 1A). Following a single transmembrane domain a short cytoplasmic domain directs the trafficking of these receptors between the plasma membrane and the endosomal apparatus. The MR is unique within the family in that its CysR domain has a lectin activity, being able to bind sulfated oligosaccharides such as those present on the pituitary hormones lutropin and thyrotropin (14, 15). Conversely, the sequence analysis of the FNII predicts that all family members will bind collagen (2). Indeed, cells expressing wild type PLA₂R bind type I and type IV collagens, while the cells expressing receptors lacking CysR and FNII domains do not have this property (16). It has also been demonstrated that Endo180 and MR play an essential role in the cellular uptake of collagen, which is recognized through their FNII domain (9–11, 17, 18). Initial characterization of the mannose receptor family described these proteins as having multiple C-type carbohydrate recognition domains. However, the majority of these domains do not have C-type lectin activity so a more accurate term to refer to these structures is CTLDs. Chicken FcRY, PLA₂R, and DEC-205 lack calcium-coordinating residues that are critical for function as calcium dependent lectins. On the other hand, Endo180 and MR bind mannose, fucose, and N-acetylglucosamine in a Ca²⁺/H⁺-dependent manner, but their sugar binding activity is restricted to CTLD2 in the case of Endo180 (19) and to CTLD4 in the case of MR (20). Accordingly, these are the only domains containing the conserved amino acids found in functional C-type lectins (17, 19, 20).

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Mannose Receptor Family Three-dimensional Structure

relevant feature of this family of receptors is that some of them interact with other proteins through their CTLD in a sugar independent fashion, as it is the case for PLA$_2$R that binds nonglycosylated forms of PLA$_2$ (12). Endo180 has been detected as part of a trimeric complex with uPAR and its receptor, uPAR (7); in this instance the exact binding site in Endo180 has not been defined, but this interaction can be inhibited by collagen suggesting the involvement of the FNII domain. This later complex is critical for the regulation of uPA-uPAR mediated cell chemotaxis (21). Therefore, the proteins in the mannose receptor family can bind sugars, collagen, and also interact with other proteins and receptors to form larger multiprotein complexes, and we predict that these biological activities can be strongly influenced by the three-dimensional organization of their extracellular domains. In agreement with this premise, FcRy displays pH-dependent binding to IgY that correlates with the presence of a compact conformation (3). The authors suggest that in this conformation the CysR-FNII region folds back to bind to the CTLDs. These pH-dependent conformational changes seem to direct delivery of IgY from endosomal compartments to the blood (3). In the case of Endo180, electron microscopy analysis of the three-dimensional arrangement of CysR, FNIII, and CTLD1–2 domains showed that they display, at neutral pH, a globular conformation in which the CysR makes contact with CTLD2 (22). These results suggest that there might be a general trend among these receptors to display complex conformations. Despite this, the hydrodynamic analysis of the MR suggests that this protein has a rigid extended conformation (23).

To solve these discrepancies we have examined the appearance of full-length soluble forms of MR and Endo180 by single-particle electron microscopy (EM) (24). Based on our EM and biochemical results we propose a model for the domain organization of mannose receptor family members in which the CTLD involved in ligand recognition (CTLD2 in the case of Endo180 and CTLD4 in the case of MR) associates with CysR. These receptors fluctuate between compact and more open conformations at different pH, indicating a possible mechanism for the regulation of ligand binding and/or selectivity.

**EXPERIMENTAL PROCEDURES**

**Expression and Purification of a Hemaglutinin-tagged Soluble Form of MR (sMR-HA)—**The expression vector pMR-HA was generated as follows. The DNA fragment encoding for a truncated form of the MR (sMR-HA) was obtained by substituting the region between the XbaI site placed at position 3496 and the EcoRI site placed at position 4250. This plasmid was digested with SalI, treated with Klenow, and digested by EcoRI. The insert was subsequently cloned into the position 4250. This plasmid was digested with SalI, treated with Klenow, and digested with EcoRI. Purified plasmid was transfected into 293T cells using genejuice (Stratagene) following the manufacturer’s instructions. Cells were kept in serum-free medium (Opti-MEM; Invitrogen) after transfection, and supernatant was collected after 3 days. Purification of sMR-HA was performed by affinity chromatography using an anti-MR monoclonal antibody (Clone MRS5D3 purchased from HyCult biotechnology b.v.) conjugated to gamma-mabind plus Sepharose as described (26).

