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The differences in structural specificity for recognition and binding between asialoglycoprotein receptors of liver and macrophages

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The Gal/GalNAc-specific lectin on the surface of rat peritoneal macrophages (macrophage asialoglycoprotein binding protein, M-ASGP-BP), which consists of a single polypeptide chain of 42 kDa, can form a homooligomeric receptor exhibiting high affinity for asialoorosomucoid (ASOR) [Ozaki K., Ii M., Itoh N., Kawasaki T. (1992) *J Biol Chem* **267**: 9229–35]. In this study, the binding affinity of M-ASGP-BP was studied by using a series of synthetic or natural glycosides as inhibitors of ^{125}I-ASOR binding to recombinant M-ASGP-BP expressed on COS-1 cells (rM-ASGP-BP), and the results were compared with those of human hepatic lectin (HHL) on Hep G2 cells. Clustering of multiple Gal (or GalNAc) residues increased the binding affinity to M-ASGP-BP as well as to HHL. In contrast to HHL and other mammalian hepatic lectins, rM-ASGP-BP bound Gal residues tighter than GalNAc residues. A galactose-terminated triantennary N-glycoside, having one *N*-acetyl-lactosamine unit on the 6 branch and two *N*-acetyl-lactosamine units on the 3 branch of the trimannosyl core structure, showed affinity enhancement of ~10^5 over a monovalent ligand for HHL, while the same glycopeptide showed enhancement of about 2000-fold for rM-ASGP-BP. These results suggest that spatial arrangements of sugar combining sites and subunit organization of macrophage and hepatic lectins are different.

Keywords: asialoglycoprotein receptor, rat liver, rat macrophages, neoglycoproteins, synthetic clustered glycosides

Introduction

The asialoglycoprotein receptor or asialoglycoprotein-binding protein (ASGP-R or ASGP-BP) on hepatocytes, which is responsible for the rapid clearance of asialo (Gal/GalNAc-terminated) glycoproteins from circulation into hepatocytes, has been investigated extensively as a model for many of the events of carbohydrate mediated endocytosis [1–3]. Rat hepatic ASGP-BP (rat hepatic lectin; RHL), which consists of three polypeptides, 42 (RHL-1), 49 (RHL-2), and 54 kDa (RHL-3) [4], requires triantennary Gal-terminated N-linked oligosaccharide chains for its high affinity binding [5]. Photoaffinity labelling experiments by Rice *et al.* [6] showed that a photolyzable group on one particular terminal Gal residue in a triantennary glycopeptide ligand binds solely to the minor subunits (RHL-2/3) and the other two to the major subunit (RHL-1). These results indicated that ASGP-BP on hepatocytes binds a triantennary glycopeptide of a single defined geometry and suggest that a subtle difference in the relative spatial arrangement of carbohydrate recognition domains (CRDs) can produce significant alterations in the binding specificity.

We have shown that peritoneal macrophages contain Gal/GalNAc specific lectin (M-ASGP-BP), the properties of which are very similar to those of hepatic ASGP-BP [7–10] except that M-ASGP-BP is functionally active as a homooligomer of a single subunit of 42 kDa [11]. Since M-ASGP-BP contains an extra insert between the transmembrane portion and CRD, which can make CRD more flexible, the specificity for recognition and binding of M-ASGP-BP might be somewhat different from that of hepatic ASGP-BP. RHL is abundantly present on the hepatocyte surface (~10^5 per cell) [12], whereas much fewer M-ASGP-BP molecules (~1000) are present on the macrophage surface [7]. However, when the recombinant protein (rM-ASGP-BP) was expressed on COS-1 cells, the lectin signal was significantly amplified, and the binding protein appeared to be distributed correctly on the plasma membrane [11]. For this reason, we used the transfected COS-1 cells in the present study. In addition, the potential ambiguity due to the presence of a second lectin on the

