Glycoconjugate Journal (1997) **14:** 357–363

Difference in binding-site architecture of the serum-type and liver-type mannose-binding proteins

Reiko T. Lee and Yuan C. Lee*

Department of Biology, Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218, USA

The carbohydrate-recognition domains (CRDs) of the serum-type and the liver-type mannose-binding proteins (MBPs) from rat display different binding characteristics toward mannose-rich oligosaccharides derived from *N*-glycosides, despite the overall similarity in their binding site architecture, oligomeric status and actual binding specificity at the monosaccharide level. We found that the liver-type MBP CRD of rat (MBP-C) bound methyl glycosides of certain mannobioses and -trioses, which are part of the mannose-rich *N*-glycoside, more tightly than methyl α-mannopyranoside. In contrast, the serum-type MBP CRD of rat (MBP-A) bound all the methyl glycosides of manno-oligosaccharide and methyl α-mannopyranoside with similar affinities. The mannobiose and -triose most strongly bound to MBP-C CRD were Manα(1-2)Manα-OMe and Manα(1-2)Manα(1-6)Manα-OMe, respectively. From these and other data, we postulate that the binding site of MBP-C has an extended area of interaction, probably the size of a mannotriose, while MBP-A interacts essentially with one mannose residue.

***Keywords*: Mannose-binding proteins, binding specificity**

***Abbreviations*: MBP, mannose-binding protein; CRD, carbohydrate-recognition domain; BSA, bovine serum albumin; TFA-ah, 6-(trifluoroacetyl)aminohexyl; PNP, *p*-nitrophenyl**

Introduction

Two types of mannose-binding protein (MBP), a serum type and a liver type, circulate in the sera of many higher animals. These MBPs belong to the C-type lectins [1], which possess a unique polypeptide domain called the carbohydrate-recognition domain (CRD) that is responsible for the binding of sugars in a calcium dependent manner (C-type). In both MBPs, the CRD is located at the C-terminal end and this domain is connected to a collagenous domain by a short polypeptide. There is a short segment of a cysteine-rich polypeptide at the N-terminal end. There are other proteins with such a polypeptide construct, e.g., pulmonary surfactant proteins A and D, and these are collectively called collectins [2]. Although monomeric units of the two MBPs are quite homologous, they differ in the oligomeric organization; the serum-type MBP exists in large aggregates of 18–20 monomers, while the liver-type MBP is composed of six monomers [3].

The serum-type MBP, the more abundant of the two, is a pre-immune defense molecule, and is identical to one of the bactericidal factors known as Ra factors that are conserved in vertebrates throughout evolution [4]. It is an acute-phase protein, having a heat-shock promotor sequence and two glucocorticoid-responsive promotors on the 5′ flanking side of its gene [5]. In rats and mice, the two forms of MBP are produced by separate genes, and only the serum-type MBP, known as MBP-A, qualifies as an acute-phase protein [6]. At the moment, the biological function of the liver-type MBP, or MBP-C, is unknown.

Although known as mannose-binding proteins, both types of MBP recognize many other sugars such as L-Fuc, GlcNAc, and Glc [7, 8]. The X-ray crystallographic structures of both MBPs show a shallow binding area for sugars, with a main anchoring force being generated by an extensive network of hydrogen bonds and coordination bonds interconnecting 3-OH and 4-OH groups of mannose with a calcium ion and amino acid residues in the binding site. This architecture of the binding area agrees well with the observed broad specificity of MBPs, and suggests that any sugar with equatorial, vicinal hydroxyl groups in the (+)syn-clinal configuration would probably bind to MBP as well as Man [9].

Despite such similarity in the binding area architecture and actual monosaccharide-binding characteristics, the two

*To whom all correspondence should be addressed. Tel: 1-410-516-7041; Fax: 1-410-516-8716; E-mail: Bio_zycl@huvms.hcf.jhu.edu.

0282–0080

MBPs appear to bind branched oligosaccharide structures differently. Simply put, MBP-C (liver type) binds oligosaccharide structures belonging to the mannose-rich type and some Gal- or GlcNAc-terminated complex biantennary type more strongly than MBP-A (serum-type) [10]. These studies were carried out using cloned fragments of MBPs that contain a small piece of connecting region in addition to CRD, but are devoid of the collagenous domain. Despite the lack of the collagenous domain, both fragments were shown to exist as trimers [11]. Therefore, it is most likely that the difference in the binding emanates from CRD itself, and is not dependent on the oligomeric state.

