Ligand Binding Specificities of the Two Mannose 6-Phosphate Receptors

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Two mannose 6-phosphate (Man-6-P) receptors (MPRs) direct the vesicular transport of newly synthesized lysosomal enzymes that contain Man-6-P from the Golgi to a prelysosomal compartment. In order to understand the respective roles of the $M_p = 46,000$ cation-dependent (CD-) MPR and the $M_p = 300,000$ cation-independent (CI-) MPR in lysosomal targeting, an assay has been developed that simultaneously measures the relative affinity of each MPR for multiple ligands. Glycoproteins containing Man-6-P were affinity-purified from the metabolically labeled secretions of mutant mouse fibroblasts lacking both MPRs. They were incubated with purified MPRs, and the resulting receptor-ligand complexes were immunoprecipitated by anti-MPR monoclonal antibodies coupled to agarose beads. Ligands were eluted with Man-6-P and then quantified following SDS-polyacrylamide gel electrophoresis. Saturating concentrations of CI-MPR resulted in the complete recovery of each Man-6-P glycoprotein in receptor-ligand complexes. Apparent affinity constants ranged between 1 and 5 nM for the individual species. Ligands precipitated by the CD-MPR appeared identical to those bound by the CI-MPR, with apparent affinity constants ranging between 7 and 28 nM. The binding affinities of the two receptors for different ligands were not correlated, indicating that the two MPRs preferentially recognize different subsets of lysosomal enzymes. In addition, saturating levels of CD-MPR resulted in the precipitation of only 50% of the total input ligands, suggesting that the CD-MPR binds a subpopulation of the Man-6-P glycoproteins bound by the CI-MPR. These results provide a biochemical mechanism, which, in part, may explain the interaction of the two MPRs with overlapping yet distinct subsets of ligands in vivo.

Most newly synthesized lysosomal hydrolases are targeted to the lysosome by a mannose 6-phosphate (Man-6-P)$^1$-dependent pathway (1, 2). Man-6-P residues are added to select N-linked oligosaccharides in a pre-Golgi compartment and are specifically recognized by Man-6-P receptors (MPRs), which direct intracellular trafficking of lysosomal enzymes to a prelysosomal compartment. The low pH of this compartment causes the complex to dissociate, releasing the lysosomal enzymes and allowing the MPRs to recycle back to the Golgi. In the absence of MPRs, most Man-6-P-containing glycoproteins are secreted from the cell. The Man-6-P-dependent pathway therefore allows for the redirection of newly synthesized proteins from the secretory pathway to the lysosomal targeting pathway.

Most mammalian cells contain two distinct MPRs. The cation-independent MPR (CI-MPR) is a $M_p 300,000$ transmembrane glycoprotein, which, in addition to targeting newly synthesized lysosomal enzymes, can bind extracellular ligands, resulting in their subsequent endocytosis and transport to the lysosome. The $M_p 46,000$ cation-dependent MPR (CD-MPR) is an oligomeric transmembrane glycoprotein that also participates in the intracellular targeting of lysosomal enzymes. Like the CI-MPR, the CD-MPR also cycles to the cell surface, but it does not bind extracellular ligands containing Man-6-P under physiological conditions in vitro.

It is not known why most mammalian cells contain two MPRs. Mutant mice expressing only one of the two MPRs are viable (3–6) in the appropriate genetic background, while disruption of both MPRs results in early death (7), indicating that each receptor can functionally compensate for the loss of the other. However, such compensation is, at best, partial. Transgenic mice lacking the CD-MPR have elevated levels of urine Man-6-P glycoproteins and plasma lysosomal enzymes (5), suggesting that the CI-MPR cannot completely substitute for loss of CD-MPR function. In addition, cultured cells lacking either MPR exhibit increased secretion of multiple lysosomal enzymes (4, 5, 8, 9).

Incomplete functional compensation suggests a selectivity in MPR-ligand interaction for which the molecular basis remains to be elucidated. The most obvious possibility is that the MPRs simply have intrinsically different affinities for different Man-6-P glycoproteins, which is reflected by selective targeting in vivo. Thus, the loss of one MPR would result in mistargeting of all ligands that are specifically bound by that MPR, while targeting of ligands common to both MPRs would be directed by the remaining MPR.