sMR-HA Binding Assay—Binding of purified sMR-HA to carbohydrates was assessed as described (26). Briefly, polysaccharides were coated onto the wells of enzyme-linked immunosorbent assay plates (Nunc; Maxisorb) by incubation in 0.154 M NaCl overnight at 37 °C (50 μl/well) using polyacrylamide (PAA)-conjugated carbohydrates (Lectivity) at 5 μg/ml and yeast mannan at 20 μg/ml. Plates were washed five times in washing buffer (10 mM Tris-HCl, pH 7.5, 10 mM CaCl$_2$, 0.154 M NaCl, and 0.05% (w/v) Tween 20). sMR-HA diluted in washing buffer was added (50 μl/well) for 2 h at room temperature. For inhibition studies, incubation of sMR-HA with carbohydrates was performed in the presence of 1 M NaCl as described (26). Bound sMR-HA was detected by incubation with an anti-MR monoclonal antibody (clone MRS5D3 from HyCult biotechnology b.v.) at 10 μg/ml for 2 h followed by detection with anti-rat IgG Fc-specific alkaline phosphatase conjugated (Chemicon). Plates were washed five times and developed with p-nitrophenyl phosphate substrate (Sigma).

**Gel Filtration of the Soluble Mannose Receptor-HA**—The sMR-HA was analyzed by gel filtration using a Superose 12 (Amersham Biosciences) gel filtration column (10 mm × 300 mm). The running buffer was 10 mM Tris, 140 mM NaCl, pH 7.4, supplemented with either 10 mM CaCl$_2$, 10 mM EDTA, 10 mM mannose or 10 mM CaCl$_2$ with pH adjusted to 5.4, in different experiments.

**Expression and Purification of a Soluble Construct of Endo18—**A soluble version of the human Endo180 protein (sEndo180-HA-HIS) was expressed in COS-1 cells and purified as described previously (22). The purified protein was shown to display C-type lectin activity by binding to a GlcNAc-Sepharose column and elution in EDTA (22).

**Electron Microscopy and Three-dimensional Reconstruction**—For the electron microscopy experiments, 5 μl of a solution containing sEndo180-HA-HIS or the sMR-HA were applied to glow-discharged carbon-coated copper-rhodium grids. sMR-HA was negatively stained with 2% (w/v) uranyl acetate and observations were conducted at a JEOL 1230 microscope operated at 100 kV. Images were obtained on Kodak 4489 film at zero degrees tilt and a nominal magnification of ×40,000. Images were also taken with the specimen holder tilted to increase the angular coverage during the three-dimensional reconstruction. Selected micrographs were digitized in a Dimage Scan Multi Pro scanner (Minolta) with a final sampling window at the specimen of 5.3 Å/pixel. A total of 7322 particles were selected from the micrographs with the boxer program from EMAN (27). Particles were initially classified using model-free algorithms to generate a collection of reference-free averages used to build an initial volume by means of common lines. A Gaussian blob generated with EMAN was also used as starting model for further refinement (27). Both starting volumes were found to converge into a similar solution. Several parameters during image processing were adjusted to exclude those particles not consistent with the overall volumes. Also, averages were analyzed during refinement and shown to cover all Fourier space and were therefore adequate to generate a three-dimensional reconstruction. Resolution was estimated to be 33 Å by Fourier Shell Correlation of two independent volumes using half of the data and a cut off value of 0.5. Handedness was adjusted to match that already defined for Endo180 (22). The volumes were rendered to show 100% of their mass assuming an average density of 1.35 g/ml.

