The structural basis of carbohydrate recognition by rat liver mannose-binding protein (MBP-C) has been explored by determining the three-dimensional structure of the C-type carbohydrate-recognition domain (CRD) of MBP-C using x-ray crystallography. The structure was solved by molecular replacement using rat serum mannoside-binding protein (MBP-A) as a search model and was refined to maximum Bragg spacings of 1.7 Å. Despite their almost identical folds, the dimeric structures formed by the two MBP CRDs differ dramatically. Complexes of MBP-C with methyl glycosides of mannose, N-acetylgalactosamine, and fucose were prepared by soaking MBP-C crystals in solutions containing these sugars. Surprisingly, the pyranose ring of mannose is rotated 180° relative to the orientation observed previously in MBP-A, but the local interactions between sugar and protein are preserved. For each of the bound sugars, vicinal, equatorial hydroxyl groups equivalent to the 3- and 4-OH groups of mannose directly coordinate Ca\(^{2+}\) and form hydrogen bonds with residues also serving as Ca\(^{2+}\) ligands. Few interactions are observed between other parts of the sugar and the protein. A complex formed between free galactose and MBP-C reveals a similar mode of binding, with the anomeric hydroxyl group serving as one of the Ca\(^{2+}\) ligands. A second binding site for mannose has also been observed in one of two copies in the asymmetric unit at a sugar concentration of 1.3 m. These structures explain how MBPs recognize a wide range of monosaccharides and suggest how fine specificity differences between MBP-A and MBP-C may be achieved.

Mannose-binding protein C (MBP-C) is a C-type (Ca\(^{2+}\)-dependent) lectin isolated from rat liver (1). MBP-C belongs to the collectin group of C-type lectins, which also includes the serum mannose-binding proteins and pulmonary surfactant apoproteins A and D (2). The collectins share a common arrangement of structural domains: a cysteine-rich domain at the amino terminus is followed by a collagenous domain, an oligomerization domain, and a COOH-terminal carbohydrate recognition domain (CRD). Individual polypeptides assemble into heterotrimers, which further associate into larger oligomers. Serum MBPs, such as MBP-A in rat and human MBP, consist of hexamers of trimeric building blocks (Mr, ~650,000). Serum MBPs function in antibody-independent host defense against pathogens by binding avidly to carbohydrate structures on foreign cell surfaces and inducing opsonization and complement-mediated cell lysis (3–5). MBP-C is a smaller oligomer than serum MBPs, and probably consists of two associated trimers (Mr, ~200,000) (6). Although the function of MBP-C is not known, the structural similarities between it and other collectins suggest that MBP-C may also play a part in host defense. Localization of MBP-C to the liver suggests that it may also have roles in cell-cell interactions or glycoprotein trafficking (7).

Consistent with the requirement that they recognize a variety of cell surfaces, MBPs A and C bind to a number of different monosaccharides containing vicinal, equatorial hydroxyl groups such as those found at the 3 and 4 positions of mannose (Man), including N-acetylgalactosamine (GlcNAc) and fucose (Fuc) (8). In contrast, MBPs A and C show minimal affinity for sugars that lack hydroxyl groups in this arrangement, such as galactosides and sialic acids. In addition to its characteristic monosaccharide specificity, MBP-C shows finer specificity differences for binding to complex N-linked oligosaccharides that contain Man, GlcNAc, and Fuc (9). MBP-C binds to a wide range of ligands that contain the trimannosyl core common to N-linked oligosaccharides, but the specific oligosaccharide structures recognized by MBP-C are not known. It is clear, however, that MBPs A and C have different binding specificities for complex oligosaccharides and other multivalent ligands, including neoglycoproteins (9). For example, MBP-A binds GlcNAc-BSA much more tightly than MBP-C, even though the two proteins bind comparably to free GlcNAc.

Sugar binding by MBPs A and C is mediated by the COOHterminal CRD. Related CRDs of 115–130 amino acids are present in all C-type lectins. Structure-based sequence alignments (10), as well as the crystal structures of two distantly related C-type lectins (10, 11), suggest that the overall structure of the CRD is very similar in all C-type lectins. The high-resolution crystal structure of a complex between a high-mannose oligosaccharide and the MBP-A CRD (12) shows that Man binds at a conserved Ca\(^{2+}\) site, designated site 2 (10, 12), through vicinal, equatorial 3- and 4-OH groups that form coordination bonds with the Ca\(^{2+}\) and hydrogen bonds with amino acid side chains that also serve as Ca\(^{2+}\) site 2 ligands. Sequence alignment, mutagenesis, and crystallographic studies suggest that this site is well-conserved among C-type lectins, and that the sequence of amino acids around this site determines binding specificity (11, 13–19). Modeling suggests that other
sugars known to bind to MBPs can bind in a similar manner, and the proposed mode of Fuc binding to MBP (12) has been used for modeling selectin-ligand interactions (11, 19).

Here we describe the structure of the MBP-C CRD, alone and complexed with a series of monosaccharide ligands. Although the structure of the MBP-C CRD is similar to that of MBP-A, the arrangement of protomers in the dimers formed by isolated CRDs differs drastically. The crystals formed by the MBP-A CRD (20) do not require sugar ligand for growth, thus providing a means to study a variety of sugar-protein complexes. The structures of MBP-C complexed with five different monosaccharides reveal how different sugars are recognized by MBPs and other C-type lectins. The local binding scheme between protein and sugar is similar to that observed in the oligosaccharide-MBP-A complex, but mannose is bound in a different orientation from that observed in MBP-A.

**EXPERIMENTAL PROCEDURES**

**Materials—**Unless otherwise specified, chemicals were obtained from J.T. Baker Inc. LB medium was obtained from Life Technologies, Inc. Guanidinium hydrochloride, isopropyl-1-thio-β-D-galactopyranoside, and subtilisin were obtained from Boehringer Mannheim. Polyethylene glycol 8000, BSA, Sephadex G-25, and all monosaccharides were obtained from Sigma. Sepharose 6B was obtained from Pharmacia Biotech Inc. Divinylsulfone was obtained from Aldrich. Immulon 4 microtiter plates were obtained from Dynatech Laboratories. NaHCO₃ was obtained from American Corp. Man₄GlcNAc₂ was obtained from E-Y. Laboratories.

**Protein Expression and Characterization—**MBP-C was expressed in Escherichia coli using a pUC19 derivative vector, as described previously (9) except that the amount of isopropyl-1-thio-β-D-galactopyranoside used to induce MBP-C expression was reduced to 0.5 mM. The protein was purified according to the procedure described previously (9) except that the amount of isopropyl-1-thio-β-D-galactopyranoside used to induce MBP-C expression was reduced to 0.5 mM. Values in parentheses are for the highest resolution shell: for native and α-Me-Man, resolution limits are 1.76–1.70 Å; for 1.3 × α-Me-Man, resolution limits are 1.97–1.90 Å; for other data sets, resolution limits are 1.86–1.80 Å.