This possibility can be directly addressed by comparing the affinities of the two MPRs for different Man-6-P glycoproteins. In this report, we describe an in vitro binding assay for the simultaneous measurement of the relative affinities of both MPRs for a wide range of phosphorylated lysosomal enzymes. We demonstrate that while both MPRs bind the same array of proteins, the respective affinity of each MPR for different phosphorylated glycoproteins is varied, and there is no correlation between the affinities of the MPRs for different Man-6-P glycoproteins (i.e., a relatively high affinity ligand for the CI-MPR is not necessarily a high affinity ligand for the CD-MPR).
Importantly, we also demonstrate that the CD-MPR is capable of binding only a subset of the ligands bound by the CI-MPR. This provides a biochemical basis for a number of studies demonstrating that even high level expression of the CD-MPR cannot fully compensate for loss of the CI-MPR in cultured cells.

EXPERIMENTAL PROCEDURES

Materials—Hybridoma cells producing monoclonal antibodies react-
ing against the CI-MPR and the CD-MPR were kindly provided by Dr. Doris Messner. Yeast phosphomannan was a generous gift from Dr. M. E. Sledki (U.S. Department of Agriculture, Peoria, IL). Other reagents were from Sigma and analytical grade unless stated otherwise.

Purification and Coupling of Anti-MPR Monoclonal Antibodies—
Monoclonal antibodies were purified from the media of hybridoma cells grown in vitro by passage over a Protein A-Sepharose column and low pH elution (10). For immunoprecipitation of CI-MPR-ligand complexes, a mixture of monoclonal antibodies (DM86 and DM70) were coupled together to Affi-Gel 10 (Bio-Rad) using the manufacturer’s protocol at 1.5 mg/ml bed volume of each. For the CD-MPR, DM22 was coupled at 3 mg/ml Affi-Gel 10. DM86 was also coupled for the immunodepletion of CI-MPR during the purification of CD-MPR (see below). Coupled antibodies were stored at 4 °C in phosphate-buffered saline containing 0.02% sodium azide.

Purification of MPRs—CD-MPR was purified using a modified ver-
sion of the method of Sahagian et al. (11). 200 g of bovine liver acetone powder was homogenized in 4.5 liters of 0.2 M NaCl, 0.1 M sodium acetate, pH 6.0, 10 mM EDTA, in 0.5-liter batches for 30 s in a Waring blender. The suspension was centrifuged for 30 min at 7,500 × g at 4 °C, and then the pellet was resuspended in 4.8 liters of distilled water and recentrifuged. The pellet was resuspended in 0.4 M KCl, 50 mM imidazole, pH 6.7, 1% membrane grade Triton X-100 (Boehringer Mannheim), 1 mM phenylmethylsulfonyl fluoride, and solubilized at 4 °C for 16 h with gentle stirring. The material was centrifuged at 5000 × g at 4 °C for 60 min.

The CD-MPR was purified by affinity chromatography on pentamannosylphosphate-Sepharose and immunodepletion of CI-MPR. Briefly, the supernatant was adjusted to 10 mM MnCl₂ and applied to a 180-ml bed volume column of pentamannosylphosphate immobilized on α-ami

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In vitro phosphorylation of the CI-MPR was carried out as described previously (14) with the addition of 10 mM MgCl₂ and 20 μCi/ml of [35S]methionine (1.0 mCi/ml of medium). Medium was collected and centrifuged at 5000 × g for 15 min. Outcomes were divided into 30 ml of buffer, added to the supernatant, which was then loaded onto a 2-ml bed volume Affi-Gel 10 affinity column containing 3 mg of sc-MPR. The column was washed with 10 volumes of 1 × phosphate-buffered saline containing 5 mM β-glycerophosphate and 1 mM mg/ml bovine serum albumin and eluted with the same buffer containing 10 mM Man-6-P. Eluate fractions containing the bound proteins were pooled and dialyzed three times against 1 liter of 0.1 M NaCl, 5 mM β-glycerophosphate for 15, 1, and 6 h before aliquoting and storage at −70 °C. Approximately 50% of the of the labeled proteins in the TME6 conditioned media contained Man-6-P.

Iodination of Purified MPRs—CD-MPR and CI-MPR were iodinated as described (14), except the pellet was collected for 3 × 2 h against 1000 volumes of 50 mM Tris-HCl, pH 7.5, 100 mM NaCl, 0.1% Triton X-100 prior to iodination.