For the experiments performed with sEndo180-HA-HIS at low pH, the same sample was divided into two aliquots and one was maintained at pH 7.5, while the other was adjusted to pH 5.4 with citrate buffer. The two samples were stained with 2% (w/v) uranyl acetate, micrographs taken at ×60,000 magnification, digitized with a final sampling window of 1.76 Å/pixel, and ~2000 particles extracted for each case. Each data set was processed independently with a similar strategy to the one used for the MR. The resolution of the final reconstructions was estimated to be around 25 Å by Fourier Shell Correlation using cutoff values of 0.5.
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Fitting of the X-ray Structures with the Electron Microscopy Data of MR—Since there are only three types of domains (CysR, FNII, and CTLD) in a precise arrangement within the primary sequence, CysR and FNII in the N-terminal end and 8 continuous copies of CTLDs at the C-terminal end, domain assignment of each of these domains to a region of the three-dimensional map was satisfactorily unambiguous after fitting atomic structures of isolated domains. Fitting was performed manually using UCSF Chimera package (from the Resource for Biocomputing, Visualization, and Informatics at the University of California, San Francisco, supported by National Institutes of Health Grant P41 RR-01081) (28), with the help of the information provided by the our previous work with Endo180 and visual inspection of the new volume (22). Segmentation of the volume for coloring was carried out also with Chimera. The atomic structures used for fitting were obtained from the Protein Data Bank (PDB) and are as follows: the CysR domain from MR (PDB entry 1DQO), the FNII domain from the fibronectin FN1F2 domain (PDB entry 2FN2), and CTLDs were fitted with the mannoside binding protein A (MBP-A) (PDB entry 2MSB) and CTLD4 from the mannoside receptor (PDB entry 1EGI).

RESULTS

Purification and Biochemical Characterization of the Mannose Receptor—MR was purified by affinity chromatography from the supernatants of 293T cells as described under “Experimental Procedures.” psMR-HA encodes for the complete extracellular region of the MR fused to a hemaglutinin tag (HA) at the C-terminal end. sMR-HA was found to be highly pure by SDS-PAGE (Fig. 1B) and to bind sulfated (Fig. 1C) and mannosylated carbohydrates (Fig. 1D) in solid phase binding assays. In these assays similar results were obtained using the anti-MR mAb 5D3 and an anti-HA mAb for detection of bound sMR (data not shown) indicating that the binding of the MR5D3 mAb to the CTLD4–7 region does not alter sugar binding specificity. Additionally, the oligomeric state of our purified MR preparation was further characterized by its behavior on a gel filtration chromatography in the presence of 10 mM calcium at pH 7.4 (Fig. 1E). The sample was found to resolve in two peaks: a slower migrating population compatible with a monomeric form of MR of around 180 kDa and a second faster migrating peak consistent with a higher order oligomer of the MR as previously described (33). The soluble monomeric form of the MR was found to constitute the major species under these conditions although the ratio of oligomeric/monomeric material in purified MR samples altered upon storage.

Electron Microscopy and Three-dimensional Reconstruction of the sMR-HA—The EM technique allows the observation of individual molecules of a purified protein sample using an electron microscope. Typically different molecules of the same protein interact with the support film in different orientations, so that thousands of projection images along different directions can be recorded and used to build a three-dimensional structure of the protein with the help of image processing tools (24). Purified sMR-HA was applied to grids covered by a carbon foil, negatively stained, and observed using the electron microscope. For some of the preparations, images revealed areas where aggregated/oligomeric protein was present in accordance with the chromatographic analysis of the sample, while other areas showed clean fields with an even distribution of single particles of apparent similar dimensions (Fig. 2A). Most of these particles corresponded to isolated sMR-HA monomers as revealed later through image processing. These individual molecules of the 180-kDa MR protein were easily visualized under the electron microscope with the aid of the staining agent (Fig. 2A). Since the single particle EM technique requires obtaining individual images of multiple molecules of the same protein, images belonging to the larger aggregated/oligomeric material were discarded of subsequent analyses.

A total of 7322 images of individual molecules were extracted from the micrographs and processed using the EMAN software package (27) as described under “Experimental Procedures.” Classification and refinement methods permit one to average those images belonging to projections of the molecules in a similar orientation. Such averages provide images with higher signal to noise ratio that represent different views of the molecule. After refinement, the volume converges and its theoretical projections (Fig. 2B) and the corresponding class averages (Fig. 2B) correlate well with each other. The visual inspection of these two-dimensional averages, representative of projections of the sMR-HA molecule in different directions, already suggests that the MR displays a partially globular conformation. These averages were then used to reconstruct the three-dimensional structure of the sMR-HA (Fig. 2C).