In the present study, we also used such MBP-CRD fragments, and found that they bind component di- and trisaccharide structures of Man-rich oligosaccharide differently. Our results suggest that MBP-C has an extended binding area, perhaps capable of accommodating up to a linear trisaccharide, while MBP-A recognizes only a single sugar residue.

Materials and methods

Materials

Bovine serum albumin (BSA) was from Bayer (Pentex). Methyl α- and β-D-mannopyranosides were from Sigma Chemical Co. (St. Louis, MO). Preparations of the following compounds have been previously reported: α-mannopyranoside of allyl [12] and 6-(trifluoroacetamido)hexyl (TFA-ah) [13]; *p*-nitrophenyl (PNP) α- and β-D-mannopyranosides [14] and methyl 4,6-*O*-benzylidene-α-D-mannopyranoside [15]. The α(1,2)-linked and α(1,6)-linked mannotetraoses and a mixture of mannohexaose isomers are kind gifts from Dr C. E. Ballou (University of California at Berkeley), and M_5-Asn is from Dr M.-C. Shao (Johns Hopkins University). M_9-Asn was prepared as described [16] from soy bean agglutinin. The rat MBP-CRD fragments of serum-type (A) and the liver type (C) were prepared as described [17]. A BSA-based neoglycoprotein containing 28 (average) thiomannosides per molecule was prepared as described [18]. Carrier-free $Na^{125}I$ was obtained from Amersham Life Science Corp. (Arlington Heights, IL). Radioactivity was counted using a Packard MINAXI gamma-counter.