Immunoprecipitation of Receptor-Ligand Complexes—For assay de-
velopment, MPRs were incubated with 30 μl of dialyzed TME6 secre-
tions (1.5 × 10⁵ cpm) in a total volume of 200 μl containing 60 mM MES, pH 6.3, at 4 °C, 0.115 mM NaCl, and 0.2% Tween (Pierce, Ultra grade). Optimization of this incubation buffer is described below. Incuba-
tion buffer contained 0.3% bovine serum albumin, which was equilibrated by washing with 3 × 10 volumes of incubation buffer, and 30 μl of 1% slurry was added per reaction. Reactions were incubated for a further 4 h, and then beads were collected by centrifugation at 10,000 × g at 4 °C for 15 s. Pellets were washed once with 1 ml of incubation buffer. Man-6-P glycoproteins were eluted from the beads by boiling for 5 min in reducing SDS-PAGE loading buffer (60 mM Tris-

Electrophoresis and PhosphorImager analysis (data not shown). Binding data was corrected for incomplete immunoprecipitation of MPRs, assuming equivalent precipitation of free receptor and receptor ligand complexes.

Optimization of Incubation Conditions—The immunoprecipitation assay described here for the simultaneous measurement of MPR binding affinities and capacities failed to detect any ligands bound almost
exclusively by one MPR (see "Results"). This was surprising, given previous findings (8, 9); therefore, we conducted the following series of control experiments to investigate a wide range of incubation conditions on MPR-ligand interactions. These were conducted at subsaturating receptor concentrations to detect potential effects on both affinity and specificity.

The secretions of MPR-deficient mouse fibroblasts contain a number of lysosomal enzymes, presumably including proteases and glycosidases. It was possible that these enzyme activities could be present under the ligand binding conditions and could influence MPR affinity and the molecular weight of the different lysosomal enzymes. However, no significant changes in total labeled protein content, total Man-6-P glycoproteins (determined by blotting with an iodinated CI-MPR probe (14)), or the pattern of immunoprecipitated Man-6-P glycoproteins were observed under a variety of different experimental conditions (data not shown), including pH 5.5 and 7.5, and the presence or absence of EDTA, monoclonal antibodies, phosphatase inhibitor (β-glycerophosphate), or protease inhibitors (leupeptin, pepstatin, and phenylmethylsulfonyl fluoride). The presence of manganese, while not affecting total protein content, had a significant effect on one ligand (band 9/10) and is discussed under "Results." These experiments confirmed that the monoclonal antibodies, MPR preparations, and ligand source were essentially free of protease, glycosidase, and phosphatase activities under the conditions tested. Sodium orthovanadate, a phosphatase inhibitor, inhibited MPR ligand binding, especially the CD-MPR, when present at 5 mM (data not shown).

Most studies comparing MPR-ligand specificities have used either MES or imidazole as a buffer and NaCl and MnCl2 as mono- and divalent salts, respectively. These conditions do not necessarily accurately model conditions in vivo and could potentially alter MPR specificity. We therefore evaluated a range of experimental conditions in terms of affinity and specificity of MPR-ligand interactions by comparing several buffers (MES, imidazole, PIPES, and MOPS, pH 6.5) and salts (sodium chloride, sodium acetate, potassium chloride, and potassium acetate). All proved to be equivalent for both MPRs in terms of ligand binding pattern and affinity. A number of detergents (Brij, CHAPS, Digitonin, SDS, Nonidet P-40, Tween-20, and Triton X-100) were also evaluated in terms of MPR specificity and affinity (data not shown). Since none affected the pattern of bound ligands, we routinely used 0.2% Tween 20, which gave the best signal to noise ratio in the assay.

Previous studies have generally used magnesium or manganese as a source of divalent cations for the CD-MPR in ligand binding experiments. We examined a number of divalent cations (data not shown) and found that the pattern of immunoprecipitated ligands was the same with all tested, with the exception of bands 9 and 10 (see "Results"). However, the total precipitated ligand varied; e.g. MnCl2 increased the total ligand bound by the CD-MPR approximately 1.5-fold compared with EDTA, while other divalent cations (10 mM Ca2+, Mg2+, Co2+, or Ni2+) had little effect. All divalent cations, especially Co2+, were slightly inhibitory for the CI-MPR. We also examined the effects of cytosolic levels of Ca2+ and Mg2+ on MPR-ligand interactions. Calcium was buffered using calcium chloride and the chelator BAPTA (Calbiochem) as calculated using the MaxChelator program (15). Free calcium concentrations ranging from 15 nM to 15 μM, in the presence or absence of 0.8 mM Mg2+, had little or no effect on the ligand binding specificity or affinity of either MPR (data not shown). The chelator dibromobetapta (Calbiochem) selectively inhibited CD-MPR-ligand interactions, although this effect is probably unrelated to the divalent cation requirements of this receptor, since BAPTA and EDTA had no effect compared with reactions containing no chelating agents.

