Structural Basis for Recognition of Phosphorylated High Mannose Oligosaccharides by the Cation-dependent Mannose 6-Phosphate Receptor*

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Mannose 6-phosphate receptors (MPRs) play an important role in the targeting of newly synthesized soluble acid hydrolases to the lysosome in higher eukaryotic cells. These acid hydrolases carry mannose 6-phosphate recognition markers on their N-linked oligosaccharides that are recognized by two distinct MPRs: the cation-dependent mannose 6-phosphate receptor and the insulin-like growth factor II/cation-independent mannose 6-phosphate receptor. Although much has been learned about the MPRs, it is unclear how these receptors interact with the highly diverse population of lysosomal enzymes. It is known that the terminal mannose 6-phosphate is essential for receptor binding. However, the results from several studies using synthetic oligosaccharides indicate that the binding site encompasses at least two sugars of the oligosaccharide. We now report the structure of the soluble extracytoplasmic domain of a glycosylation-deficient form of the bovine cation-dependent mannose 6-phosphate receptor complexed to pentamannosyl phosphate. This construct consists of the amino-terminal 154 amino acids (excluding the signal sequence) with glutamine substituted for asparagine at positions 31, 57, 68, and 87. The binding site of the receptor encompasses the phosphate group plus three of the five mannose rings of pentamannosyl phosphate. Receptor specificity for mannose arises from protein contacts with the 2-hydroxyl on the terminal mannose ring adjacent to the phosphate group. Glycosidic linkage preference originates from the minimization of unfavorable interactions between the ligand and receptor.

The biogenesis of lysosomes is an essential component of the degradative machinery of the cell and is mediated in part by the mannose 6-phosphate receptors (MPRs). Soluble acid hydrolases are synthesized in the rough endoplasmic reticulum where they undergo N-glycosylation along with other secreted proteins. However, in the Golgi, these acid hydrolases acquire a recognition marker on their N-linked oligosaccharides, mannose 6-phosphate (Man-6-P), which serves as a high affinity ligand for the MPRs. The MPR-hydrolase complexes are then transported to a previlysosomal compartment where the acidic pH of the compartment causes the MPR to release the hydrolase. The acid hydrolase is subsequently packaged into lysosomes, whereas the receptor is free to cycle back to the Golgi or move to the plasma membrane (1–3).

Two integral membrane glycoproteins, the 46-kDa cation-dependent MPR (CD-MPR) and the 300-kDa insulin-like growth factor-II/cation-independent MPR, have been identified that function in the recognition of Man-6-P-containing proteins. Recent studies have demonstrated that both MPRs are required for the efficient targeting of lysosomal enzymes to the lysosome (4, 5). Although the two MPRs are both capable of high affinity binding of phosphorylmannosyl residues, they exhibit only limited (~24%) sequence identity between their extracytoplasmic ligand binding regions (6, 7). Over the last 10 years, significant progress has been made in understanding the specificity of carbohydrate recognition by the MPRs. Inhibition studies using chemically synthesized oligomannosides or neoglycoproteins have shown that the presence of Man-6-P at a terminal position represents the major determinant of receptor binding (8, 9). A similar study using monosaccharides that differed from Man-6-P by a single substituent demonstrated that the phosphate group and the axial 2-hydroxyl of mannose are critical for receptor binding (10). Chemical modifications (11) and site-directed mutagenesis (12, 13) studies have been conducted to begin to identify the structural determinants of the MPRs that are critical for carbohydrate recognition. An arginine residue has been identified that is conserved in the Man-6-P binding sites of both MPRs and that when changed to either a conservative (Lys) or nonconservative (Ala) amino acid results in the loss of ligand binding activity. We have recently solved the structure of a glycosylation-deficient form of the extracytoplasmic domain of the CD-MPR (Asn87/STOP155) complexed with Man-6-P (1M6P) which has confirmed the importance of this conserved arginine residue and has identified additional residues surrounding the ligand in the binding pocket (7).