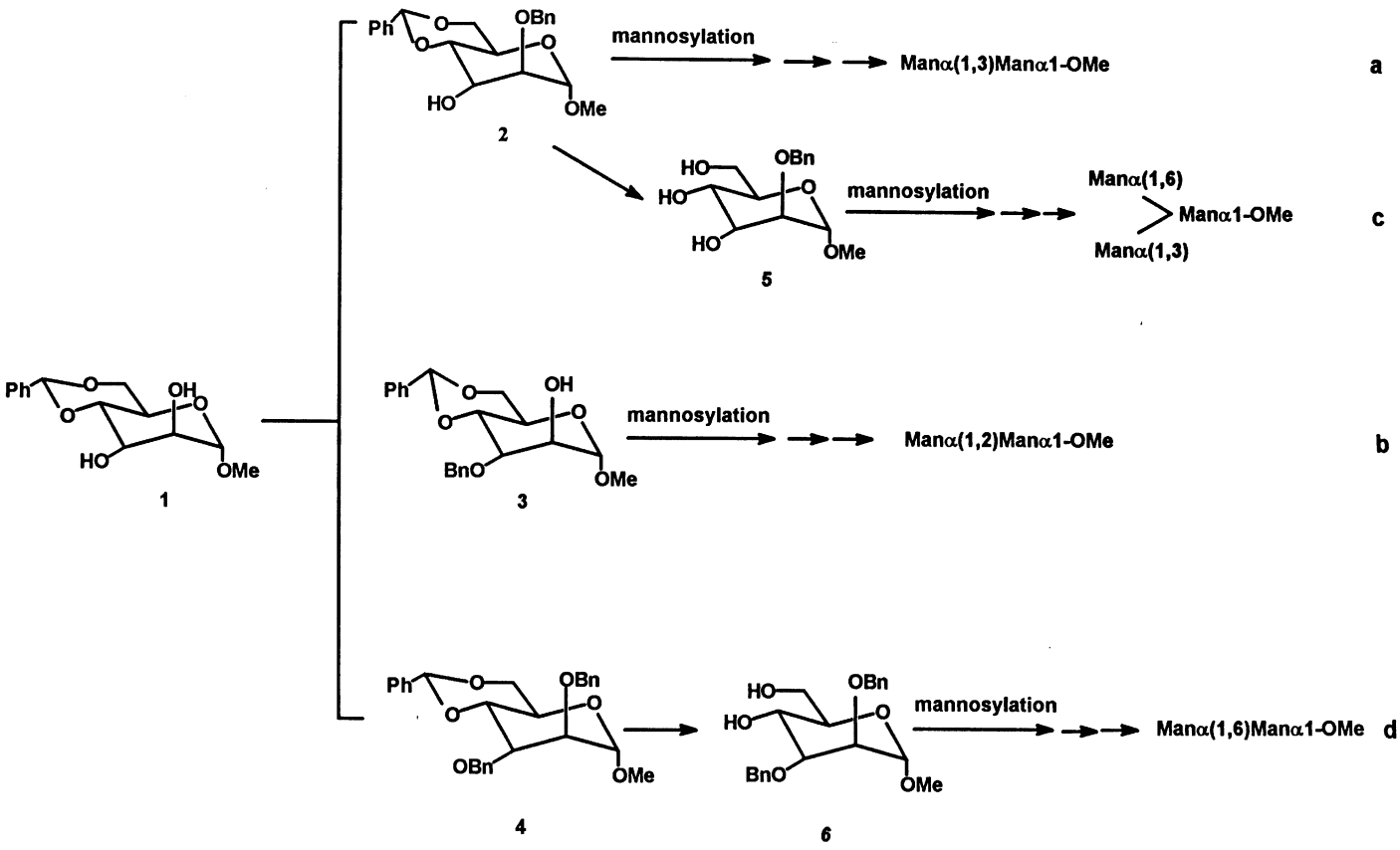
Methods

^{1}H-NMR spectra were obtained using a Bruker AMX 300 and Bruker AM 600 spectrometers. The binding activity of MBP was assayed using ammonium sulfate precipitation method as described [16]. Briefly, MBP-CRD (~40 nM) and ^{125}I-Man_{28}-BSA (~0.1 nM) in 0.3 ml of tris buffer (pH 7.8) containing 1 M NaCl and 25 mM $CaCl_2$ were incubated in an ice-water bath for 20 min and then for 1 h after addition of 0.44 ml of cold 100% ammonium sulfate solution. The mixture was then filtered through a glass fibre filter and washed. The radioactivity collected on the filter paper was measured. In order to estimate the affinity of a potential inhibitor, it was included in the incubation mixture at levels that can cause 0–100% inhibition of binding of ^{125}I-Man_{28}-BSA, and the extent of decrease in the binding was measured. A plot of percent inhibition (%) versus inhibitor concentration (logarithmic scale) produced a sigmoidal curve and the concentration that causes 50% inhibition (I_{50}) was determined from it. Radioiodination of Man_{28}-BSA was carried out using chloramine T method [19]. Typically 10 μg of Man_{28}-BSA was iodinated with 0.5 mCi $Na^{125}I$ in 30 μl total volume for 40–60 s. The iodinated protein was purified by passing the reaction mixture through a column (5-ml bed volume) of Sephadex G-25. Usually specific radioactivity of 80–100 million cpm $nmol^{-1}$ was obtained. The radioactive protein solution was mixed with 100 μg of BSA and stored in the cold.

For a select few inhibitors, K_d values were estimated using a non-linear regression program, LIGAND [20]. For this purpose, the binding isotherm curves for the interaction between Man_{28}-BSA and the two MBP CRDs were generated by carrying out the binding assays at Man_{28}-BSA concentrations in the range of 10 nM–10 μM. The curves for MBP-A and -C almost completely overlapped and produced a K_a value of $6.8 \times 10^6\ M^{-1}$ for both MBP-A and -C by the LIGAND fitting. The inhibition assays were carried out at ten different concentrations of each inhibitor that would give 0–100% inhibition, and the bound counts (without subtracting the non-specific binding) were directly fitted with the LIGAND program with K_a of Man_{28}-BSA set at 6.8×10^6. Fittings were done using one-site and two-site models.

Preparation of manno-oligosaccharide methyl glycosides

Preparation of methyl glycosides of mannobioses and mannotrioses has been reported by several groups [21–25]. Our scheme (Scheme 1), which is not identical to any of the reported procedures, utilizes a single key intermediate, methyl 4,6-*O*-benzylidene-α-D-mannopyranoside, and a controlled benzylation to obtain all the glycosyl acceptors needed for syntheses. Since the endproducts have been reported, we will limit our description to overall reaction scheme and component reactions in general terms rather than describing preparation of each end product in detail. The reactions used routinely in the scheme are as follows: (1) De-*O*-acetylation is accomplished by treating a methanolic solution of compounds with 10 mM sodium methoxide in dry methanol for 2 h at room temperature; (2) De-*O*-benzylidenation is by heating the solution of compound in 80% acetic acid at 80° for 1.5 h; (3) Alternatively, de-*O*-benzylidenation is accomplished together with de-*O*-benzylation by hydrogenolysis at atmospheric pressure in either 95% ethanol or 80% acetic acid using 10% palladium on carbon as catalyst; (4) Mannosylation was carried out in dry toluene-nitromethane mixture (1 : 1) using 2,3,4,6-tetra-*O*-acetyl-l-bromo-mannopyranose [26] and mercuric cyanide,