FIGURE 2. Electron microscopy of the sMR-HA. A, representative area of a sMR-HA micrograph. Protein density is shown black over the background. Some images of MR single molecules have been highlighted within white ovals. Scale bar represents 30 nm. B, selected projections of the final volume (Proj) and two-dimensional averages of the images within the corresponding class (Aver) as provided by EMAN (27). Protein density is shown white over the background. C, two views of the reconstructed volume of the MR. D, fitting of the atomic structures of individual domains into the EM density of the sMR-HA allowed segmentation of the three-dimensional structure. Regions of the volume assigned to each domain are shown with different colors following the legend inserted.
The overall shape of the sMR-HA three-dimensional volume (Fig. 2C) resembles the general structure described for Endo180 (22) (see below) containing a globular head followed by an extended tail region. However, the globular part of the sMR-HA is much larger in size and consequently encloses more domains than the head of Endo180 (22). Interestingly, the tail region in the MR does not display a fully extended conformation but a rather twisted S-shaped profile (Fig. 2C, side view), decorated by globular domains. These globular regions appear in two distinct sizes and probably correspond to either an isolated or a pair of CTLDs. Subsequently, to be able to better describe the structure of the MR and to quantify the number of domains enclosed within the head and tail regions, we fitted the atomic structures of CysR, FNII, and CTLD domains into the EM map (data not shown). Due to the limited resolution of the EM map, fitting was only used to assign regions within the three-dimensional medium resolution structure of the sMR-HA to specific domains (24, 29) (Fig. 2D). The extended region and most of the globular head show a collection of regular segments that have the right shape and dimensions to be easily accommodated by the atomic structure of the MBP-A protein, highly homologous to the CTLDs (Fig. 2D). Up to eight CTLD domains could be placed in the three-dimensional reconstruction, after which a larger terminal triangularly shaped region was found at one side of the globular head. The shape of this area was not adequate to enclose a CTLD and we placed both the MR CysR and the 2F2 domain from fibronectin (FNII domain) in a hypothetical arrangement (Fig. 2D, dark green). Other interpretations for the EM map are less likely, since the CysR and FNII domains cannot be placed at the opposite end of the three-dimensional reconstruction due to size limitations. Therefore, the head segment found in the sMR-HA encloses the N-terminal CysR, FNII, and CTLD1 to CTLD4 domains arranged into a compact region (Fig. 2D). Within the triangular terminal end, and as a consequence of the resolution of the structure, we were unable to define the limits or precise locations of the CysR and FNII domains. Nevertheless, these two domains are sufficient to account for all the protein density observed in this region. It is also possible that the FNII could be intimately associated with adjacent domains making difficult the resolution of the CysR and FNII domains as independent structural units.

At the bottom tip of the tail region a globular domain is differentiated, most possibly corresponding to the C-terminal end of sMR-HA, and which was found sufficient to enclose two CTLDs, and we reasoned that these should match CTLD7 and CTLD8 (Fig. 2D, orange). This segment is followed up by a smaller region that can only comprise one CTLD, CTLD6 (Fig. 2D, red), continued by another larger area that should comprise CTLD5 and CTLD4 (Fig. 2D, light green), already part of the head. The structure then twists again along a region assigned to CTLD3 (Fig. 2D, pink) and CTLD2 (Fig. 2D, yellow), whereas CTLD1 (Fig. 2D, purple) seems to be intimately associated to the FNII-CysR region (Fig. 2D, dark green). The atomic structure of the MR CTLD4 has been solved and shows structural similarity to the core domain of MBP-A but with an extended loop not being present in other CTLDs (2, 30). This domain may represent a unique structure of the MR CTLD4. We do not have the resolution to specifically place the loop of MR CTLD4 within the structure of the sMR-HA. Nevertheless, it seems clear in the three-dimensional reconstruction of the sMR-HA that CTLD4 contacts with the region comprising the CysR and FNII domains.