**Crystallization—**Lyophilized sub-MBP-C was dissolved in water at 40 mM/g. The protein was neutralized with 1 M Tris-Cl, pH 9.5 (final concentration of Tris, 10 mM; final pH, 7.5; final concentration of protein, 12–20 mM), and CaCl₂ was added to a final concentration of 10 mM. 1 μl of protein was mixed with 1 μl of Solution A (11% (w/v) polyethylene glycol 8000, 100 mM Tris-Cl, pH 7.4, 100 mM NaCl, 12 mM CaCl₂, 2 mM NaN₃), and the hanging drop was allowed to equilibrate over 1 ml of Solution A at 22°C. Clusters of crystals occasionally appeared within a few days. These crystals were crushed and washed in fresh Solution A to provide a stock of seed crystals. Serial dilutions of seed crystals in Solution A were prepared. A 1-μl drop from an appropriate dilution was mixed with 1 μl of protein, and the hanging drop was allowed to equilibrate over 1 ml of Solution A at 22°C. Single crystals appeared within 1 h, and crystals reached full size (typically 1.0 × 0.2 × 0.2 mm) within 2 days. The symmetry and unit cell dimensions of these crystals were determined by precession photography.

**X-ray Diffraction Measurements and Calculations—**The data used for molecular replacement and the initial rounds of refinement were measured at room temperature from a single crystal mounted in a glass capillary (Rcryst = 6.8%; completeness = 96% over 40–2.0 Å; average redundancy = 3.5). Subsequently, data were collected from crystals flash-frozen at ~100 K. Only data measured from frozen crystals were used for the final rounds of refinement. Crystals were prepared for freezing by transfer through a series of solutions containing Solution A plus 0, 5, 10, 15, and 20% MDP. Crystals were left for 15 min at each concentration of MDP. A crystal adapted to 20% MDP was suspended in a rayon loop and flash-cooled under a stream of nitrogen at ~100 K. For the ligand complexes, sugars were introduced at a concentration of 200 mM to the final two soaks (15 and 20% MDP) before freezing. For galactose, MDP was omitted, and a crystal was adapted to solution A plus 0, 5, 10, 15, 20, and 25% Gal before freezing and data collection. For the analysis of Man binding at 13 Å, a crystal was adapted to solution A plus 0, 5, 10, 15, 20, and 25% α-O-methyl mannoside (α-Me-Man) before freezing and data collection.

Data were measured on an R-AXIS IIIC imaging-plate detector (Rigaku), using CuKα radiation from a rotating anode (Rigaku; 50 kV, 90 mA, graphite monochromator, 0.3-mm collimator) at a crystal-to-detector distance of 85 mm. Lorentz-polarization-corrected integrated intensities were obtained using DENZO (21), and redundant measurements were scaled and merged with SCALEPACK (21). Each data set was put on a quasi-absolute scale using TRUNCATE (22) and scaled to the data from the sugar-free crystal using RSTATS (22). Data processing statistics are presented in Table I. X-PLOR (23) was used for molecular replacement, reciprocal space refinement, and electron density map calculations. Model building was performed using O (24). Throughout the course of refinement, regions where the model fit the electron density poorly or had poor geometry were omitted and rebuilt according to simulated annealing omit maps (25). The geometry of the

| Crystal | Maximum resolution | Independent reflections | Completeness | Average redundancy | Rcryst | Rfree |
|---------|--------------------|-------------------------|--------------|-------------------|-------|-------|
| Native  | 1.7                | 26,829                  | 90.3 (63.0)  | 3.1 (1.5)         | 22.645| 20.1  |
| α-Me-Man| 1.7                | 28,080                  | 94.5 (74.1)  | 3.2 (1.6)         | 22.126| 20.3  |
| α-Me-GlucAc | 1.8            | 23,205                  | 92.3 (71.4)  | 2.9 (1.9)         | 19.301| 20.3  |
| α-Me-Fuc | 1.8              | 24,379                  | 97.8 (91.9)  | 3.0 (2.5)         | 20.202| 19.6  |
| β-Me-Fuc | 1.8              | 23,087                  | 92.7 (84.3)  | 2.7 (2.1)         | 19.072| 19.4  |
| Gal     | 1.8                | 23,364                  | 93.7 (84.3)  | 2.7 (1.9)         | 19.350| 21.4  |
| α-Me-Man | 1.9              | 20,777                  | 96.6 (93.9)  | 3.0 (2.6)         | 17.049| 19.1  |

| Table I Crystallographic data |
|-------------------------------|
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Recent changes in the agarose as supplied by the manufacturer (20) may reflect altered properties of the affinity resin following the lack of tight binding to the affinity resin (see "Experimental Procedures")

Preparation, Characterization, and Crystallization of the MBP-C CRD—A bacterial expression system for a trimeric 156-residue COOH-fragment of MBP-C, consisting of the neck and CRD (Fig. 1), has been described previously (9). Approximately 2 mg of this fragment was obtained per liter of bacterial culture. As in previous studies on MBP-A (20), proteolytic digestion of MBP-C was used to remove the neck from the expressed protein. The results of limited proteolysis with subtilisin are shown in Fig. 2. Digestion results in a reduction of approximately 5 kDa in the size of the fragment. Following preparative digestion, the truncated fragment (sub-MBP-C) was isolated by affinity chromatography on Man-Sepharose.

Subtilisin digestion of MBP-C. Aliquots of purified, bacterially expressed MBP-C were digested with 0, 5, 10, 20, 40, and 80 μg/ml subtilisin in 1.25 w NaCl, 25 mM Tris-Cl (pH 7.8), and 25 mM CaCl2 at 37°C for 1 h. Following electrophoresis on a SDS-polyacrylamide gel (17.5%), digest products were detected by staining with Coomassie Blue. The sizes of molecular weight standards (in kDa) are shown at the left.

and does not necessarily indicate lower affinity of the MBP-C CRD for Man.2

Sequence analysis of purified sub-MBP-C indicates some heterogeneity in the NH2-terminal sequence. Relative amounts of different fragments are indicated in Fig. 1. The unique site of cleavage in MBP-A corresponds to Val112 of MBP-C, suggesting that a similar region of the two proteins is exposed to digestion. The oligomeric state of sub-MBP-C was investigated by gel filtration chromatography and chemical cross-linking. The fragment elutes with an apparent molecular weight of 21 kDa from a gel filtration column (data not shown). Since the polypeptide molecular weight is 13 kDa, the elution position suggests that the fragment is dimeric. This conclusion is supported by cross-linking data (Fig. 3), in which the fragment containing neck and CRD forms trimers, while the CRD alone forms only dimers. Thus, like the CRD of MBP-A, the MBP-C CRD appears to undergo a rearrangement in oligomer geometry upon removal of the adjacent neck.