Scheme 1. Utilizes a single-key intermediate, methyl 4-6-*O*-benzylidene-*a*-D-mannopyranoside, and a controlled benzylation to obtain all the glycosyl acceptors needed for syntheses.

both in 1.6-fold molar excess over the glycosyl acceptors (**2**, **3**, and **6**). For glycosyl acceptor **5**, the glycosylating reagents were used in 3.2-fold molar excess. Glycosylation products were size-fractionated on a column (5 × 190 cm) of Sephadex LH-20 using 95% ethanol as eluant. The column separated mono-, di-, and tri-saccharide completely. Fractions containing trisaccharides and disaccharides were combined separately, and each oligosaccharide fraction was further purified by silica gel chromatography, if needed.

The first step of the reaction scheme (Scheme 1) is a partial benzylation of methyl 4,6-*O*-benzylidene-α-D-mannopyranoside (**1**) in dry *N*,*N*-dimethylformamide using equimolar amounts of NaH and benzyl bromide. Three products, methyl 2-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**2**), methyl 3-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**3**), and methyl 2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-mannopyranoside (**4**), and the remaining starting material (**1**) were separated by silica gel chromatography using toluene–ethyl acetate (2:1) as solvent. De-*O*-benzylidenation of **2** and **4** yielded methyl 2-*O*-benzyl-α-D-mannopyranoside (**5**) (silica gel purification with ethyl acetate-acetone, 4:1) and methyl 2,3-di-*O*-benzyl-α-D-mannopyranoside (**6**) (silica gel purification with toluene–ethyl acetate, 1:2), respectively. Mannosylation of **2**, **3**, **5**, and **6** (reaction sequences a, b, c, and d, respectively in Scheme 1) followed by purification, de-*O*-acetylation and hydrogenolysis produced methyl 3-*O*-(α-D-mannopyranosyl)α-D-mannopyranoside [Manα(1-3)Manα1-OMe], methyl 2-*O*-(α-D-mannopyranosyl)α-D-mannopyranoside [Manα(1-2)Manα1-OMe], methyl 3,6-di-*O*-(α-D-mannopyranosyl)α-D-mannopyranoside (tri-Man core methyl glycoside), and methyl 6-*O*-(α-D-mannopyranosyl)α-D-mannopyranoside [Manα(1-6)Manα1-OMe], respectively. Completely deprotected products were purified on a column (2.5 × 140 cm) of Sephadex G-15 in 0.1 M acetic acid, if necessary.

In addition to the expected products, both the reaction sequences c and d produced a trisaccharide byproduct (separated by Sephadex LH-20 column), which upon deprotection yielded Manα(1-2)Manα(1-6)Manα1-OMe. Formation of such byproduct having Manα(1-2)Manα-linkage in lieu of simple Manα- linkage occurring in certain mannosylation reactions has been reported [27]. Two other linear trisaccharide glycosides, Manα(1-2)Manα(1-2)Manα1-OMe and Manα(1-2)Manα(1-3)Manα1-OMe, were prepared by the pentenyl coupling method [28], and will be described elsewhere.

The ^{1}H-NMR spectra of di- and tri-saccharide methyl glycosides agreed well with reported spectra or chemical shift values [29]. In addition, linkage positions were

confirmed by NMR spectra of per-*O*-acetylated products and decoupling experiments of deprotected products.

Results

The I_{50} values of various inhibitors, which are inversely related to affinity, are presented in Table 1. Three key inhibitors were chosen to determine their K_a values by non-linear regression as described in Materials and methods. The two-site fitting produced two K_a values which were both similar to the value obtained with the one-site fitting with little improvement in the fitting. For the MBP-A binding of the di- and tri-saccharides (Table 2), the two-site model failed to fit the data. For this reason, only the results of the one-site fitting are presented in Table 2. Examination of the tables leads to the following observations and conclusions:

(1) The I_{50} values of all the compounds are quite similar for MBP-A, generally falling within a twofold range of one another. In contrast, the I_{50} values of the same set of inhibitors for MBP-C are more varied, difference between the best and the worst inhibitor being $\sim$17-fold.