**The Mannose Receptor Experiences Large Conformational Changes under Low pH**—The chicken yolk sac IgY receptor (FcRY) has been recently described to conform into a compact conformation where the CysR-FNII domains interact with the CTLD region under acidic pH, a condition required for ligand (IgY) recognition. Surprisingly, this receptor undergoes large conformational changes at pH 8.0 that disrupt this compact arrangement and impair ligand binding (3). Significantly, all the receptors in the MR family are continuously recycled between the plasma membrane and the endosomes, the low pH compartment where extracellularly bound target ligands are released (2). Consequently, we reason that pH could be a general driving force in the mannose receptor family, and we tested to see whether similar pH-dependent transitions could be an intrinsic property of the family. Gel filtration chromatography was employed to determine whether sMR-HA could undergo conformational changes under different conditions (Fig. 3). The migration of the monomeric sMR-HA was not altered in the presence or absence of calcium (Fig. 3, A and B) or in the presence of mannose (together with calcium) (Fig. 3C). In contrast, under pH 5.4 (Fig. 3D) the chromatogram is drastically altered, and only one peak with a significant shift in its elution profile was observed. This implies that conformational changes have occurred to influence the elution time and that the MR can vary its overall compactness under different conditions. This reflects that the conformational dynamics of the MR are probably much more complex to be accounted by a very simple model, which may contribute to explain our discrepancies with the extended conformation predicted for the MR by previous reports (23).

**Visualization of Full-length sEndo180-HA-HIS Receptor**—To be able to generalize a structural model for the mannose receptor family, we decided to try to define the three-dimensional structure of full-length Endo180. In a previous study by our group using the complete extracellular region of Endo180 (sEndo180-HA-HIS), we were able to contrast and visualize only the CysR, FNII, CTLD1, and CTLD2 domains (22). In the three-dimensional structure obtained, the polypeptide chain is bent, and the CTLD2 domain contacts the CysR region to build a globular structure, with a triangular shape when looked at from one side (see three-dimensional reconstruction in the top of schematic in Fig. 4D) (22). Nevertheless the actual conformation of the complete receptor remained unknown. To visualize the full-length sEndo180-HA-HIS the same protein preparation was negatively stained with a different staining protocol using high concentration (16%) ammonium molybdate and examined by electron microscopy (Fig. 4A). Views similar to those found before (22) were observed (asterisks in Fig. 4A), but among these, some images displayed longer dimensions (within circles in Fig. 4A and enlarged in Fig. 4, B and C). These particles represent side views of the whole Endo180 molecule having a compact triangular portion followed by a thin extended region (Figs. 1A and 4D). It is likely that this extended component of the molecule corresponds to the domains of the protein missing in our previous reconstructed volume (CTLD3 to CTLD8) (22). Therefore, we can now conclude that, under experimental conditions compatible with substrate binding (22), Endo180 displays an extended conformation tipped by a head segment which is built by N-terminal domains (Fig. 4D). The CysR domain contacts the CTLD2, the only functional CTLD in Endo180, to form a globular conformation at the position of the receptor implicated in ligand recognition (19, 22).

**Electron Microscopy and Three-dimensional Reconstruction of sEndo180-HA-HIS under Low pH**—In our hands, the soluble Endo180-HA-HIS sample was found to render more clear and reproducible images observed by the electron microscope than the sMR-HA sample. Consequently, we used sEndo180-HA-HIS as a target to analyze the possible conformational effect of pH. To determine the conformation of Endo180 in a low pH environment typical for the endosomal apparatus, one aliquot of sEndo180-HA-HIS sample was split in two halves, one kept at neutral pH and the other diluted in a citrate buffer to the final pH of 5.4 (Fig. 5B). Both preparations where then applied to grids covered by a carbon foil, negatively stained with uranyl acetate, observed under
the electron microscope, and processed to obtain two-dimensional averages (Fig. 5A, see projections of the final volume and corresponding averages), and three-dimensional reconstructions were obtained in parallel but independently (Fig. 5, C and D). As found before, only the N-terminal globular tip of the protein was contrasted by the staining agent (22). The particle images extracted from the sample at neutral pH generated a volume identical to the one already published (22), whereas at low pH some significant differences were observed. First, the functional CTLD2 is rotated in relation to its position found in the protein under neutral pH (compare Fig. 5, C and D) becoming parallel to CTLD1. Second, the interaction between CTLD2 and CysR is disrupted at low pH. We can therefore conclude as the most likely conformational
conversion that CTLD1 and CTLD2 arrange in parallel under low pH, whereas CTLD2 rotates and interacts with CysR at neutral pH.