Despite these recent findings, it remains to be determined how the MPRs interact with newly synthesized acid hydrolases, a heterogeneous population of more than 40 enzymes that differ in a number of properties including the extent of phosphorylation of their N-linked oligosaccharide chains. Although it is clear that the terminal Man-6-P is the major determinant of receptor binding, several studies indicate that the MPRs recognize an extended oligosaccharide structure, including the Man-6-P and other substituents within this region (6, 7). Over the last 10 years, significant progress has been made in understanding the specificity of carbohydrate recognition by the MPRs. Inhibition studies using chemically synthesized oligomannosides or neoglycoproteins have shown that the presence of Man-6-P at a terminal position represents the major determinant of receptor binding (8, 9). A similar study using monosaccharides that differed from Man-6-P by a single substituent demonstrated that the phosphate group and the axial 2-hydroxyl of mannose are critical for receptor binding (10). Chemical modifications (11) and site-directed mutagenesis (12, 13) studies have been conducted to begin to identify the structural determinants of the MPRs that are critical for carbohydrate recognition. An arginine residue has been identified that is conserved in the Man-6-P binding sites of both MPRs and that when changed to either a conservative (Lys) or nonconservative (Ala) amino acid results in the loss of ligand binding activity. We have recently solved the structure of a glycosylation-deficient form of the extracytoplasmic domain of the CD-MPR (Asn87/STOP155) complexed with Man-6-P (1M6P) which has confirmed the importance of this conserved arginine residue and has identified additional residues surrounding the ligand in the binding pocket (7).

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Structure of CD-MPR Bound to Pentamannosyl Phosphate

Data collection statistics

| Parameters                              | Values                          |
|-----------------------------------------|---------------------------------|
| Resolution range (Å)                    | 40–1.85 (1.90–1.85)*             |
| Total number of reflections             | 48273                           |
| Unique reflections                      | 28822                           |
| Rsym (%)                                | 5.1 (29.2)                      |
| Completeness (%)                        | 85 (62.3)                       |
| Space group                             | P2_1                            |
| Number of molecules/asym. unit          | 2                               |
| Unit cell dimensions                    |                                 |
| a (Å)                                   | 42.8                            |
| b (Å)                                   | 79.3                            |
| c (Å)                                   | 55.6                            |
| β (°)                                   | 100.3                           |
| Final R factor (%)                      | 21.1                            |
| Rmeas (%)                               | 25.2                            |
| Number of water molecules               | 172                             |
| Deviations in rms geometry              | 0.006                           |
| Bond angles (°)                         | 1.6                             |
| Average B factors (Å²)                  | 25.9                            |
| Main chain                              | 28.2                            |
| Side chain                              | 38.5                            |
| Pentamannosyl Phosphate                 | 61.6                            |
| N-acetylglucosamine                     |                                 |
| Mn^{2+}                                 |                                 |
| Monomer A                               | 73.1                            |
| Monomer B                               | 54.1                            |
| Water                                   | 38.4                            |
| * Numbers in parentheses are for the highest resolution shell of the data. |

TABLE II

| Molecular replacement solutions         |                  |
|-----------------------------------------|------------------|
| Rotation function                      | Patterson function |
| θ₁, θ₂, θ₃ degrees                     |                  |
| Rotation function                      |                  |
| a = 12.81                               |                  |
| b = 13.67                               |                  |
| c = 22.64                               |                  |

Overall Structure—The bovine CD-MPR consists of a 28-residue amino-terminal signal sequence, a 159-residue extracytoplasmic domain, a single 25-residue transmembrane domain, and a 67-residue carboxyl-terminal domain (25). The CD-MPR exists as a dimer (7, 26, 27) and is glycosylated at four of its five potential N-glycosylation sites (17). In many species the presence of divalent cations, such as Mn^{2+}, enhances the binding affinity of the receptor (10, 26, 28). We have previously shown that a truncated, glycosylation-deficient form of the bovine CD-MPR (Asn^{81}/STOP^{155}) binds β-glucuronidase with an affinity identical to that of the full-length wild-type receptor (18). Asn^{81}/STOP^{155}, which consists of residues 1–154 of the mature protein, has four out of its five potential N-glycosylation sites (17). In many species the presence of divalent cations, such as Mn^{2+}, enhances the binding affinity of the receptor (10, 26, 28).