(2) For MBP-C, glycosides of mannose with a large and hydrophobic aglycon of α-orientation (e.g., benzyl and TFA-ah) were much better inhibitors than the ones with small α-aglycon (e.g., methyl and allyl). In contrast, the nature of aglycon did not influence the affinity for MBP-A. These results suggest that the binding site of MBP-A recognizes primarily one mannose residue, while that of MBP-C has an extended area of interaction for aglycon, which appears to be hydrophobic in nature. Two examples of β-glycoside (methyl and PNP) suggest that a β-aglycon does not interact with the putative extended binding site of MBP-C.

(3) When the C-1 substituent is another mannose, which is considerably more hydrophilic than benzyl or TFA-ah, the I_{50} values varied considerably for MBP-C depending on the linkage. The least effective inhibitor of the three disaccharide sequences was Manα(1-3)Man, which had an I_{50} $\sim$3.5-fold higher than that of the best disaccharide inhibitor, Manα(1-2)Manα1-OMe. The I_{50} of the latter is comparable to those of hydrophobic glycosides. As for trisaccharides, Manα(1-2)Manα(1-6)Manα1-OMe with an I_{50} of 0.25 mM was the best linear, synthetic inhibitor for MBP-C. The fact that its I_{50} is lower than that of disaccharide or hydrophobic glycosides suggests that the area of interaction of the MBP-C binding site may be at least as large as a trisaccharide.

(4) For the I_{50} values to approximate the K_d values, the inhibition experiments should be performed using concentrations of both the protein and the reference ligand below 1/10 K_d. In the present study, in order to obtain a good signal to background ratio, the MBP concentration of 40 nM was used, which is only slightly lower than the K_d of the MBP-Man_{28}-BSA interaction. Despite this limitation, the I_{50} values for MBP-A were quite similar to K_d values, as a comparison of data in Tables 1 and 2 shows. For instance, K_d of the three compounds are in the 0.5–0.6 mM range, while the corresponding I_{50} values are in the 0.8–1 mM range. However, the I_{50} values for MBP-C were considerably higher than the corresponding K_d values. For example, K_d of Me α-Man is 0.6 mM, which is the same as MBP-A, while the corresponding I_{50} is 4.44 mM. However, the ratios of I_{50} values for MBP-C nonetheless appear to reflect the relative potency of the inhibitors reasonably well. For instance, K_a values indicate that affinity of Manα(1-2)Manα1-OMe and Manα(1-2)Manα(1-6)Manα1-OMe is three- and ninefold higher than Me α-Man, while the corresponding affinity increments calculated from I_{50} values are five- and 18-fold. The K_a values indicate that Manα(1-2)Manα1-OMe and Manα(1-2)Manα(1-6)Manα1-OMe bind three- and eightfold tighter to MBP-C than to MBP-A, respectively. The overall free energy of interaction (ΔG) and the free energy contribution by each Man residue ($\Delta\Delta G$) calculated from the K_a values are also presented in Table 2.

(5) Of the two tri-branched Man-rich glycopeptides tested, M_9-Asn is the best inhibitor of all the compounds listed in Table 1, whereas M_5-Asn, a smaller Man-rich glycopeptide, is appreciably inferior by comparison. The structure of M_9-Asn and M_5-Asn (Table 1, footnote b) both contain three terminal Man residues, but only M_9-Asn contains unsubstituted, linear trisaccharide segments, including the highest affinity-triaccharide sequence of Manα(1-2)Manα(1-6)Man.

Discussion

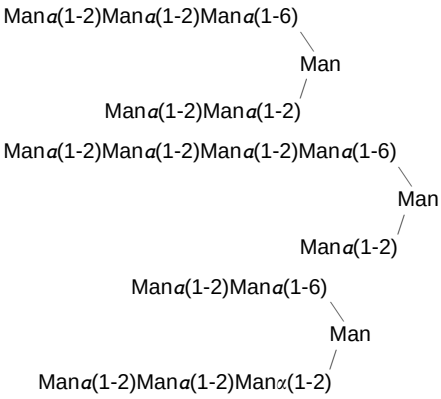
Despite the similarity in their monomer polypeptide construct, state of oligomerization and binding characteristics at the monosaccharide level, MBP-A and MBP-C CRDs show dissimilar binding specificity toward some oligosaccharides derived from *N*-glycosides [10]. Although quantitative data are not available in the study referenced above, the results seem to show that MBP-C CRD binds Man-rich oligosaccharides and the trimannosyl core structure, while MBP-A CRD does not under a similar set of conditions. In trying to decipher the basis for this difference in the binding specificity, we discovered recently, using quantitative inhibition assays, that the two CRDs behave differently toward a series of neoglycopeptides having two terminal mannose residues, and concluded that MBP-C CRD contains two mannose binding sites per monomer, while MBP-A CRD has only one* [30]. The putative second site of MBP-C is of a considerably weaker affinity, and is postulated to bind mannose but not GlcNAc, in contrast to the primary site which binds both Man and GlcNAc. A recent report on