RESULTS

Structure of MBP-C CRD

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Crystals of sub-MBP-C were grown in the presence of Ca2+ at neutral pH. Precession photography revealed the space group to be P212121. Based upon the unit cell dimensions, the calculated molecular weight of a protomer (13 kDa), and a partial specific volume of 0.73 g/cm3 for the protein, the crystal would contain either 77, 54, 31, or 8% solvent if it contained one, two, three, or four protomers/asymmetric unit. Because space group P212121 does not contain a pure 2-fold rotation axis, if a 2-fold axis exists in the sub-MBP-C dimer, the asymmetric unit would most likely consist of a single dimer. If the dimer lacks a 2-fold axis, there could be from one to three protomers/asymmetric unit. Self-rotation functions calculated from the data used for molecular replacement did not reveal the presence of a noncrystallographic rotation axis.

Structure Solution by Molecular Replacement—The search model for molecular replacement calculations consisted of the CRD from the refined structure of MBP-A (12) in which all conserved side chains were retained, but nonconserved side chain atoms past the β carbon were deleted. Temperature factors from the refined MBP-A model were retained. Cross-rotation searches with the MBP-A dimer model yielded a weaker solution (6.9 s above the mean; next highest solution 4.3 s). Cross-rotation searches with the MBP-A dimer model

2 K. K.-S. Ng, W. I. Weis, and M. E. Taylor, unpublished observations.
the rotation function solution obtained with the MBP-A protomer gave a correlation coefficient of 0.12 (next highest 0.06). This solution was used to calculate a translation function using data from 10 to 4 Å. The translation function yielded two solutions (7.3 and 6.6 Å above the mean; next highest solution, 5.2 Å) with identical x and y coordinates but a difference of 7 Å in z. A native Patterson synthesis showed a very strong peak (33 Å above the mean) corresponding to a direct translation of 7 Å in z and 31 Å in x (½ of a). These results indicated that the asymmetric unit consists of a dimer with a noncrystallographic 2-fold axis parallel to the crystallographic a axis but offset from it by 3.5 Å in z. The combination of the noncrystallographic 2-fold and the crystallographic 21 axes generates a nearly-perfect direct translational repeat within the unit cell. This repeat is manifested in pseudo-centering seen along a* in a precession photograph of the hko zone (data not shown). The parallel arrangement of noncrystallographic and crystallographic symmetry axes explains the lack of a peak in the self-rotation function, since origin peaks generated by crystallographic symmetry obscure the peak from the noncrystallographic 2-fold axis.

When a single protomer was positioned according to either of the two translation function solutions, the R-factor was unusually high (0.62). When the second protomer was positioned according to the repeat shown in the native Patterson and translation functions, however, the R-factor was 0.48, a value more typical of a correct molecular replacement solution. This behavior arises because the interference function generated by the repeat along x cannot be modeled with a single protomer. Visual inspection indicated that the molecular packing in the crystal was reasonable. Finally, the model was refined first as a single rigid body and then with individual protomers as separate rigid bodies, yielding an R-factor of 0.41 for data between 10 and 2.8 Å.

Refinement—The model was initially refined against data measured from a crystal at room temperature. Final rounds of refinement were carried out against data measured from a crystal flash-frozen at ~100 K (Table I). A random 10% of reflections were selected as a test set for calculations of the free R-factor (29) before refinement commenced, and this quantity was monitored throughout refinement. Noncrystallographic symmetry restraints were not imposed at any point during refinement.

Initial rounds of map calculation and refinement used data from 10 to 2.8 Å. Calcium ions, side chains not present in the search model, and main chain positions that differed significantly from the search model were manually positioned using 2Fo – Fc and Fo – Fc electron density maps. After a round of positional refinement, resolution limits were changed to the range of 5–2.5 Å, and resolution-dependent weighting was introduced. A round of simulated annealing refinement at 400 K was followed by manual model building. Resolution limits were then extended to 5–2.3 Å, and individual isotropic temperature factors were reset (22 Å² for main chain atoms and 26 Å² for side chains) and refined. Additional rounds of positional and temperature-factor refinement were carried out against data from 5–2.0 Å, and solvent molecules were added. Finally, atomic positions and temperature factors were refined against data from 10–1.7 Å, when alternate side chain conformations and weaker solvent sites were added to the model. The final temperature factors and occupancies of solvent atoms were refined by resetting the temperature factor to a value equal to the average of all hydrogen-bonded neighbors plus 5 Å² and by iteratively refining occupancy and temperature factor. Only solvent atoms visible at the 1.0 σ contour of 2Fo – Fc maps, and which were capable of making at least one hydrogen bond with either a protein atom or another solvent atom, were retained in the model. A representative portion of the final electron density map is shown in Fig. 4.

The final model contains all of sub-MBP-C except residues 111–115 at the NH₂ terminus and residue 226 at the COOH terminus. Electron density is weak or absent for the following surface side chains: in protomer 1, Tyr₁₁⁶, Lys₁⁵⁷, Arg₁₈⁰, and Arg₁²₂ in protomer 2, Tyr₁₁⁶, Gln₁₆⁷, Arg₁²₂, and Glu₁₈⁰. The side chains of five residues (Met₁¹⁸ in both protomers, Arg₁²₂ in protomer 1, and Ser₁³⁴ and Thr₁³⁹ in protomer 2) were modeled in two conformations. All main chain torsion angles fall within allowed regions of the Ramachandran plot. Model geometry and temperature-factor statistics are summarized in Table II. Coordinate error was assessed by several methods. Luzzati analysis (30) suggests that the coordinate error is in the range 0.15–0.25 Å, although the behavior of the R-factor with scattering angle does not follow the theoretical curves very well (not shown). As an alternative estimate of coordinate precision, the final structure was subject to two simulated annealing runs at 400 K followed by minimization. Superposition of the structures gave an overall root mean square deviation of 0.30 Å for atoms whose temperature factors are less than 50 Å². This is most likely an overestimate, since the molecular dynamics trajectory is not well constrained by the data for surface residues. Finally, superposition of the main chain atoms of the two independent copies in the asymmetric unit gives a root mean square deviation of 0.24 Å if regions clearly different due to lattice contacts (see below) are removed. Thus, the coordinate error appears to be on the order of 0.20–0.25 Å.