RESULTS AND DISCUSSION

of the extracytoplasmic domain of the CD-MPR complexed with pentamannosyl phosphate.

EXPERIMENTAL PROCEDURES

Materials—All chemicals unless otherwise specified were purchased from Sigma. Phosphomannan from Hansenula holstii was the kind gift of Dr. M. E. Slodki of the Northern Regional Research Center (Peoria, IL).

Purification of Pentamannosyl Phosphate—Penta-D-mannose 6-monophosphate [α-D-Man6PO_4(1,3)-α-D-Man1,3-α-D-Man1,3-α-D-Man1,2-α-D-Man] was prepared from the high molecular weight phosphomannan of H. holstii (Y. 2448) as described previously (16).

Protein Purification and Crystallization—The glycosylation-deficient extracytoplasmic domain (Asn^{81}/STOP^{155}) of the bovine CD-MPR was generated as described previously (17) and expressed in Trichoplusia ni 5B1-4 (High Five) insect cells. Recombinant Asn^{81}/STOP^{155} was purified to near homogeneity by pentamannosyl phosphate-agarose affinity chromatography as described previously (18). CD-MPR was extensively dialyzed against buffer containing 150 mM NaCl, 10 mM MnCl_2, 5 mM β-glycerophosphate, and 50 mM imidazole, pH 6.4, to remove the Man-6-P present from the above purification. The protein was then concentrated to ~1.8 mg/ml prior to incubation overnight at 4 °C in the presence of 100 mM pentamannosyl phosphate. Crystallization was carried out at 19 °C by vapor diffusion using the hanging drop method (19) by mixing equal volumes (1 μl:1 μl) of the purified bovine CD-MPR (10 mg/ml) with the precipitating solution ((25% (w/v) poly(ethylene glycol) 5000 monomethyl ether (Fluka, Milwaukee, WI), 0.2 M ammonium acetate in 0.1 M cacodylate buffer pH 6.5)).

Data Collection—The crystal was mounted in a thin-walled glass capillary tube, and diffraction data were collected at 4 °C on an R-axis IIc image plate detector system with a Rigaku RU200 rotating anode generator operating at 50 kV and 100 mA with a graphite monochromator. Still photographs indicated that the crystal belongs to the monoclinic space group P2_1, with unit cell parameters a = 42.8 Å, b = 79.3 Å, c = 55.6 Å, and β = 100.3 °. Assuming two monomers/asymmetric unit, the calculated Matthews’ coefficient is 2.7 A³/Da (20). A single native data set was collected to 1.85 Å resolution at 4 °C. The diffraction data were processed using all different data between 15 and 4 Å resolution. The rotation search produced two peaks that are related by the 2-fold axis of the dimer molecule that corresponds to the noncrystallographic symmetry in the asymmetric unit (Table II). The translation search was carried out using a solution with the highest rotation function value of 6.37. This gave an initial R factor of 34.7%. Ten cycles of rigid body refinement were subsequently carried out treating each monomer (A and B) as a rigid unit. This lowered the R value to 33.0% using 10 to 3 Å resolution data.

Structure Refinement—At this stage, the structure was refined using all data to 1.8 Å resolution using X-PLOR with manual adjustments between refinement cycles on a Silicon Graphics workstation using Turbo graphics software (24). In general, one round of refinement consisted of Powell positional refinement, simulated annealing from 3000 to 300 K, and a second positional refinement. Temperature factor refinement was also employed in the later cycles. Bulk solvent correction for the reflection data was applied in later cycles of refinement.