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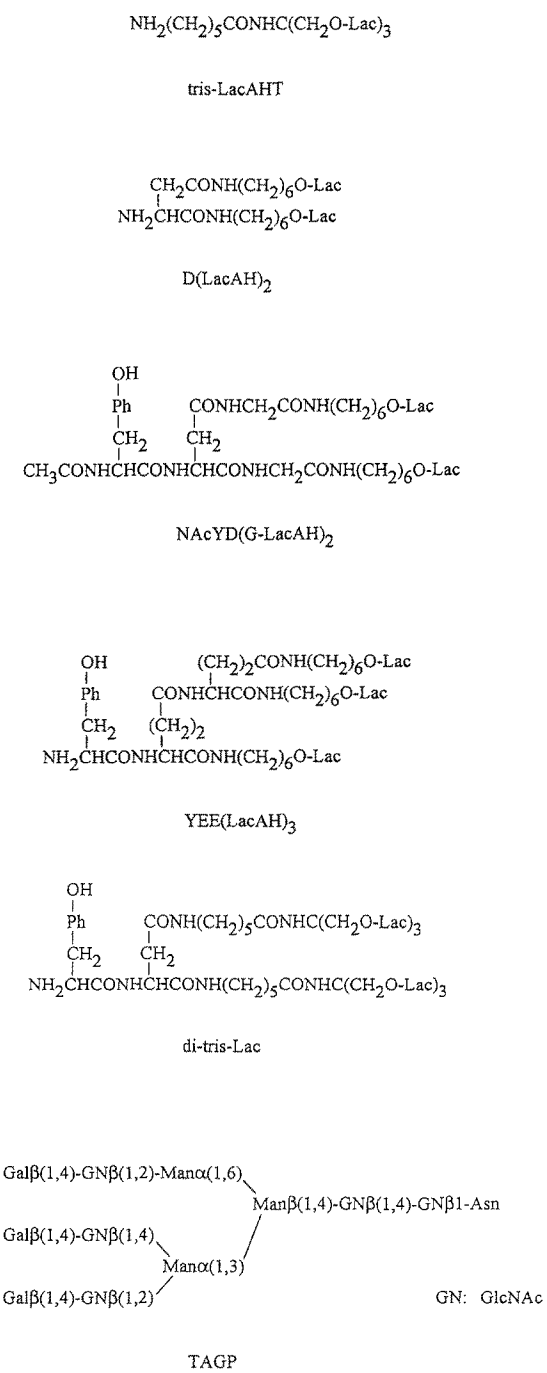
macrophage surface, i.e. Man/L-Fuc-lectin [9] with a rather broad specificity, is circumvented by the use of the transfected COS-1 cells. A human hepatoma cell line, Hep G2 [13], which carries human hepatic lectin (HHL) on its surface, is used in parallel experiments.

In this paper the binding specificities of the macrophage and hepatic lectins were studied by inhibition assay of ^{125}I-ASOR binding to rM-ASGP-BP on COS-1 cells and to HHL on Hep G2 cells using a series of neoglycoproteins, Gal-BSAs and GalNAc-BSAs, and synthetic cluster glycosides as inhibitors. The observed differences in the binding specificity appear to reflect the innate difference of the sugar-combining sites as well as the difference in organization of the combining sites.

Experimental procedures

Materials

ASOR was prepared as previously described [14] from orosomucoid provided by Dr M. Wickerhauser, the American Red Cross Research Center. Sodium [^{125}I] iodide (carrier-free) was obtained from Amersham Corp. Gal$_n$-AI-BSA and GalNAc$_n$-AI-BSA, which carry on the average n residues of sugar per molecule of bovine serum albumin (BSA) in Sugar-S-$CH_2C(=NH)NH$- linkage to the amino group of BSA, were prepared as described [15]. Syntheses of 6-(trifluoroacetamido) hexyl β-D galactopyranoside (Gal-AH-TFA) and 6-(trifluoroacetamido)hexyl 2-acetamido-2-deoxy-β-D-galactopyranoside (GalNAc-AH-TFA) have been reported [16, 17]. The following cluster glycosides: D(LacAH)$_2$, D(G-LacAH)$_2$, NAcYD(GG-LacAH)$_2$, NAcYD(GGG-LacAH)$_2$, NAcYD(G-GalNAcAH)$_2$, YEE(LacAH)$_3$, YEE(GalNAcAH)$_3$, tris-LacAHT, di-tris-Lac were synthesized as described [18–20]. In these shorthand structure designations, D, E, G, and Y are one-letter amino acid abbreviations for Asp, Glu, Gly and Tyr, all in L-configuration. The first five divalent glycosides were constructed by attaching an ω-amino-containing glycoside to each of the carboxyl group of Asp. The ω-amino glycosides used were 6-aminohexyl β-glycoside of lactose (LacAH) and GalNAc (GalNAcAH), and their elongation derivatives which were prepared by attaching up to three residues of glycine. Similarly, trivalent ligands were prepared by attaching LacAH or GalNAcAH to three carboxyl groups of tyrosyl-γ-glutamylglutamic acid (YEE). Structures of representative cluster glycosides are shown in Scheme 1. TAGP, a particular triantennary glycopeptide having three terminal Gal residues as shown in Scheme 1 was prepared from bovine fetuin [21].