**DISCUSSION**

In this work, we have defined the three-dimensional organization of the MR and Endo180, two of the four members of the mannose receptor family in mammals so that some common principles for the whole family can be obtained (Fig. 6). Strikingly, Endo180 and MR are structurally very different but simultaneously very similar in their conceptual three-dimensional arrangement of domains. In both receptors, the N-terminal CysR domain intimately contacts the only CTLD to be functional in sugar binding (CTLD4 in MR and CTLD2 in Endo180). Such a configuration must certainly sustain some functional implications in ligand binding. Taking our results together, with the data from others (2, 3, 31), we propose a model for the conformation of members of the MR superfamily in which the N-terminal end is bent to generate a globular head comprising several domains. In Endo180 and the MR, the extent of this globular region is determined by the specific CTLD to be functional in each receptor (Fig. 6). In addition, the chicken FcRY has been shown to recognize IgY exclusively when in a compact folded configuration where the N-terminal CysR and FNII domains interact with the CTLDs (3). We propose that adoption of a bent or compact conformation under certain conditions is the general trend in the mannose receptor family. The three-dimensional structure of the MR also reveals that some CTLD domains are packed in pairs (CTLD4–5 and CTLD7–8) connected by single CTLD domains (CTLD3 and CTLD6). These connecting CTLDs appear to twist the structure and determine some points of the molecule that could potentially be more reachable by proteases. In this regard, regions accessible for proteolytic cleavage for the MR have been identified by Napper and colleagues (23) and they are highlighted with black arrows. In an acidic environment it is predicted that Endo180 switches to a more conformation. For the MR, conformational changes could modify its overall compactness by either an opening or closing of the observed conformation.

The model of an extended structure of MR proposed after hydrodynamic analysis of the MR (23) is in contrast with the “bent” three-dimensional structure of MR and Endo180 demonstrated here as well as with the structure inferred for the physiologically active FcRY (3). One explanation for this discrepancy is that, as demonstrated here, the conformation of the receptors can be modified under different conditions and that the extended conformation observed previously (23) may represent just one of a range of possible conformations. In this respect, it is important to note that the bent three-dimensional conformation observed here was obtained under functional buffer conditions using samples shown to be competent in ligand binding (22) (Fig. 1). Furthermore, the collection of conformations in the mannose receptor family might even be additionally affected by differential glycosylation of the receptors (33).

Extensive work on Endo180 and the MR and PLA2R (6, 19, 26, 31) has demonstrated that individual domains (CysR, FNII, and specific CTLDs) are necessary and sufficient to bind their specific ligands (sugars or collagens). Nevertheless, the fact that bent conformations are found under ligand binding conditions strongly suggests that such a configuration must have an important functional significance, and it might be important to regulate ligand recognition in their cellular con-
text. Importantly, one of the ligand binding domains in the mannose receptor family is found in the middle of the polypeptide chain, an atypical arrangement for most cellular receptors (23). Therefore, the bent conformation could serve to project ligand binding competent domains closer to their possible substrates and also to concentrate binding domains. Another important functional feature of these receptors is that they each can recognize multiple ligands. In this respect, Endo180 can, for instance, form a trimolecular complex with the uPA and its receptor (uPAR). Such a complex is disrupted after incubation with collagen indicating that collagen recognition somehow modifies the uPA/uPAR recognition sites (7). In our model, ligand binding domains are fully interconnected in the three-dimensional structure of these receptors, which might be a mechanism to regulate and coordinate the interaction with a diverse set of substrates. In accordance with this model, our MR sample migrates within at least two distinct peaks in a gel filtration chromatography (Fig. 1F) with each peak displaying differential selectivity toward ligands as described by Su and colleagues (33).

Finally, another characteristic of these receptors is that they all have life cycles where pH transitions take place. They are rapidly internalized from the plasma membrane into the endosomal system where the bound ligand is released and the receptor recycled back to the membrane. Furthermore, it has recently been demonstrated that the chicken FcγR alternates between a compact conformation at acidic pH, capable of interaction with IgY, and an extended configuration at basic pH, which releases the substrate (3). We have shown here that under acidic pH conditions both the MR and Endo180 undergo significant conformational changes. In Endo180, such re-arrangements result in a more open protein configuration after a displacement of the functional CTLD2. It is quite likely that an alternation between compact and more open conformations, regulated by the difference in the pH, might serve as a “conformational switch” to regulate ligand binding or selectivity. Similar behavior has already been described during integrin activation as a “conformational switch” to regulate ligand binding or selectivity. We propose that the presence of different conformational states in the mannose receptor family could serve as a molecular mechanism to regulate ligand selectivity in different compartments and functional states.

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