After each round of refinement, both F_A - F_C and F_B - F_C difference Fourier maps were calculated. When attempting to clarify residues 38–43, which were not well defined in the initial electron density map, alanine residues were initially substituted. These residues were changed to the appropriate native residues upon improvement of the electron density maps. The individual mannanose rings of pentamannosyl phosphate were added at various stages of refinement based on the clarity of the electron density map. Two N-acetylglucosamine molecules were fitted to each monomer mid-way through the refinement procedure. Finally, water molecules were assigned when densities greater than 3.5 σ and within 3.2 Å of a potential hydrogen-bonding partner were observed in the F_A - F_C electron density map.

The structure of the bovine Asn^{81}/STOP^{155} CD-MPR bound to pentamannosyl phosphate has been refined to 1.85 Å with good geometry. Table I summarizes the data collection and
refinement statistics. Analysis of the final structure by PROCHECK (30) yielded all nonproline and nonglycine residues in either the most favored or additionally allowed regions of the Ramachandran plot. The first residue with visible electron density is Glu\(^1\), and the electron density for both peptide chains of the dimer is continuous through the carboxyl-terminal Ser\(^{154}\). Only the two N-acetylglucosamine residues of the oligosaccharide chain at Asn\(^{82}\) are visible, suggesting that the remainder of the oligosaccharide is flexible. Although the exact structure of this oligosaccharide is not known, removal of the carbohydrate by endo-β-N-acetylgalactosaminidase\(^{H2}\) demonstrates that the oligosaccharide contains two N-acetylgalactosamine and at least four mannose residues. A comparison of the two peptide chains of the dimer reveals that they are virtually identical with a root mean square deviation between backbone atoms of 0.55 Å for the entire polypeptide chain.

**Influence of Bound Ligand on the Structure of the CD-MPR**—We have recently reported the structure of the bovine CD-MPR bound to Man-6-P (7). The overall structure of the CD-MPR bound to pentamannosyl phosphate is very similar to that of the receptor bound to Man-6-P (Fig. 1). The root mean square deviation between the corresponding monomers of the two structures, excluding loop A, is 0.66 Å and demonstrates that the structures are virtually identical. The protein is comprised of an amino-terminal α-helix that leads into a four stranded, antiparallel β-sheet oriented orthogonally over another β-sheet of the remaining five β-strands of the protein. The loops between strands 1 and 2 (loop A), 3 and 4 (loop B), and 6 and 7 (loop C) enclose the ligand binding pocket. The six cysteine residues of the molecule are involved in three disulfide bonds (Cys\(^{6}-\)Cys\(^{52}\), Cys\(^{106}-\)Cys\(^{141}\), and Cys\(^{119}-\)Cys\(^{153}\)). The most striking difference between these structures lies in loop A (residues 38–43). The previously reported structure utilizing the smaller Man-6-P ligand showed that this region was not well defined, indicating its dynamic nature. In the presence of the larger ligand, loop A adopts a more ordered structure, as demonstrated by its clearly discernable electron density, and is drawn toward the binding pocket by 1–3 Å. The C loop containing residues 102–105, which is located in the region near the phosphate in the binding pocket, has also shifted toward the phosphate group, and the main chain amide group of Asn\(^{104}\) has rotated by approximately 80° (ψ = 28 from ϕ = -50), thereby altering some of the contacts between ligand and receptor.

The Extended Binding Site of the CD-MPR—Previous inhibition studies have indicated that the MPRs recognize at least two mannose residues of the oligosaccharide chain (8, 9). To analyze the interaction of the MPRs with an oligosaccharide, we have now solved the structure of the Asn\(^{82}\)/STOP\(^{155}\) CD-MPR complexed to pentamannosyl phosphate. Fig. 2 depicts the electron density map (2Fo – Fc) of the ligand contoured at 1σ. The phosphate group and terminal three sugar rings exhibit clearly observable electron density. The electron density of the fourth mannose ring is only partially visible at the 1σ level. Modeling of a sugar ring into this region results in a slight (0.4, 0.1% increase in R and R\(_{free}\) values upon refinement. This ring also appeared to make no contacts with the polypeptide, and therefore, it was omitted from the final structure. In this structure we show the phosphate moiety is essentially buried in the protein with only 12 Å\(^2\) (8%) solvent accessible. The mannose rings become progressively more solvent-accessible as their location is more distal (terminal mannose (I) = 3%, penultimate mannose (II) = 38%, and prepenultimate mannose (III) = 62%) from the phosphate group. The prepenultimate mannose ring extends to the protein surface (Figs. 3 and 4).