*Quesenberry, MS, Lee, RT and Lee, YC, manuscript in preparation.

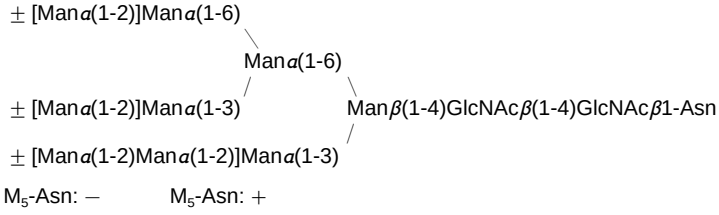
Table 1. Inhibition potency of Man-containing compounds.

	I_{50} (mM)	
	MBP-A CRD	*MBP-C CRD*
Glycosides of Man		
Methyl α	0.94	4.44
Methyl β	1.9	7.69
Allyl α	1.23	4.19
Benzyl α	0.62	0.59
TFA-ah α	1.18	0.49
PNP α	1.09	0.43
PNP β	0.4[c]	1.3[c]
Disaccharide glycosides		
Manα(1-2)Manα1-OMe	0.94	0.86
Manα(1-3)Manα1-OMe	1.95	2.96
Manα(1-6)Manα1-OMe	0.99	1.80
Trisaccharide glycosides		
Manα(1-6)-[Manα(1-3)]Manα1-OMe	0.94	0.99
Manα(1-2)Manα(1-2)Manα1-OMe	0.59	0.99
Manα(1-2)Manα(1-3)Manα1-OMe	1.21	1.36
Manα(1-2)Manα(1-6)Manα1-OMe	0.86	0.25
Man oligosaccharides		
$[Manα(1-2)Manα(1-2)]_2$	0.86	1.08
$[Manα(1-6)Manα(1-6)]_2$	0.94	2.34
Manno-hexaose mixture[a]	0.74	0.86
High-Man glycopeptides		
M_5-Asn[b]	0.85	0.73
M_9-Asn[b]	0.5[c]	0.1

[a] This mixture is likely to be composed of:



[b] Structure of Man_5-Asn and Man_9-Asn is shown below:



M_5-Asn: − M_5-Asn: +

[c] Due to limited solubility or availability, these numbers are estimates from partial inhibition curves.

Table 2. Association constants of three inhibitors.

	MBP-A				*MBP-C*			
	K_a $\times 10^3$ M^{-1}	K_d mM	ΔG kcal mol^{-1}	$\Delta\Delta G$ kcal mol^{-1}	K_a $\times 10^3$ M^{-1}	K_d mM	ΔG kcal mol^{-1}	$\Delta\Delta G$ kcal mol^{-1}
Mal*α*1-OMe	1.7	0.59	4.10		1.68	0.60	4.09	
Man*α*(1-2)Man*α*1-OMe	1.7	0.59	4.10	0	5.0	0.2	4.69	0.6
Man*α*(1-2)Man*α*(1-6)Man*α*1-OMe	2.1	0.48	4.21	0.11	16.0	0.06	5.33	1.24

x-ray crystallographic structure of MBP-C CRD [31] alsoidentified a second methyl α-Man binding site of weaker affinity. This site, unlike the primary site, engages the axial 2-OH of mannose in the binding process, suggesting that GlcNAc which has an equatorial 2-NHAc may not bind. This weaker binding site, which is ~ 25 Å away from the primary site, may well be the secondary site identified in our work with divalent ligands.