RESULTS

Previous studies have measured the affinity of both or either MPRs for a number of mannose 6-phosphorylated ligands, including monosaccharides, oligosaccharides, and some lysosomal enzymes (reviewed in Ref. 2). However, to date, a direct comparison of the affinities of the two MPRs for a wide range of naturally occurring ligands has not been conducted. This was the primary objective of this study, with the aim of shedding light on MPR targeting specificity in vivo.

Assay Specificity—Potentially confounding effects in the assay could include contamination of one MPR with the other, nonspecific binding of 35S-labeled proteins to the Affi-Gel or cross-reaction between the antibodies and the labeled proteins in the TME6-conditioned media. In Fig. 1, we address these possibilities and demonstrate that the antibodies are completely specific for their respective MPRs. Incubation of MPRs in the absence of coupled antibody does not result in precipitation of labeled proteins. A small quantity of the starting secretions nonspecifically coprecipitated with either coupled antibody alone or antibody incubated with the heterologous MPR. This demonstrates that the monoclonal antibodies are specific but also indicates some nonspecific entrapment. In view of this, a standard control for background quantitation of precipitated ligands was a reaction containing coupled antibody but no MPR.

An additional concern was that the binding of the antibody could influence MPR specificity. However, the pattern of precipitated ligands using soluble/solubilized MPR and coupled antibody was identical to the pattern of proteins detected using iodinated CI- or CD-MPR as an affinity probe in a blotting assay (14), indicating that this is not the case (data not shown).

pH Dependence of MPR-Ligand Interactions—The pH binding profiles at 4°C of total phosphorylated lysosomal enzymes for both MPRs were determined using total labeled secretions from TME6 cells as a source of Man-6-P glycoprotein ligands (Fig. 2). Maximal binding of CD-MPR to phosphorylated lysosomal enzymes was at approximately pH 6.2 and dropped off steeply on either side of this peak. Binding was essentially abolished at pH > 8.2. In contrast, the CI-MPR had a similar pH optimum but retained binding activity at higher pH values; for example, at pH 8.0, the CI-MPR retained about 70% of its maximal ligand binding capabilities. Immunoprecipitation efficiency of both MPRs was unaffected by pH (tested at pH 5, 7, and 9; data not shown). These results are consistent with other studies of the effects of pH on the ligand interactions of the MPRs determined using different methods (16–20). The pattern of ligands bound by each MPR was identical at each pH, indicating that pH is not a modulator of relative MPR-ligand affinity (data not shown). Pohlmann et al. (9), using immobilized MPR affinity chromatography, also demonstrated that the pattern of ligands bound by each MPR was not influenced by pH.
Immunoprecipitation of MPR-Ligand Complexes—Development of the equilibrium binding assay was carried out using total labeled secretions of TME6 MPR-deficient embryonic mouse fibroblasts as a source of Man-6-P glycoproteins (Figs. 1 and 2). In contrast, apparent MPR binding affinities for different ligands were determined using Man-6-P glycoproteins that were further purified by affinity chromatography on immobilized scCI-MPR. This additional step removed components in the media that could potentially modulate MPR affinities (e.g. insulin-like growth factor II; reviewed in Ref. 21) and also allowed a known starting concentration of ligands to be used for definitive binding studies. Earlier studies (data not shown) suggested that the high concentration of coupled CI-MPR (~6 μM) would be sufficient to bind all Man-6-P glycoproteins in the media. For the purposes of this study, however, it was vital to ensure that no CD-MPR-specific ligands were being selectively lost. This was achieved by receptor binding assays on material that flowed through the affinity column (Ligand Source M, Fig. 3). In reactions containing CI-MPR and affinity-purified Man-6-P glycoproteins, most of the labeled ligands were immunoprecipitated as part of MPR-ligand complexes. No labeled proteins were immunoprecipitated with the CD-MPR when the column flow-through was a potential source of ligands, indicating that CD-MPR-specific ligands were not lost during purification of the labeled Man-6-P glycoproteins. In support of this conclusion, the pattern of Man-6-P glycoproteins precipitated by both MPRs was identical when total labeled secretions of TME6 cells, rather than purified labeled Man-6-P glycoproteins, were used as a source of ligands (e.g. compare Figs. 1 and 3).