Fig. 5 shows the various contacts between pentamannosyl phosphate and the CD-MPR (Fig. 5A) and between Man-6-P and the CD-MPR (Fig. 5B). The interactions between the polypeptide and the terminal phosphomannose moiety are essentially the same in the two structures, except there is a minor difference in the distance between the C-4 hydroxyl of the terminal sugar and the terminal NH of the guanidine group of Arg\(^{135}\). From comparison of the bonding schemes we may conclude that the presence of additional mannose rings does not affect the binding of the terminal residue. However, as discussed above, the presence of the additional rings results in a slight collapse of the binding pocket because of the change in positioning of loops A and C. Loop A is tethered in place by residues Asp\(^{43}\) and Tyr\(^{45}\), which make hydrogen bond contacts with the penultimate sugar ring.

The structures of several other mannose binding lectins have been determined in both the presence of a monosaccharide as well as a di- or trisaccharide. Concanavalin A is an extensively studied legume lectin that specifically binds the trimannoside core found in all N-linked glycans. Naismith and Field (31) reported the structure of a concanavalin A-trimannoside complex (2.3 Å resolution); when this structure was compared with the structure of concanavalin A complexed to methyl a-mannoside at 2.0 Å resolution (32), the root mean square deviation

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\(^{2}\) N. M. Dahms, unpublished results.
between the two structures for all c atoms was 0.28 Å (31). It was observed that residues 118–123 as well as the loops at residues 161 and 204 remained disordered in the presence of the trisaccharide (31). However, none of these regions are involved in binding ligand unlike the case of loop A in the CD-MPR, which becomes more ordered in the presence of an oligosaccharide and does in fact contain residues that interact with the longer ligand. In the case of the legume lectins, isolectin I of *Lathyrus ochrus* and pea lectin, there is no discernable change in structure upon binding a tri- or disaccharide compared with a monosaccharide (33, 34). Therefore, when compared with other mannose binding lectins, the CD-MPR appears to be distinct in that the presence of an oligosaccharide orders the binding site.

A bound oligosaccharide may have a greater influence on the binding site structure of CD-MPR compared with other mannose binding lectins because of the depth of the binding pocket. The binding site of CD-MPR penetrates into the molecule, whereas the other mannose binding lectins interact with the oligosaccharide on the surface. In the case of *Erythrina coral-lodendron* lectin, only the penultimate sugar makes one direct hydrogen-bond contact with the protein (35). On the other hand, in the structure of isolectin I of *L. ochrus*, the prepenultimate and penultimate sugars of a trisaccharide do not directly contact the protein. Only the terminal mannose establishes direct hydrogen bonds to this lectin, whereas the remaining sugar interactions are mediated through water molecules (33).

Residues upon binding, but it does so along the surface of the protein in a shallow groove rather than a cleft (31) as do many of the legume isolectin I lectins (36).

The binding site architecture of the CD-MPR appears to be unique among the lectins. The binding site is a deep cleft with the phosphate and terminal mannose positioned against the bottom. The bottom of the cleft is formed by residues Gln66, Arg111, and Tyr143. These residues are located in β-strands 3, 7, and 9, respectively. The sides of the cleft are formed by residues in loops A (Asp43 and Tyr45), B (Gln68), and C (Asp103, Asn104, and His105) in addition to the loop connecting β-strands 8 and 9 (Arg135) (Fig. 3).