Scheme 1.

Cell culture and DNA transfection

Hep G2 cells (American Type Culture Collection) and COS-1 cells (Japanese Cancer Research Resources Bank) were maintained in 35 mm wells containing Dulbecco's modified Eagle's medium (Nissui Pharmaceutical Co., Ltd) supplemented with 10% fetal calf serum (GIBCO) at 37°C under a 5% CO_2 atmosphere. The expression vector containing M-ASGP-BP cDNA, pdKCR-M-ASGP-BP, purified by two cycles of CsCl gradient centrifugation was transfected by means of the calcium-phosphate precipitation method into COS-1 cells as described previously [11].

Inhibition assays of ^{125}I-ASOR binding

ASOR was iodinated by the chloramine-T method described by Greenwood *et al.* [22]. Transfected COS-1 cells at 60 h post transfection and Hep G2 cells in a confluent phase (1×10^5) in

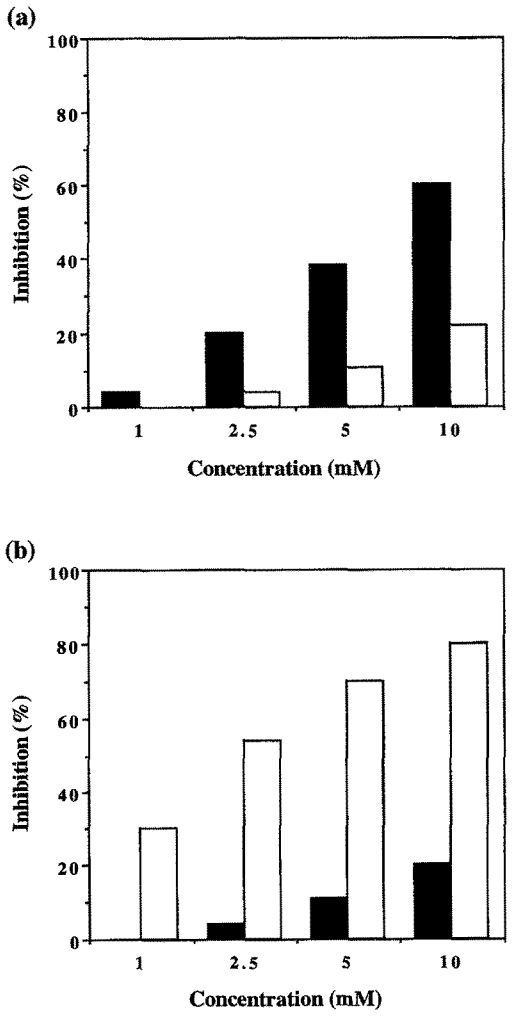


Figure 1. Inhibition of ^{125}I-ASOR binding to rM-ASGP-BP and HHL by monosaccharide glycosides. Inhibition of ^{125}I-ASOR binding to (a) rM-ASGP-BP on COS-1 cells and (b) HHL on Hep G2 cells by monosaccharide glycosides, Gal-AH-TFA (closed bar) and GalNAc-AH-TFA (open bar), was determined as described in Experimental procedures.

35 mm wells were used for inhibition assay. After preincubation for 10 min at 4°C, the cells were incubated with 10nM of ^{125}I-ASOR for 1 h at 4°C in the presence of various inhibitors at the different concentrations given in the Figures. Medium was removed by aspiration, and the wells were rinsed three times with 1 ml of phosphate buffered saline (PBS). To each well, 1 ml of 0.1 M NaOH was added to lyse the cells, and then the radioactivity bound to the cells was determined with a Beckman γ-5000 counter. Nonspecific binding was determined in the presence of a 100-fold molar excess of unlabelled ASOR or 5 mM EDTA. Control binding was measured under the same conditions in the absence of added inhibitors. The concentration of the inhibitor causing 50% inhibition of ^{125}I-ASOR binding to the lectin was referred to as I_{50}.