Structure of the MBP-C CRD Dimer—The structure of the MBP-C CRD is very similar to that of MBP-A, reflecting the fact that MBP-A and MBP-C share 58% sequence identity in the CRD, and more importantly, share all of the residues that form the disulfide bonds, core hydrophobic packing interactions, and Ca²⁺-binding sites. The path of the polypeptide backbone within a single protomer is nearly identical in the two proteins (Fig. 5); when a single protomer of MBP-C is superimposed on a protomer from MBP-A, the root mean square deviation for all main chain atoms is 0.70 Å. However, despite the similarity in protomer structure, the structures of the MBP-A and C dimers differ dramatically (Fig. 5). Differences in sequence of the NH₂-terminal strand give rise to very different packing in the dimer interfaces. The positions of corresponding residues in the NH₂-terminal strands differ by over 1 Å when protomers from the two proteins are superimposed. Fig. 4 shows the central region of the MBP-C dimer interface. Leu₁³⁶ is buried in a pocket formed by Met₁¹⁸, Leu₁³², Leu₁³⁶, and...
Cys\textsuperscript{222} (disulfide bonded to Cys\textsuperscript{133}) of the other protomer. The corresponding residues in MBP-A are at the periphery of the MBP-A dimer interface and form very different contacts (10). The asymmetric unit of the MBP-C crystal consists of a noncrystallographic dimer that corresponds to the dimer observed in solution (Fig. 5). When one protomer is superimposed on the other, the root mean square deviation of all main chain atoms is 0.32 Å. Significant differences between the two protomers are restricted to residues near different lattice contacts in the two independent copies of the protomer: residues 153–154 (\(\alpha_2\)), 177–181 (loop 3), and 195–196 (loop 4) (see Weis et al. (10) for secondary structure assignments). The amount of buried surface in the dimer is roughly 650 Å\textsuperscript{2}/protomer, which is one-eighth of the total surface area of the protomer. The amount of buried surface is comparable with that seen in the MBP-A dimer (10) as well as other protein dimers (31).

A noteworthy difference between MBP-A and MBP-C is the lack of the third Ca\textsuperscript{2+} site seen in both rat MBP-A (12, 32) and human MBP (33). In MBP-A, three of the Ca\textsuperscript{2+} site 3 ligands come from the side chains of Glu\textsuperscript{165} and Asp\textsuperscript{194}, which also serve as ligands for Ca\textsuperscript{2+} site 1; Asp\textsuperscript{194} provides two of the ligating oxygens (see Fig. 2b of Ref. 12). In MBP-C, Asn\textsuperscript{199} is equivalent to Asp\textsuperscript{194} of MBP-A, and apparently cannot provide the requisite ligands for Ca\textsuperscript{2+} site 3. Based on the fact that carbohydrate binding and adoption of a protease-resistant conformation by MBP-A requires two Ca\textsuperscript{2+} (20), it has been argued that Ca\textsuperscript{2+} site 3 is adventitious and is observed due to the high concentrations of Ca\textsuperscript{2+} used for crystallization (12, 32).
saccharides were soaked into sub-MBP-C crystals at concentrations of 0.2 or 1.3 M. The higher concentration was used only for Gal and for locating the second binding site of α-Me-Man. Different sugars could be soaked into MBP-C crystals without affecting lattice contacts, since Ca²⁺ site 2 is clear of lattice contacts. As a result, the unit cell parameters vary less than 1% in the presence of different sugars, and scaling R-factors are less than 20% when comparing data collected from unliganded and liganded crystals (Table III). F_o(sugar) – F_o(native) difference electron density maps also showed that the largest change between the sugar complexes and the native crystals was the presence of a single sugar bound at Ca²⁺ site 2.

The starting model for refining different sugar-protein complexes was derived from the fully refined model for the unliganded protein. The positions of 246 water molecules were independently determined for the unliganded protein and the α-Me-Man and α-O-methyl-N-acetylglucosamine (α-MeGlcNac) complexes. For all other complexes, these solvent positions were transferred, refined, and carefully checked against 2F_o – F_c electron density maps. For each complex, the starting model without sugar was refined against data from 10 Å to the highest resolution measured (1.7–1.9 Å) (Table I) before an F_o – F_c difference electron density map was calculated. Unbiased, positive F_o – F_c difference electron density defined the entire sugar molecule bound at Ca²⁺ site 2; however, the aglycon was not visible in any case except for α-O-methyl fucoside (α-Me-Fuc) and one copy of α-Me-Man. A model for the bound sugar with regular geometry could be placed unambiguously into positive F_o – F_c difference electron density, and water molecules near the sugar were then added. The model was subject to further rounds of positional and temperature factor refinement, and the temperature factors and occupancies of the water molecules were iteratively refined as described for the unliganded protein. Representative electron density for a bound sugar at Ca²⁺ site 2 is shown in Fig. 6c, and model geometry and temperature-factor statistics are presented in Table II.

Mannoside Binding—α-Me-Man binds to MBP-C at Ca²⁺ site 2 in a manner analogous to that first seen in the MBP-A-Man ocrycrystal structure (Fig. 7) (12). As seen in MBP-A, the vicinal, equatorial 3- and 4-OH directly coordinate the Ca²⁺ and form hydrogen bonds with the Ca²⁺ ligands. The 3-OH donates a hydrogen bond to O₂ of Glu198 and accepts a hydrogen bond from N₅ of Asn192, while the 4-OH donates a hydrogen bond to O₁ of Glu198 and accepts a hydrogen bond from N₁₂ of Asn192. The remaining lone pair of electrons on each hydroxyl group forms a coordination bond with the Ca²⁺. The only other interaction between α-Me-Man and protein is a van der Waals’ contact between C-3 of the pyranose ring and the side chain of Val194 (Fig. 8). The interaction is slightly different in the two different copies in the asymmetric unit: in one copy, the C-3 ring carbon is 3.8 Å from C₂ of Val194, while in the other copy, C-3 is 4.4 Å from C₁ and 4.2 Å from C₁₂. It should be noted that although the 2-OH of Man is 3.3 Å from Asn192 N₅, the angle formed by O-2, N₁₂, and O-3 is 55°, indicating

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**FIG. 6.** Displacement of water upon Man binding. Electron density (contoured at 1.0 σ) from unliganded MBP-C superimposed on models for unliganded MBP-C (a) and the model of α-Me-Man bound to MBP-C (b). In c, the electron density (contoured at 1.0 σ) for the complex of α-Me-Man and MBP-C is superimposed on the model for that complex. The 3- and 4-OH of the bound mannoside superimpose on the water molecules bound to the unliganded protein. The figure was prepared using O (24).
that a hydrogen bond between O-2 and N$_2$ could not be formed. Surprisingly, the positions of the 3- and 4-OH groups of α-Me-Man are reversed in MBP-C when compared with MBP-A (Fig. 7) (12). A dyad axis relates the vicinal, equatorial hydroxyl groups and the carbon atoms to which they are directly bonded, so the local geometry of hydrogen and coordination bonds is the same for both orientations. Modeling indicates that the binding sites of MBPs A and C would allow Man to bind in either orientation, but the electron density clearly shows that Man is bound in a single orientation in both cases (Fig. 6) (12). Moreover, in both dimer structures there are two independent copies in the crystallographic asymmetric unit, which argues against effects of lattice environment on the orientation. In the case of MBP-A, however, the orientation of Man is known from the structure of a cocrystal of a high mannose oligosaccharide and the sub-MBP-A dimer (12). The oligosaccharide cross-links the crystal such that the terminal Man residues of two nonequivalent branches (α-(1,2) and α-(1,3)) are bound to the two independent copies. It is possible that other sugars, including other mannosides, bind to MBP-A in the orientation observed in MBP-C.