It is of interest that a purified Man-6-P glycoprotein of approximately 37 kDa (denoted band 9 in Fig. 4) appears to be converted to a lower molecular weight form (band 10) during the course of the incubation, and this conversion is more pronounced in the presence of manganese (Fig. 3; compare total fractions from reactions containing CI-MPR and reactions containing CD-MPR/manganese). This conversion is diminished when ligands are incubated with the CD-MPR in the absence of manganese (data not shown). This may represent the action of a selective, manganese-dependent protease or glycosidase. Interestingly, the processed form appears to have lower affinity
for the two receptors. No changes in the relative levels of any other purified ligands were observed, although a band of approximately 42 kDa in the flow-through fraction is also diminished upon incubation with manganese (Fig. 3, compare lanes 1 and 5 with lanes 3 and 7).

SDS-PAGE of immunoprecipitated MPR-ligand complexes resolved at least 11 major \(^{35}S\)-labeled glycoproteins (Figs. 1, 3, 4). The overall pattern of Man-6-P glycoproteins bound by both MPRs is very similar and clearly comparable with the pattern of Man-6-P glycoproteins secreted by TME6 fibroblasts lacking MPR (Fig. 4). The overall pattern of Man-6-P glycoproteins bound by both MPRs (8). (For the sake of comparison, we have adopted the same numbering system used in Ref. 8.) This result was unexpected, given the differences observed in ligands secreted by fibroblasts lacking either MPR (8) in the absence of manganese. This does not necessarily preclude the existence of a population of very low affinity ligands for the CD-MPR that would not be detected in the assay described here. A saturating concentration of CD-MPR in the absence of manganese could not be determined. Importantly, saturating concentrations of CI-MPR resulted in the precipitation of all input Man-6-P glycoproteins, whereas saturating CD-MPR, in the presence of manganese, bound only approximately 50% of the total labeled ligands. This indicates that the CD-MPR is capable of binding only a subset of the ligands bound by the CI-MPR (at least at relatively high affinity).

In a sequential receptor binding experiment, the CI-MPR effectively bound the Man-6-P glycoproteins contained within the immunoprecipitation supernatant of a binding reaction containing saturating CD-MPR (data not shown). This control confirms that binding of the CD-MPR to a restricted set of the ligands bound by the CI-MPR is an intrinsic property of the CD-MPR and does not reflect ligand loss during incubation (e.g., by the action of contaminating protease or phosphatase activities in the CD-MPR preparation).
From Fig. 5, the apparent dissociation constants (Kd) of the CD- and CI-MPR for total Man-6-P glycoproteins were estimated to be >30 and 2 nm, respectively. The addition of MnCl2 decreased the Kd of the CD-MPR for total phosphorylated lysosomal enzymes to approximately 11 nm.

In Fig. 5, binding of total Man-6-P glycoproteins with each MPR is quantitated, providing an overall picture of MPR-ligand interaction. An important question remains whether the CD-MPR binds 50% of all ligands bound by the CI-MPR or binds relatively more of some and less of others. In order to resolve this question, we quantitated MPR-ligand interactions and constructed binding curves, which allow determination of both the affinity and capacity of each MPR for the individual ligands.

Binding curves are presented in Fig. 6, from which apparent Kd values and relative binding capacities (Bmax) were estimated (Table I). (Bands 9 and 10, which do not remain in stable equilibrium during the incubation (see above), were excluded from this analysis.) Kd values for binding to the CI-MPR were relatively similar for all ligands and varied between 1 and 5 nm. In the absence of manganese, Kd values for binding to the CD-MPR were >25 nm. In the presence of manganese, Kd values for the CD-MPR varied between 7 and 28 nm. The dissociation constants for the two MPRs for different ligands are comparable with those determined for individual lysosomal enzymes using different techniques (β-galactosidase binding for the CI-MPR, 20 nm (22) and 25 nm (23) and to the CD-MPR, 270 nm (21); β-glucuronidase binding to the CD-MPR, 0.28 nm (18) and 4.4–5.1 nm (20)).

Most, if not all, of the input ligands were associated with the CI-MPR at saturating concentrations, with Bmax values ranging from 78 to 111%. This MPR is therefore capable of binding most or all of the purified Man-6-P glycoproteins, with relatively similar affinities (1–5 nm). In contrast, saturating levels of CD-MPR bound a maximum of between 28 and 75% of the available ligands, depending on which particular ligand is examined. The CD-MPR, therefore, differs from the CI-MPR in that both its binding affinities and capacities are markedly different from one Man-6-P glycoprotein to another.