Results

Use of monosaccharides with aglycon as inhibitors

Earlier we reported that GalNAc is a slightly more potent inhibitor than Gal for the uptake of ^{125}I-ASOR by M-ASGP-BP: the I_{50} values of the uptake at 37°C were 10 and 17 mM for GalNAc and Gal, respectively [11]. In this study we compared the relative affinities of Gal and GalNAc in more detail by using simple glycosides, Gal-AH-TFA and GalNAc-AH-TFA, as well as cluster ligands and neoglycoproteins (see below). As shown in Fig. 1, ^{125}I-ASOR binding to rM-ASGP-BP on transfected COS-1 cells was inhibited by 50% in the presence of 8 mM Gal-AH-TFA, while 10 mM GalNAc-AH-TFA showed only 20% inhibition. In contrast, ^{125}I-ASOR binding to HHL was inhibited by 50% in the presence of 2 mM GalNAc-AH-TFA, while 10 mM Gal-AH-TFA showed less than 20% inhibition. These results suggest that macrophage ASGP-BP prefers Gal over GalNAc, while HHL prefers GalNAc over Gal. The previous study with reducing sugars as inhibitors [11] may not have given an accurate picture, since reducing sugars can exist in many different forms.

Use of neoglycoproteins as inhibitors

In order to study the effects of sugar density on the binding affinity, inhibition assays were performed in the presence of neoglycoproteins, Gal_n-BSA and $GalNAc_n$-BSA, at different concentrations. In Fig. 2, the percentage of inhibition caused by an inhibitor was plotted against inhibitor concentration on a logarithmic scale. The inhibition curves of Gal_{31}-BSA and $GalNAc_{35}$-BSA for ^{125}I-ASOR binding to rM-ASGP-BP on COS-1 cells and to HHL on Hep G2 cells are shown in Fig. 2(a) and (b), respectively. Other neoglycoproteins, Gal_8-, Gal_{10}-, Gal_{23}-, Gal_{41}-BSAs and $GalNAc_8$-, $GalNAc_{16}$-, $GalNAc_{26}$-BSAs, also exhibited similar curves (data not shown). In Fig. 3, the I_{50} values estimated from such curves were plotted against the density of sugar residues on BSA. The I_{50} values of Gal_8-BSA and Gal_{10}-BSA for the binding to rM-ASGP-BP were 3 and 0.7 μM, respectively, while those of Gal_{23}- and Gal_{30}-BSAs were ~0.05 μM. Thus, about two to three fold increase in Gal density produced more than 50-fold increase in the binding affinity. A similar effect was also produced by GalNAc-BSAs. Being consistent with the aforementioned results, M-ASGP-BP preferred Gal to GalNAc and HHL preferred GalNAc to Gal regardless of the sugar density.

Use of synthetic cluster glycosides as inhibitors

In the previous studies, so-called short-hand cluster glycosides which contain only terminal monosaccharide or disaccharide units and no internal sugars (see Scheme 1) were found to produce strong enhancement of binding affinity for the mammalian and avian hepatic lectins [19, 20, 23]. Such small ligands were used in the inhibition assays for rM-ASGP-BP on COS-1 cells and HHL on Hep G2 cells.