The paucity of interactions between the protein and α-Me-Man reflects the openness of the sugar-binding site, which can be described as a shallow trough. As shown in Fig. 9, one edge of this trough is formed by Val$_{194}$. Binding of α-Me-Man to MBP-C buries approximately 130 Å$^2$ (40%) of sugar and 80 Å$^2$ of protein solvent-accessible surface area (calculated with a probe radius of 1.4 Å (34)). For comparison, binding of the terminal Man to MBP-A buries 160 Å$^2$ of sugar and 90 Å$^2$ of protein (12). Interestingly, the less polar surface of α-Me-Man faces Val$_{194}$ (Figs. 8 and 9). Although this surface is largely exposed to solvent, no ordered water molecules are visible near it. In contrast, an extensive network of water molecules extends along the other surface of the sugar. Except for six water sites closest to the sugar itself, the network of water sites in this binding trough is not disrupted by binding of the sugar.

Superposition of liganded and unliganded structures, as well as isomorphous difference Fourier maps, reveal that the binding of α-Me-Man does not detectably affect the conformation of the protein. Moreover, the temperature factors of residues in the sugar-binding site do not change significantly upon sugar binding. The most notable effect of sugar binding is the displacement of two water molecules, which form the same set of coordination and hydrogen bonds as described for the sugar (Fig. 6, Table IV). The sugar hydroxyl groups superimpose perfectly over the displaced water molecules, preserving the 8-fold coordination of the calcium ion.

N-Acetylglucosaminide Binding—α-Me-GlcNAc binds to
MBP-C at Ca$^{2+}$ site 2 in a manner similar to that of Man (Fig. 7). The 3- and 4-OH groups coordinate Ca$^{2+}$ and form the same hydrogen bonds with amino acid side chains that serve as Ca$^{2+}$ ligands as seen in the α-Me-Man complex (Fig. 7). The orientation of GlcNAc when bound to MBP-C is the same as that of Man: the B-face (for naming conventions see (35, 36)) of each sugar faces Val194, and the same atoms of the pyranose rings superimpose. Also like α-Me-Man, α-Me-GlcNAc forms a van der Waals' contact between C-3 of the pyranose ring and the side chain of Val194 (Fig. 8). α-Me-GlcNAc forms an additional van der Waals' contact between the acetamido methyl carbon and C$_{31}$ of Pro$_{113}$ (Fig. 7).

Fucoside Binding—α-Me-Fuc and β-Me-Fuc bind to MBP-C in a manner similar to that of Man and GlcNAc. The predominant chair conformation adopted by L-Fuc, 1C4, matches the predominant chair conformation of D-Man and D-GlcNAc, 4C1, by superimposing the ring oxygens of each sugar, and C-1, C-2, C-3, C-4, and C-5 of L-Fuc over C-5, C-4, C-3, C-2, and C-1, respectively.

### Table IV

| Sugar atom | Protein atom/Ca$^{2+}$ | Distances | Type of interaction |
|------------|------------------------|-----------|-------------------|
|             |                        | MBP-C | Water-protein | MBP-A | Sugar-protein |
| O-3 (O-1)$^b$ | Ca$^{2+}$ 2           | 2.56 ± 0.04 | 2.59 | 2.60 | Coordination |
| O-3 (O-1)$^b$ | Glu198 O$_1$          | 2.53 ± 0.05 | 2.61 | 2.60 | Hydrogen bond |
| O-3 (O-1)$^b$ | Asn192 N$_{12}$       | 2.99 ± 0.07 | 3.06 | 2.87 | Hydrogen bond |
| O-4 (O-2)$^c$ | Ca$^{2+}$ 2           | 2.53 ± 0.05 | 2.69 | 2.44 | Coordination |
| O-4 (O-2)$^c$ | Glu190 O$_2$          | 2.54 ± 0.04 | 2.57 | 2.52 | Hydrogen bond |
| O-4 (O-2)$^c$ | Asn192 N$_{12}$       | 2.82 ± 0.04 | 2.88 | 2.90 | Hydrogen bond |
| C-3 (C-1)$^d$ | Val194 C$_{32}$ (copy 1) | 3.78 ± 0.06 | 2.88 | van der Waals' |
| C-3 (C-1)$^d$ | Val194 C$_{31}$ (copy 2) | 4.24 ± 0.03 | 2.88 | van der Waals' |
| C-3 (C-1)$^d$ | Val194 C$_{32}$ (copy 2) | 4.40 ± 0.07 | 2.88 | van der Waals' |

$^a$ Distances for water molecules in positions equivalent to that of a sugar hydroxyl group.

$^b$ O-3 atom of all sugars except Gal, where O-1 is in the equivalent position; for MBP-A, the O-4 atom of the bound mannose, Glu$_{193}$, and Asn$_{205}$ take equivalent positions.

$^c$ O-4 atom of α-Me-Man and α-Me-GlcNAc; O-2 atom of α-Me-Fuc, β-Me-Fuc, and Gal; for MBP-A, the O-3 atom of the bound mannose, Glu$_{185}$ and Asn$_{197}$ take equivalent positions.

$^d$ C-3 atom of all sugars except Gal, where C-1 is in the equivalent position.

**Fig. 8.** Van der Waals' contacts between MBP-A or MBP-C and Man. View of the interactions between the terminal Man residue of a bound oligosaccharide and MBP-A (a) (12) and a view of the interactions between α-Me-Man and MBP-C (b). In a, the remaining portion of the oligosaccharide has been omitted for clarity. The view is roughly 90° away from the view presented in Fig. 7. Atoms are shaded as in Fig. 7. Van der Waals' contacts are denoted by dashed lines. The figure was prepared using MOLSCRIPT (49).