In the present study, we discovered yet another difference in the ligand-binding characteristics between MBP-A and -C. We confirmed as proposed earlier [8] that the serum-type MBP (MBP-A) interacts only with a monosaccharide, since all the mannose oligosaccharides of both linear and branched structures as well as various glycosides of Man exhibited inhibition potency similar to Me α-Man. In contrast, the same set of inhibitors exhibited more varied inhibition potencies toward MBP-C, the smallest ligand, Me α-Man, being the weakest inhibitor. Of the three disaccharide glycosides, Manα(1-2)Manα1-OMe had the highest affinity (I_{50}, approximately fivefold lower than that of Me α-Man), while its positional isomer, Manα(1-3)Manα1-OMe, hardly showed any affinity increase over Me α-Man. The preferred conformations of the disaccharides determined by molecular mechanics [32] show that two Man rings tend to be in a stacked conformation in the case of the α(1-2)-linked disaccharide, whereas the two rings extend out with little overlap in the α(1-3)-linked Man disaccharide. Manα(1-6)Manα1-OMe, conformationally the most flexible isomer of the three, can easily assume both overlapping and extended conformations, granted these conformations are not identical to those of α(1-2)- and α(1-3)-linked disaccharides. The fact that this most flexible, α(1-6)-linked isomer was less inhibitory than the α(1-2)-linked disaccharide suggests that the relationship of sugar rings presented by Manα(1-2)Manα1-OMe is most complementary to the binding site. We postulate, therefore, that the secondary interacting area of MBP-C is located below the ring of the terminal Man residue to match the contour of the Manα(1-2)Manα1-OMe structure. The affinity increase by Manα(1-2)Manα1-OMe is quite similar to or slightly less than that afforded by a large hydrophobic aglycon (e.g., benzyl), suggesting that nature of the interaction in this area may be mainly hydrophobic. The fact that β-glycosides bind to MBP-C much less strongly than the corresponding α-glycosides also suggests that the secondary interacting region of MBP-C is located below the plane of the sugar ring.

The only trisaccharide structure with substantially higher affinity than Manα(1-2)Manα1-OMe is Manα(1-2)Manα(1-6)Manα1-OMe. All other trisaccharides and oligomannosides of six or fewer Man residues had inhibition potency similar to or weaker than Manα(1-2)Manα1-OMe. This suggests the existence of a third sub-site, albeit of relatively weak interaction, which can best accommodate the trisaccharide sequence of Manα(1-2)Manα(1-6)Man.

In order to assess the binding energy at each of the postulated subsites, the association constant of three key inhibitors was determined by a non-linear regression program, LIGAND (Table 2). Although the X-ray crystallographic study [31] and the inhibition study using some divalent ligands* [30] suggested the presence of a second Man-binding site of considerably weaker affinity than the primary site, fitting of the binding data of the three ligands did not suggest the presence of a site with a much weaker affinity in either of the MBPs, perhaps due to insufficient data points at higher concentration range. Therefore, the parameters obtained from the fitted data in the present study are assumed to be for the primary site. Thus, the ΔG value at subsite 1 of the primary site of MBP-C is 4.09, and the addition of successive carbohydrate moieties at subsites 2 and 3 increases the free energy by 0.6 and 0.64 kcal mol^{-1}. Although the extra energy provided by subsites 2 and 3 is quite small individually, their additive effect resulted in the K_a of the trisaccharide binding by MBP-C becoming one order of magnitude larger than that of MBP-A. Since M_9-Asn, the best inhibitor in this study for MBP-C, contains the high-affinity, linear trisaccharide segment, Manα(1-2)Manα(1-6)Manα1-OMe, whereas M_5-Asn does not (see Table 1, footnote b), it may be reasonable to postulate that the major portion of the M_9-Asn interaction with MBP-C resides in this trisaccharide sequence. This interaction is perhaps further augmented by the binding of another

*Quesenberry, MS, Lee, RT and Lee, YC, manuscript in preparation.

terminal mannose residue on the 3-branch of the core structure at the weaker second binding site about 25 Å away from the primary site [31] to produce the affinity of Man_9-Asn which is stronger than that of the trisaccharide segment.

The K_d values of the three compounds for MBP-C (Table 2) are considerably smaller than the corresponding I_{50} values, meaning that the present inhibition assay from MBP-C considerably underestimates the affinity. Since this trend would likely hold true for all the compounds, MBP-C in fact would have, somewhat higher affinity for most of the Man oligosaccharides in Table 1 than MBP-A. The higher affinity generated by MBP-C probably results from the interaction with its extended binding area. The degree of affinity increase is much dependent on the oligosaccharide structure. For example, M_9-Asn exhibited the largest affinity enhancement of 44-fold over Me α-Man, while this factor for the trimannosyl core structure was only 4.5-fold. Various naturally occurring Man-rich oligosaccharide structures and some GlcNAc-substituted core structures would be expected to show a gradient of affinity toward MBP-C depending on their fine oligosaccharide structure.