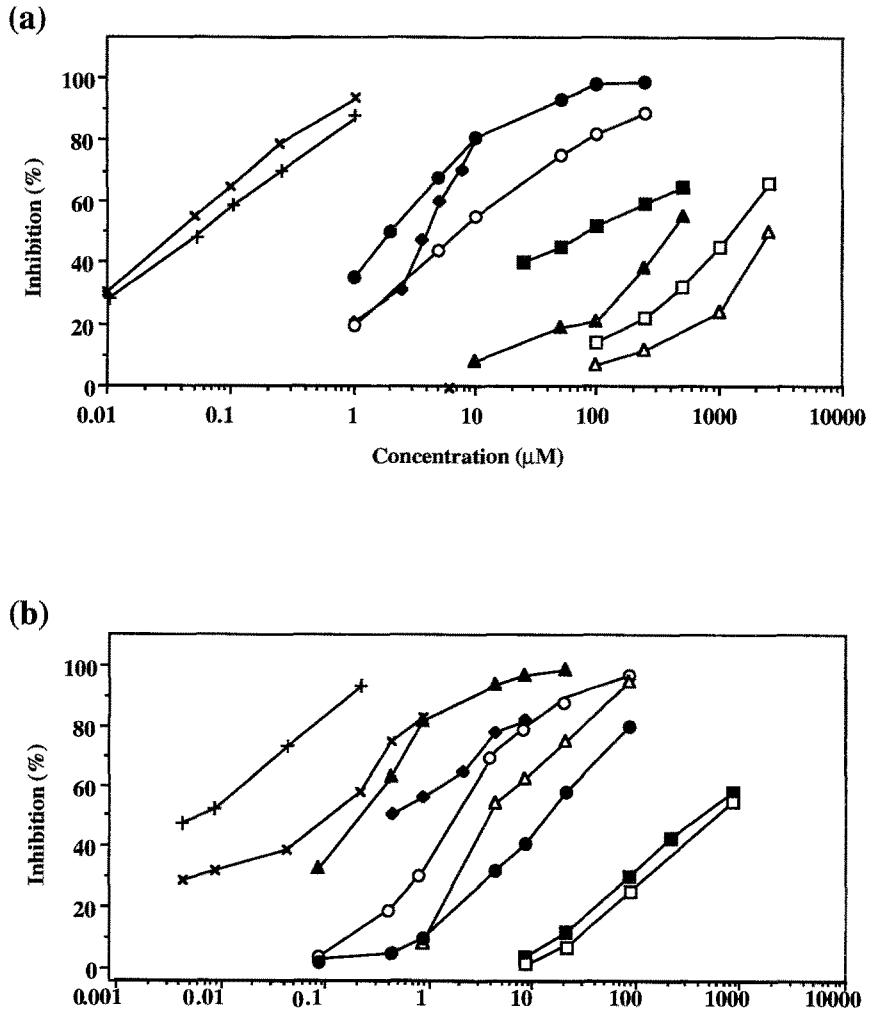


Figure 2. Inhibition of ^{125}I-ASOR binding to (a) rM-ASGP-BP on COS-1 cells (b) HHL on Hep G2 cells by neoglycoproteins and cluster glycosides was determined as described in 'Experimental procedures'. Gal_{31}-BSA (x), $GalNAc_{35}$-BSA (+), di-tris-Lac (○), YEE(LacAH)$_3$ (●), YEE(GalNAcAH)$_3$ (▲), TAGP (◆), tris-LacAHT (□), D(LacAH) (■), NAcYD(G-GalNAc)$_2$ (△).□

Small synthetic multivalent ligands used in this study can be divided into two types. The first type is glycosylation products of *tris*-hydroxymethylaminomethane [18] and thus has sugar residues in close proximity to each other. An example of this type is tris-LacAHT (Scheme 1), in which inter-Gal distances are ~1.7 nm. The second type is structurally based on aspartic acid or γ-glutamyl glutamic acid and has longer and more flexible arms between sugars and the branching point. Most of the ligands listed in Table 1 belong to this type (Scheme 1). For divalent ligands of this type, the length of the arm was varied by linking one to three glycyl residues in each arm so that the maximum inter-Gal distance ranged from 2.8 nm to 5.0 nm (Table 1). Di-tris-Lac, a hexavalent glycoside, contains elements of both types.

Inhibition curves for these cluster ligands are shown in Fig. 2(a) and (b). The I_{50} values obtained from these curves are presented in Table 1. Table 1 also includes the data on RHL [20] for comparison, and affinity enhancement factor for each Gal-terminated cluster ligand, which was calculated on the basis of the affinity of Gal-AH-TFA set as 1. For this purpose, I_{50} values of Gal-AH-TFA were estimated from Fig. 1 to be ~ 8mM for rM-ASGP-BP and ~ 50 mM for HHL, although the accuracy of the latter value is questionable. It is quite evident that for all three lectins, multivalent ligands showed a strong 'cluster effect', which is defined as affinity enhancement over and beyond what would be expected from the concentration increase of the determinant sugar in a multivalent ligand. A sole exception may be tris-LacAHT binding to rM-ASGP-BP on COS-1 cells.

As shown in Fig. 2(a) and Table 1, for rM-ASGP-BP on COS-1 cells a trivalent Gal-terminated ligand, YEE(LacAH)$_3$, was the best small ligand being bound about 50-fold tighter than D(LacAH)$_2$, a divalent Gal-containing glycoside. Interestingly, D(LacAH)$_2$ was a stronger inhibitor than the trivalent tris-LacAHT, suggesting the importance of geometrical positioning of Gal residues (see Discussion). Fig. 2(b)

Table 1. Inhibition constant (I_{50}) and enhancement factor of cluster glycosides.