**Fig. 9.** Water structure in the MBP-C-Man complex. Stereo view of protein residues 190–198 and 210–213 of MBP-C, α-Me-Man, and all water molecules within 5.0 Å of a sugar atom. Atoms are shaded as in Fig. 7. Long-dashed lines denote coordination bonds with Ca$^{2+}$, medium-dashed lines denote hydrogen bonds, and short-dashed lines denote van der Waals' contacts. All protein-water, sugar-water, and water-water hydrogen bonds are shown. The figure was prepared using MOLSCRIPT (49).
respectively, of \( \alpha \)-Man or \( \beta \)-GlcNAc. When bound to MBP-C, both \( \alpha \)-Me-Fuc and \( \beta \)-Me-Fuc superimpose on Man and GlcNAc in this manner (Fig. 7). Both fucosides make the same set of coordination and hydrogen bonds seen in the Man and GlcNAc complexes, with the 2- and 3-OH of the \( \alpha \)-sugar adopting the same stereochemistry as the 3- and 4-OH of Man and GlcNAc ((+)-syn-clinal conformation (37)) (Fig. 7, Table IV). The only other interaction between the fucosides and MBP-C is the van der Waals' contact between C-3 and the side chain of Val194. As in the case of the Man 2-OH discussed above, the equivalent 1- and 2-OH of Fuc is 3.3 \( \AA \) from Asn192 N\( \beta \) (Fig. 7) but is not properly disposed for hydrogen bond formation. Similarly, the anomeric oxygen of \( \alpha \)-Me-Fuc is 3.3 \( \AA \) from Asn192 N\( \beta \) but is not suitably positioned to form a hydrogen bond.

Galactose Binding—When soaked at 1.3 \( \text{M NaCl} \) into MBP-C crystals, Gal binds at Ca\(^{2+}\) site 2 similar to the other monosaccharides, forming the same set of coordination bonds with Ca\(^{2+}\), hydrogen bonds with the Ca\(^{2+}\) ligands, and van der Waals' contact with Val194. Gal is unusual, however, in that while the free sugar binds with low affinity, the methyl glycoside does not bind at all (Table V). The structure of the Gal-MBP-C complex differs from the complexes formed by the other monosaccharides in that the 1- and 2-OH groups of the \( \beta \)-anomer are the only pair of vicinal hydroxyls that can adopt the (+)-syn-clinal conformation required to interact with Ca\(^{2+}\) and its ligands at Ca\(^{2+}\) site 2 (Fig. 7). Thus, in galactosides, the 1-OH group is not available to form the same coordination and hydrogen bonds as the free sugar. Hence, the selectivity of MBPs against methyl galactosides and galactoside-containing oligosaccharides can be explained by the strict requirement for equatorial, vicinal hydroxyl groups in the (+)-syn-clinal configuration.

The significantly weaker binding of free galactose relative to the other sugars studied here, despite the same number of contacts formed with the protein, may be due to the unique electronic properties of the anomeric oxygen that make it a relatively poor hydrogen bond acceptor (38). This property may also make the coordination bond between the anomeric oxygen and Ca\(^{2+}\) relatively weak. In addition, since only the \( \beta \)-anomer of Gal has the correct disposition of 1- and 2-OH for binding, and the ratio of \( \alpha \)- to \( \beta \)-Gal is approximately 1:2 in solution (39), the apparent affinity for Gal is diminished by a factor of 1.7.

**Monosaccharide Recognition by MBP-C**

The affinity for \( \alpha \)-Me-Man and \( \beta \)-Me-Fuc is 5-fold higher than for \( \beta \)-Me-Fuc, and this difference in affinity can be eliminated by mutations at position 189 (17). These observations led to the suggestion that Fuc is oriented in MBP-A with the anomeric oxygen near position 189, opposite to that originally proposed based on the orientation of Man (12) but like that seen in the high-resolution crystal structures (40, 41). It should be noted that the only restraint on Ca\(^{2+}\)-oxygen distances used in refinement was the nonbonded van der Waals' energy term (42), terms for coordination bond lengths and angles, hydrogen bonds, and electrostatic energies were not included. Sugar-protein distances for different complexes are also not biased to a common starting model, because each sugar was placed separately into F\( _{o} \) – F\( _{c} \) electron density maps and refined independently.

Aside from the interactions with Ca\(^{2+}\) and Ca\(^{2+}\) ligands, the only other interaction between sugar and protein common to all complexes is the van der Waals' contact between C-3 of the pyranose ring and the side chain of Val194 (Fig. 6). In all complexes, this interaction is slightly different in the two different copies in the asymmetric unit, although each protomer makes the same interactions with each of the bound sugars, as noted above for the \( \alpha \)-Me-Man complex (Table IV). The small number of interactions between sugar and protein attests to the importance of the interactions between (+)-syn-clinal hydroxyl groups on bound sugars and Ca\(^{2+}\) site 2, as well as the openness of the sugar binding site.

Despite the open nature of the MBP-C binding site, all monosaccharides are bound in a single orientation relative to the Ca\(^{2+}\) site 2 ligands. Modeling shows that if any of the sugars is rotated \( 180^\circ \) relative to Ca\(^{2+}\) ligands, steric clashes do not occur between sugar and protein, and the (+)-syn-clinal hydroxyl groups can still make the same coordination and hydrogen bonds. However, there is no evidence in the electron density maps for a mixture of two orientations in any of the complexes. A similarity among all bound sugars, which may account for the selection of a preferred binding orientation is that the less polar surface of each sugar faces Val194 (B face of Man, GlcNAc, and Gal; A face of Fuc) (Figs. 7-9).

**Binding Affinities**—Monosaccharide binding affinities were measured using an assay in which tight binding to a multivalent ligand, Man\( _{37}\)BSA, is competitively inhibited by different monosaccharides (Table V). Measurements for MBP-A are consistent with previous studies (17) and show that sugars with (+)-syn-clinal hydroxyl groups are bound with roughly the same affinity, except for free galactose, which is discussed below. The present results show that the same monosaccharides that bind to MBP-A also bind to MBP-C, although the relative affinities of different sugars are somewhat different. The open nature of the MBP-C binding site and the paucity of interactions between sugar and protein explain how MBPs are able to bind a wide range of sugars with roughly the same affinity. Unfortunately, the crystal structures do not indicate the origin of subtle differences in affinity for different sugars, reflecting the difficulty of relating structural and thermodynamic data. For example, \( \alpha \)-Me-GlcNAc makes an additional van der Waals' contact but is bound more weakly than \( \alpha \)-Me-Man. Likewise, both \( \alpha \)- and \( \beta \)-Me-Fuc make the same number of contacts with the protein yet bind with different affinities. The relative strength of coordination and hydrogen bonds formed by the sugar versus water, the energy of desolvation of the sugar ligands and other factors undoubtedly have a role in determining the strength of binding in each case.