Acknowledgements

This work was supported by NIH Research Grant DK 09970. We thank Drs L. X. Wang and C. Abeygunawardana and Ms L. Neeley for obtaining NMR spectra and helping us in assignment of NMR signals.

References

1 Drickamer K (1988) *J Biol Chem* **263**: 9557–60
2 Sastry K, Ezekowitz RA (1993) *Curr Opin Immun* **5**: 59–66
3 Oka S, Ikeda K, Kawasaki T, Yamashina I (1988) *Arch Biochem Biophys* **260**: 257–66.
4 Matsushita M, Takahashi A, Hatsuse H, Kawakami M, Fujita T (1992) *Biochem Biophys Res Commun* **183**: 645–51.
5 Summerfield JA (1993) *Biochem Soc Trans* **21**: 473–78.
6 Sastry K, Zahedi K, Lelias J-M, Whitehead AS, Ezekowitz AB (1991) *J Immunol* **147**: 692–97.
7 Kawasaki N, Kawasaki T, Yamashina I (1989) *J. Biochem* **106**: 483–89.
8 Lee RT, Ichikawa Y, Fay M, Drickamer K, Shao MC, Lee YC (1991) *J Biol Chem* **266**: 4810–15.
9 Weis WI, Drickamer K, Hendrickson WA (1992) *Nature* **360**: 127–34.
10 Childs RA, Feizi T, Yuen C-T, Drickamer K, Quesenberry MS (1990) *J Biol Chem* **265**: 20770–77.
11 Weis WI, Crichlow GV, Murthy HMK, Hendrickson WA, Drickamer K (1991) *J Biol Chem* **266**: 20678–86.
12 Lee RT, Lee YC (1974) *Carbohydr Res* **37**: 193–201.
13 Weigel PH, Naoi M, Roseman S, Lee YC (1979) *Carbohydr Res* **70**: 83–91.
14 Schneider J, Lee YC (1973) *Carbohydr Res* **30**: 405–8.
15 Buchanan JG, Schwarz JCP (1962) *J Chem Soc*: 4770–77.
16 Lee RT, Ichikawa Y, Kawasaki T, Drickamer K, Lee YC (1992) *Arch Biochem Biophys* **299**: 129–136.
17 Drickamer K (1989) *Biochem Soc Trans* **17**: 13–16.
18 Lee YC, Stowell CP, Krantz MJ (1976) *Biochemistry* **15**: 3956–63.
19 Greenwood FC, Hunter NM, Glover JS (1963) *Biochem J* **89**: 114–23.
20 Munson PJ, Rodbard D (1980) *Anal Biochem* **107**: 220–39.
21 Reichert CM (1979) *Carbohydr Res* **77**: 141–47.
22 Ogawa T, Sasajima K (1981) *Carbohydr Res* **93**: 53–66.
23 Winnik FM, Brisson J-R, Carver JP, Krepinsky JJ (1982) *Carbohydr Res* **103**: 15–28.
24 Ogawa T, Nukada T (1985) *Carbohydr Res* **136**: 135–52.
25 Khan SH, Piskorz CF, Matta KL (1994) *J Carbohydr Chem* **13**: 1025–35.
26 Kartha KPR, Jennings HJ (1990) *J Carbohydr Chem* **9**: 777–81.
27 Lee RT, Lee YC (1995) *Carbohydr Res* **271**: 131–36.
28 Fraser-Reid B, Udodong UE, Wu Z, Ottosson H, Merritt JR, Rao CS, Roberts C, Madsen R (1992) *Synlett*: 927–42.
29 Ogawa T, Sasajima K (1981) *Carbohydr Res* **97**: 205–27.
30 Quesenberry MS, Lee RT, Lee YC (1994) *Glycobiology* **4**: 723.
31 Ng KK-S, Drickamer K, Weis WI (1996) *J Biol Chem* **271**: 663–74.
32 Imberty A, Gerber S, Tran V, Perez S (1990) *Glycoconjug J* **7**: 27–54.

Received 29 July 1996, revised 2 October 1996, accepted 7 October 1996