![Fig. 3. A, stereo diagram of the ligand binding pocket of CD-MPR complexed to pentamannosyl phosphate. The ligand moiety is shaded, and the mannose rings are numbered as discussed in the text. B, an enlarged view of the Mn$^{2+}$ coordination found in the B monomer.](image-url)
hydrogen bond interactions with the phosphate group. We also find the 2-hydroxyl group participates in hydrogen bonds to both the guanidinium nitrogen atom of Arg\textsuperscript{111} and the $\gamma$N of Gln\textsuperscript{66}. Studies on the human CD-MPR indicate that this arginine is essential for Man-6-P binding (11, 12). Mutagenesis studies conducted in our laboratory have shown that mutations of either Arg\textsuperscript{111} (Lys) or Gln\textsuperscript{66} (Asn) disrupts the binding of the receptor to pentamannosyl phosphate columns\textsuperscript{3}. Taken together, these results support an important role for Arg\textsuperscript{111} and Gln\textsuperscript{66} in sugar recognition and are likely candidates to confer the specificity of the receptor for mannose over glucose. Superimposition of a model of a glucose 6-phosphate molecule onto mannose 6-phosphate in the binding site reveals that the hydrogen bond contact with Arg\textsuperscript{111} is lost, whereas it is maintained with Gln\textsuperscript{66}. However, the 2-hydroxyl group of glucose 6-phosphate is also in position to act as a hydrogen bond donor to the $\gamma$ amide oxygen of Gln\textsuperscript{66}. Previous inhibition studies conducted by Tong and Kornfeld (10) indicated a $K_c$ value for glucose 6-phosphate about 3 orders of magnitude greater than the $K_d$ for Man-6-P (Table III). This indicates that the hydrogen bonding interaction between Arg\textsuperscript{111} and the 2-hydroxyl of Man-6-P is primarily responsible for the considerably higher affinity for Man-6-P compared with that of glucose 6-phosphate. However, in their same studies, Tong and Kornfeld showed that both glucose 6-phosphate and 2-deoxyglucose 6-phosphate have the same $K_c$ values (Table III). This suggests that the presence of the 2-OH at the equatorial position has no beneficial or deleterious effect. A possible explanation for this observation is that the binding energy gained by the hydrogen bond between the 2-OH of glucose 6-phosphate and Gln\textsuperscript{66} is balanced by the loss of a hydrogen bond with a water molecule. Although our data do not show a bound water molecule in this region, it is possible for a water molecule to be present in the vicinity of where an equatorial 2-OH might lie upon binding of glucose 6-phosphate. Modeling studies provide additional insight con-

\textsuperscript{3} L. J. Olson and N. M. Dahms, unpublished data.
cerning the recognition of other phosphorylated monosaccharides by the receptor. The CD-MPR has a similar $K_a$ value ($1 \times 10^{-2}$ M) for fructose 1-phosphate as the reported $K_a$ ($8 \times 10^{-6}$ M) for Man-6-P. This is not surprising when one considers the similarities in the structures of α-D-mannopyranose 6-phosphate and β-D-fructofuranose 1-phosphate. The 2-, 3-, and 4-hydroxyls along with the phosphate group can be superimposed. However, there is a difference: the anomeric hydroxyl group of mannopyranose superimposes with the hydrogen of C-5 of the fructofuranose and the anomeric hydroxyl of fructopyranose superimposes with the C-5 hydrogen of mannopyranose, where it is unable to make any additional hydrogen bond contacts to the protein. In the currently reported structure, the anomeric hydroxyl of mannose has one hydrogen bond contact with Tyr45. The loss of this contact and the presence of the anomeric hydroxyl of mannose has one hydrogen bond contact to the protein. In the currently reported structure, the anomeric hydroxyl of mannose has one hydrogen bond contact with Tyr45. The loss of this contact and the presence of the anomeric hydroxyl of mannose has one hydrogen bond contact to the protein.