While the structures of MBP-C-monosaccharide complexes do not explain subtle differences in binding affinity, the structures shed light on certain differences observed in MBP-A. In MBP-A, the affinity for \( \alpha \)-Me-Fuc is 5-fold higher than for \( \beta \)-Me-Fuc, and this difference in affinity can be eliminated by mutations at position 189 (17). These observations led to the suggestion that Fuc is oriented in MBP-A with the anomeric oxygen near position 189, opposite to that originally proposed based on the orientation of Man (12) but like that seen in the Table V

| Competing sugar | MBP-A | MBP-C |
|-----------------|-------|-------|
|                 | Ki    | Ki/\( K_i/\text{mutant} \) | Ki   | Ki/\( K_i/\text{mutant} \) |
| \( \alpha \)-Me-Man | 8.0 \( \pm \) 0.3 | 1.0 | 5.7 \( \pm \) 0.6 | 1.0 |
| \( \alpha \)-Me-GlcNAc | 12.7 \( \pm \) 1.3 | 1.6 \( \pm \) 0.1 | 14.4 \( \pm \) 2.9 | 2.5 \( \pm \) 0.3 |
| \( \alpha \)-Me-Fuc | 2.9 \( \pm \) 0.3 | 0.4 \( \pm \) 0.04 | 8.8 \( \pm \) 1.1 | 1.5 \( \pm \) 0.1 |
| \( \beta \)-Me-Fuc | 12.3 \( \pm \) 0.8 | 1.6 \( \pm \) 0.1 | 21.7 \( \pm \) 3.4 | 3.8 \( \pm \) 0.4 |
| Gal | 212 \( \pm \) 9 | 27 \( \pm \) 2 | 114 \( \pm \) 20 | 20 \( \pm \) 1 |
| \( \beta \)-Me-Gal | >500 | >60 | >500 | >80 |

\( ^a \) No inhibition observed at 500 \( \text{mNaCl} \).
MBP-C-Fuc complexes (Fig. 7). The present results confirm that this binding orientation is possible, although a definitive answer requires the determination of MBP-A-Fuc complex structures.

Second α-Me-Man Binding Site—A molecule of α-Me-Man binds at a second site in one of the two independent MBP-C protomers when crystals are soaked in a solution containing 1.3 M α-Me-Man (Figs. 5 and 10). When bound at the second site, the Man 2-OH, 6-OH, and the ring oxygen form two bifurcated hydrogen bonds with N1 of Lys130 (Fig. 10). No other hydrogen bonds are formed between sugar and protein. Van der Waals’ contacts are formed between the aglycon methyl group and Sγ of Cys214 (3.3 Å) and between O-1 and Cα of Gln167 (3.5 Å). Electron density for the sugar is clear only for one of the two copies in the asymmetric unit, even though there are no direct contacts between symmetry-related molecules and this region in either copy. In the copy where the electron density does not define an entire sugar, there is continuous electron density only for the positions corresponding to O-6, C-6, C-5, O-5, C-1, O-1, and O-1-methyl atoms in the well defined copy (not shown). It is uncertain whether this density represents partially ordered solvent or sugar. In this copy, the nearest symmetry mate is farther away than in the first copy, where an ordered network of solvent atoms extends between the sugar and the nearest symmetry mate. Thus the water network between the sugar and the nearby symmetry copy may help stabilize the orientation of the bound sugar in this case. When α-Me-Man is soaked at 0.2 M, electron density in this region does not define an entire sugar molecule in either copy, although there is density corresponding to the positions occupied by the C-5, O-5, C-1, O-1, and O-1-methyl atoms of the sugar bound in the ordered copy of the second binding site. At the same contour level in the electron density map of the unliganded structure, there is diffuse density only near positions corresponding to the O-1 and O-1-methyl atoms.

**DISCUSSION**

**Monosaccharide Specificity of MBP-C**—The limited number of interactions between MBPs and bound sugars contrasts with those observed in other carbohydrate-binding proteins. Bacterial toxins, viral and plant lectins, carbohydrate-binding antibodies, bacterial periplasmic binding proteins, and enzymes with carbohydrate substrates have more extensive hydrogen bonding and van der Waals’ contacts between the bound sugar and protein (43, 44). Of these classes of proteins, the bacterial toxins and other lectins have weak (mM) affinities for monosaccharides comparable with the C-type lectins, despite a larger number of hydrogen bonds and van der Waals’ contacts. These observations suggest that sugar-Ca2+ coordination bonds provide much of the binding energy for C-type lectins. The direct coordination of Ca2+ by sugar hydroxyl groups seen in MBPs is commonly found in crystals of sugars formed in the presence of Ca2+ (40) but has not been observed in other protein-sugar complexes.

MBPs require a broad monosaccharide specificity in order to recognize a variety of cell surfaces. The structures described here, as well as the previous structure of an MBP-A-oligosaccharide complex (12), demonstrate that MBPs meet this requirement by having a very open binding site that is specific only for a minimal subset of functional groups on the ligand. The site selects ligands containing vicinal equatorial hydroxyl groups with the same stereochemistry as that of the 3- and 4-OH of Man, resulting in the formation of two coordination bonds with Ca2+, four hydrogen bonds with Ca2+ ligands, and a single apolar van der Waals’ contact. The absence of interactions between other portions of the sugar and protein permits binding to a variety of sugars, including Man, Fuc, and GlcNAc.

**Orientation of Mannose at the Binding Site**—The most surprising aspect of monosaccharide recognition by MBPs A and C is the different orientation of Man when bound to the two proteins, despite the very similar structure of their binding sites (Fig. 7). The most likely determinants of mannose orientation are His189 and Ile207 of MBP-A and the corresponding residues Val194 and Val212 of MBP-C, which are the only residues that both differ in the two proteins and make direct interactions with bound Man. In MBP-A, C-4 of Man contacts C6 of His189, equivalent to C-3 of Man contacting C5 of Val194 in MBP-C. Also, the 2-OH of Man forms a van der Waals’ contact with an imidazole ring carbon of His189 (12), an interaction with no equivalent in MBP-C (Fig. 8). Similarly, Ile207 forms a van der Waals’ contact with the exocyclic carbon of Man in MBP-A (12), while Val212 of MBP-C does not contact the sugar. A reasonable rationalization of the observed orientation is that in MBP-C Val194 provides a relatively apolar environment for the less polar face of each pyranose, whereas the orientation seen in MBP-A is due to favorable contacts formed between the imidazole ring of His189 and the 2-OH of Man and between the exocyclic C-6 of Man and C5 of Ile207. Although binding and mutagenesis data indicate that Man binds to MBP-A with approximately the same affinity when these contacts are removed (17), it is possible that very small differences in binding energy would favor the observed orientation. Structures of each protein with the appropriate substitutions will be required to understand the origin of the preference for a particular orientation of the bound sugar.

The presence of alternative binding orientations for Man in two highly related proteins highlights the pitfalls of homology modeling. In particular, the orientation of Fuc proposed for
MBP-A based on the Man$_\text{p}$ complex structure (12) has formed the basis for modeling of fucosylated ligand binding to the selectins (11, 14–16, 19). The structures presented here show that slight changes in protein structure can effect significant changes in the orientation of the bound sugar.