In Fig. 7, A and B, the binding capacity and apparent Kd values are compared for each ligand and are found to be unrelated for both MPRs. This confirms that the highest MPR concentrations were actually saturating for the high affinity ligands; if not, we would expect that at limiting MPR concentrations, ligands would compete for receptor binding, and thus the relative proportion of any given ligand bound by the MPR would be proportional to the affinity of the MPR for that ligand. The relative binding capacities of the MPRs for the different ligands were also uncorrelated (data not shown).
Interestingly, another third of the CI-MPR ligands bound weakly to the immobilized CD-MPR, and the other third was unbound, suggesting that the ligands bound by the CI-MPR represent three categories of ligands for the CD-MPR (high, low, and very low affinity). It is worth stating that when the binding was conducted at pH 6.3, 90% of the input ligands bound to the immobilized CD-MPR. One possible explanation is that under optimal conditions, even weak ligands are bound by the high concentration of immobilized receptor, thus obscuring even marked differences in affinity. Thus, in our study, it is possible that the subset of ligands bound by submicromolar concentrations of CD-MPR are equivalent to the high affinity ligands detected previously.

The molecular basis for the differences in ligand binding between the two MPRs also remains to be elucidated. One possibility is that determinants at the amino acid level could influence the affinity of different ligands for either MPR. For example, cathepsin L contains polypeptide components that influence the affinity of different ligands for either MPR. For instance, cathepsin L contains polypeptide components that influence the affinity of different ligands for either MPR. For example, cathepsin L contains polypeptide components that influence the affinity of different ligands for either MPR.

The respective roles of the MPRs have also been examined by monitoring the effects of altering the expression of either or both MPRs on the sorting of lysosomal enzymes in cultured cells. Many of these studies have used mouse L cells, which are deficient in the CI-MPR but contain endogenous CD-MPR. These cells secrete 60% of their newly synthesized β-glucuronidase but secrete only 5% when transfected with the CI-MPR (18). However, even when overexpressing high levels of transfected CD-MPR, these cells still secrete 30–40% of the β-glucuronidase (18, 20). Similar results have been obtained with cathepsin D (26, 27). In other studies, CD-MPR function has been blocked by adding anti-CD-MPR antibodies to cultured cells (28). Such immunodepletion in CI-MPR-deficient cells results in increased secretion of lysosomal enzymes, demonstrating that the CD-MPR has some capacity for targeting. However, immunodepletion of the CD-MPR in cells containing the CI-MPR has no effect on lysosomal enzyme sorting (28). Finally, lysosomal enzyme targeting has also been studied in transgenic mouse fibroblasts lacking both or either MPRs (8, 9). Secretion of lysosomal enzymes was considerably more pronounced in the absence of the CI-MPR than the CD-MPR, although loss of both MPRs resulted in the greatest missorting (9). In addition, while the pattern of secreted Man-6-P glycoproteins in the absence of either MPR was very similar, there were subtle differences depending on which MPR was absent, suggesting that the two MPRs target overlapping but distinct populations of ligands in vivo (8, 9).

Our finding that the CD-MPR binds only a subset of the ligands bound by the CI-MPR provides a rational explanation for the failure of the CD-MPR to compensate for loss of the CI-MPR. Our results also predict that the CI-MPR should be capable of fully compensating for loss of the CD-MPR in vivo. However, while the CI-MPR, in general, has a greater capacity for substituting for CD-MPR function in terms of lysosomal enzyme secretion than vice versa (9), it is clear from the studies described previously that this prediction is not borne out. This indicates that either MPR targeting specificity in vivo is regulated by more than just receptor-ligand affinity or that MPR-ligand affinities in vivo differ from those measured here. The fact that we observe in vitro binding of the same ligands to both MPRs, whereas in cultured cells, several Man-6-P glycoproteins are secreted only in the absence of one MPR (8, 9) only serves to further illustrate the potential complexities of the targeting process. Nonetheless, our results quantitatively demonstrate that the two MPRs have different affinities and capacities for Man-6-P glycoproteins in vivo, which is very likely to influence their targeting specificities in vivo.

Finally, the ligand binding assay described here should prove to be a valuable analytical tool in the analysis of Man-6-P glycoproteins bound preferentially by either MPR. Identification of these ligands and characterization of their respective carbohydrate structures will provide a direct approach for understanding the selectivity in MPR-ligand interactions at the molecular level, which, in turn, should shed light on the respective functions of the MPRs in vivo.

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