The coordination of the metal appears to be the same whether the receptor is bound to ligand comprised of one or five mannose rings. The relatively high $B$ values for the Mn$^{2+}$ (Table I) indicate there is a low occupancy of this site in both of the monomers. This could account for the findings of Tong and Kornfeld (10). They reported only a 4-fold decrease in binding affinity of the receptor for pentamannosyl phosphate in the absence of MnCl$_2$ and the presence of EDTA. The Mn$^{2+}$ is located in an electrostatically negative region at the base of loop C located at the edge of the binding pocket (Fig. 6A). The cation is coordinated to one of the carboxylate oxygens of Asp103, the most solvent-accessible oxygen (O1) of the phosphate group, and to four water molecules (Fig. 3B). Our data suggest that the presence of a metal cation enhances binding of the phosphate group by shielding the solvent accessible oxygen from the negative electrostatic surface of the tip of loop C (Asp103) of the binding pocket (Fig. 6A). In contrast to the CD-MPR, several other classes of lectins have an absolute requirement for divalent cations for function. The legume lectins require both Ca$^{2+}$ and Mn$^{2+}$ to stabilize the binding site, and the metals also form coordination bonds with the amino acid side chains. In addition, the C-type lectins require Ca$^{2+}$ to form direct coordination bonds with the hydroxyls of the sugar moiety (38).

**Modeling of Naturally Occurring Phosphorylated Oligomannose Ligands in the Binding Site**—The CD-MPR has been shown to bind phosphorylated high mannose oligosaccharides found on lysosomal enzymes. Fig. 7 illustrates the five possible sites of phosphorylation of the oligosaccharide; studies have shown that only 20% of the population of oligosaccharides contain more than one Man-6-P residue (15). Studies by Distler et al. (8) have previously shown that mannoses with a single phosphate ester moiety located on the terminal mannose ring are substantially better inhibitors of β-galactosidase binding to

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**Table III**

| Compound                  | $K_a^a$ | $K_a^b$ | Inhibition of β-galactosidase binding relative to M6P |
|---------------------------|---------|---------|-----------------------------------------------------|
| Mannose                   | 1–5 $\times 10^{-2}$ | 0.002   |                                                     |
| Mannose 6-phosphate       | $8 \times 10^{-6}$  | 1.00    |                                                     |
| Glucose 6-phosphate       | 1–5 $\times 10^{-2}$ | 0.54    |                                                     |
| 2-deoxyglucose 6-phosphate| 1–5 $\times 10^{-2}$ | 0.82    |                                                     |
| Fructose 1-phosphate      | $1 \times 10^{-5}$  | 0.82    |                                                     |
| P-Manα1,3M                | 5.35    | 5.35    |                                                     |
| P-Manα1,6M                | 17.69   | 17.69   |                                                     |
| P-Manα1,2M Mα1,6M-X       | 16.43   | 16.43   |                                                     |
| P-Manα1,2M Mα1,3M-X       | 29.49   | 29.49   |                                                     |
| P-Manα1,3M Mα1,3M Mα1,3M | $6 \times 10^{-6}$ | 29.49   |                                                     |
| Mnα1,2M                   | 45.00   | 45.00   |                                                     |

$^a$ Ref. 10.  
$^b$ Ref. 8.
The values for each monomer are shown, with the values for the A monomer enclosed in parenthesis. The angles are defined as follows based on the work of Imberty et al. (40): $\tau = \theta (C1-O1-C3), \phi = \theta (O5-C1-O1-CX), \psi = \theta (C1-O1-CX-CX+1).

| Glycosidic linkage | $\tau$ | $\phi$ |
|-------------------|-------|-------|
| P-Man(1,3)Man(1,6) | 113 (116) | 70 (85) | -165 (160) |
| Man(1,3)Man(1,6) | 128 (119) | 57 (75) | 138 (101) |
| P-Man(1,3)Man(1,6) | 108 | 96 | 161 |
| Man(1,3)Man(1,6) | 108 | 179 | 120 |

a The glycosidic linkages were measured from our crystallographic structure.

b The glycosidic linkage is between the terminal and penultimate mannose.

c The glycosidic linkages were measured from our modeling studies.

d The glycosidic linkage is between the penultimate and prepenultimate mannose.
Structure of CD-MPR Bound to Pentamannosyl Phosphate

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