The different orientations of Man bound to MBPs A and C may have implications for detailed specificity differences between these two proteins. A previous study has shown that MBP-C binds a different set of oligosaccharides than MBP-A, even though the two proteins bind a similar spectrum of monosaccharides (9) (Table V). In particular, binding to neoglycolipid blots suggests that MBP-C preferentially recognizes the internal Man$_3$ core structure common to N-linked oligosaccharides (residues 3, 4, and 4' in Scheme I), while MBP-A prefers terminal Man, Fuc, and GlcNAc residues.

\[
\begin{align*}
\text{Man}_{\ell-1} & \rightarrow \text{Man} - \text{Me-Man} \\
\text{GlcNAc} - \text{GlcNAc} & \rightarrow \text{GlcNAc} - \text{GlcNAc} \\
\text{Asn} & \rightarrow \text{Asn}
\end{align*}
\]

**Scheme I.**

The results in the present study indicate that direct binding of MBP-C to the Man$_3$ core would require interaction with Man residues 4 and/or 4', since the 3-OH group of Man residue 3 is substituted in a glycosidic linkage. Similarly, further substitution of Man residue 4' at the 3-OH, as occurs in the case of high-mannose oligosaccharides, would preclude binding of MBP-C to this residue, although interactions with 2-substituted Man residues further out on this branch would still be possible.

If the orientation of the sugar at Ca$^{2+}$ site 2 is reversed between MBPs A and C, the disposition of other sugars on an oligosaccharide chain with respect to the protein would differ greatly and give rise to different interactions. When a Man$_6$ oligosaccharide binds to MBP-A, the penultimate sugar of each branch interacts with the protein through ordered water molecules, forming at most one direct contact with the protein (a poorly ordered lysine residue) (12). Thus an extended site is formed using water molecules, and in this case there is no site on the protein specific for a second sugar residue. The energetics of these water-mediated sites are unclear, however, since this oligosaccharide binds to MBP-A with the same affinity as Man (8). Nonetheless, it is clear that certain oligosaccharide conformations can be sterically excluded from binding, if the orientation of the sugar at the primary binding site is reversed between MBPs A and C.

**Specificity Differences between MBPs A and C—Aside from subtle differences in monosaccharide specificity, MBPs A and C have strikingly different specificities for oligosaccharides and neoglycoproteins (9). As discussed above, the orientation of the sugar bound at Ca$^{2+}$ site 2 may contribute to these differences. In addition, an oligosaccharide may bind to either an extended site or to a second site distinct from Ca$^{2+}$ site 2. While MBP-C-monosaccharide structures do not address the nature of an extended binding site, a second binding site distinct from Ca$^{2+}$ site 2 has been detected in one protomer of MBP-C in the presence of 1.3 M α-Me-Man (Figs. 5 and 10). Arguments for a second site in C-type CRDs have been made based on binding data for RHL-1, a galactose-specific C-type lectin (45), and experiments on MBP-C. Binding at the crystallographically observed second site is not detectable when any of the sugars studied here are present at 0.2 M, which suggests that it has very low affinity for monosaccharides. It is possible, however, that the second site described here is part of an extended site that possesses significant affinity only for ligands larger than a monosaccharide. Precedent for such a site is found in lectin IV of Griffonia simplicifolia, which has no significant affinity for monosaccharides but is specific for the Lewis b tetrasaccharide (46), and the galectins, which bind galactose poorly but interact strongly with lactose and lactosamine (47). It is also notable that α-Me-Man is bound at the second site in an orientation that would allow an oligosaccharide to extend from Ca$^{2+}$-site 2 to the second site. The shortest path over the surface of the protein is about 25 Å, a distance that could be bridged by six to seven sugar residues.

In addition to the second site observed here, it is possible that there is yet another monosaccharide binding site in MBP-C, which is blocked by a lattice contact in our crystal form. This would be surprising, however, because sub-MBP-C crystals remain intact, retain the same unit cell dimensions, and diffract to 1.8 Å after being soaked in 1.3 M α-Me-Man for several hours. If a second monosaccharide-binding site were blocked by a lattice contact, the crystal might be expected to crack or at least change cell dimensions when soaked in a high concentration of Man. As noted above, however, a second binding site specific for a ligand larger than a single monosaccharide may not be detected by soaking monosaccharides into an intact crystal. Ultimately, the determination of the structure of a complex between MBP-C and an oligosaccharide will be needed to see whether oligosaccharide binding requires an extended site or multiple sugar binding sites.

**Relationship of the MBP-C Dimer to the Native MBP-C Oligomer—Removal of the neck domain of MBP-A by subtilisin digestion exposes hydrophobic residues of the CRD that form the interface with the neck. Dimerization results from burial of these hydrophobic residues (32). The structure of the sugar binding site is identical in both dimeric and trimeric forms of MBP-A (12, 32), indicating that the mode of monosaccharide binding is independent of the oligomeric structure. In contrast, high avidity, multivalent oligosaccharide binding to intact MBPs A and C depends critically on the oligomerization state. For example, the carbohydrate-binding sites at Ca$^{2+}$ site 2 are separated by 53 Å in the native MBP-A trimer, a distance too large to be bridged by the terminal Man residues on a single N-linked oligosaccharide (32). As a result, MBP-A has only modest affinity for a single N-linked oligosaccharide, but has high affinity for larger arrays of sugars, such as yeast mannan (8).

The relationship of protomers in the MBP-A dimer differs significantly from the relationship of protomers in the trimer, but the same residues of the CRD form the interfaces of both the dimer and trimer (32). In this regard, it is noteworthy that the central contact in the MBP-C dimer interface, which occurs between Leu$^{136}$ and a pocket formed by the other protomer (Fig. 4), is very similar to the central contact of the called-coil neck-CRD interface in the MBP-A trimer. In that case, Phe$^{101}$ from the neck domain is buried in a pocket formed by Thr$^{114}$, Asn$^{115}$, Leu$^{127}$, Leu$^{131}$, and Cys$^{217}$ of the CRD of a different protomer (32). The residues that form this pocket are equivalent to those that form the pocket in the MBP-C dimer interface (Met$^{118}$, Leu$^{132}$, Leu$^{136}$, and Cys$^{222}$), and Phe is present at position 106 of MBP-C, corresponding to Phe$^{101}$ of MBP-A.

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3 M. S. Quesenberry, R. T. Lee, and Y. C. Lee, personal communication.
Therefore, it is likely that Phe106 forms an integral part of the neck-CRD interface of MBP-C, and that the neck-CRD interaction seen in the MBP-A trimer is similar in MBP-C. On the other hand, the fact that the MBP-A and MBP-C dimer structures are so different suggests that sequence differences in the neck-CRD interface may also produce significant differences in the relative disposition of the neck and CRD between the MBP-A and MBP-C trimers. This would in turn create a different spacing of carbohydrate-binding sites in the two proteins and give rise to different avidities that depend on the spacing of monosaccharides in multivalent ligands. Resolution of this important issue awaits determination of a trimeric MBP-C structure.

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