Compound	*Maximum inter-sugar distances* (nm)	*rM-ASGP-BP* (COS-1)		*HHL* (Hep G2)		*RHL* (*Hepatocytes*)	
		I_{50} (μM)	*Enhancement factor*	I_{50} (μM)	*Enhancement factor*	I_{50} (μM)	*Enhancement factor*
Monovalent ligand							
Gal-AH-TFA		8000	1	5.0×10^4	1	600	1
Divalent ligand							
$D(LacAH)_2$	2.8	90	89	400	125	5.5	109
$D(G\text{-}LacAH)_2$	3.5	22	360	50	1000	7	86
$NAcYD(G\text{-}G\text{-}LacAH)_2$	4.2	160	50	150	330	14.5	41
$NAcYD(G\text{-}G\text{-}G\text{-}LacAH)_2$	5.0	110	73	100	500	11	55
$NAcYD(G\text{-}GalNAcAH)_2$	3.3	2500		4		0.003	
Trivalent ligand							
tris-LacAHT	1.7	1200	7	500	100	4	150
$YEE(LacAH)_3$	3.3, 4.1, 4.3	2	4000	15	3300	0.05	1.2×10^4
TAGP		4	2000	0.5	1×10^5	0.007	8.6×10^4
$YEE(GalNAcAH)_3$	2.7, 3.3, 3.4	400		0.25		0.0002	
Hexavalent ligand							
di-tris-Lac		7	1140	2	2.5×10^4	0.012	5.0×10^4

I_{50} refers to the concentration of an inhibitor to cause 50% inhibition of ^{125}I-ASOR binding. Enhancement factor was estimated by dividing the I_{50} values for the monovalent reference ligand (Gal-AH-TFA) by those for multivalent Gal-containing compounds. Data on the rat hepatocytes were from [20].

shows inhibition curves of synthetic glycosides to HHL on Hep G2 cells. A trivalent GalNAc-containing ligand, $YEE(GalNAcAH)_3$, was the best small ligand which bound approximately 20-fold tigher than $NAcYD(G\text{-}GalNAcAH)_2$, a divalent GalNAc-containing glycoside. Here again, preference of GalNAc over Gal by hepatic lectin and the reverse preference of Gal over GalNAc by the macrophage lectin was evident. For example, the trivalent GalNAc-containing glycoside [$YEE(GalNAcAH)_3$] was a 60-fold better inhibitor than the trivalent Gal-containing ligand [$YEE(LacAH)_3$] for HHL. In fact, $YEE(GalNAcAH)_3$ was an even better inhibitor than di-tris-Lac, which contained six terminal Gal residues. In contrast, $YEE(LacAH)_3$ was a much better inhibitor (200-fold) than $YEE(GalNAcAH)_3$ for M-ASGP-BP.

It is interesting to note that TAGP was bound more tightly to HHL than to rM-ASGP-BP, despite the fact that the affinity of most Gal-terminated ligands to rM-ASGP-BP was equal to or slightly better than to HHL (see Discussion for detail).

Discussion

In this paper, we attempted to characterize the binding mode of the Gal/GalNAc-specific binding protein from rat peritoneal macrophage (M-ASGP-BP). The polypeptide structure of this lectin shares a strong homology with the major subunit (RHL-1) of rat hepatic lectin (RHL), which is also known as asialoglycoprotein receptor (ASGP-R). The main structural difference between RHL-1 and M-ASGP-BP is that the neck region of the latter is considerably longer (24 amino acid residues) [10]. Although M-ASGP-BP shares many properties with ASGP-BP on hepatocytes consisting of two subunits, it can form a homooligomeric receptor.

For RHL and other mammalian hepatic lectins, only the terminal Gal (GalNAc) residues were likely to be involved in the binding, and GalNAc residues had a higher affinity than Gal residues (2). Binding of monosaccharide residues by these lectins is rather weak, the dissociation constants being in the range of 0.1–1 mM. The binding force that is relevant to their biological function (K_d~nM), i.e. binding of a ligand at cell surface followed by endocytosis of the ligand-receptor complex, is generated in the hepatic lectins by clustering of subunits which allows binding of multiple determinant sugar residues. For this reason, in the present study we have concentrated our effort on multivalent ligands, both of neoglycoprotein type and small, synthetic ligands that carry up to six terminal Gal or GalNAc residues.

The relative affinities of various ligands were estimated by an inhibition assay performed with plated COS-1 cells expressing M-ASGP-BP. Hep G2 cells. which carry human hepatic lectin (HHL) on their surface, are used in parallel experiments. As seen in Table 1, I_{50} values for RHL are considerably lower than the corresponding values for HHL. It appears that the I_{50} values obtained with the confluent plated cells are significantly higher than the values obtained with single cells in a well-suspended state, which is the method used for the rat hepatocyte experiments [20]. Because of this, all conclusions are made from the relative inhibitory potencies within a particular cell type, rather than on the basis of the I_{50} values.

As mentioned in Results, rM-ASGP-BP clearly prefers Gal over GalNAc. This tendency is exactly opposite in HHL, which

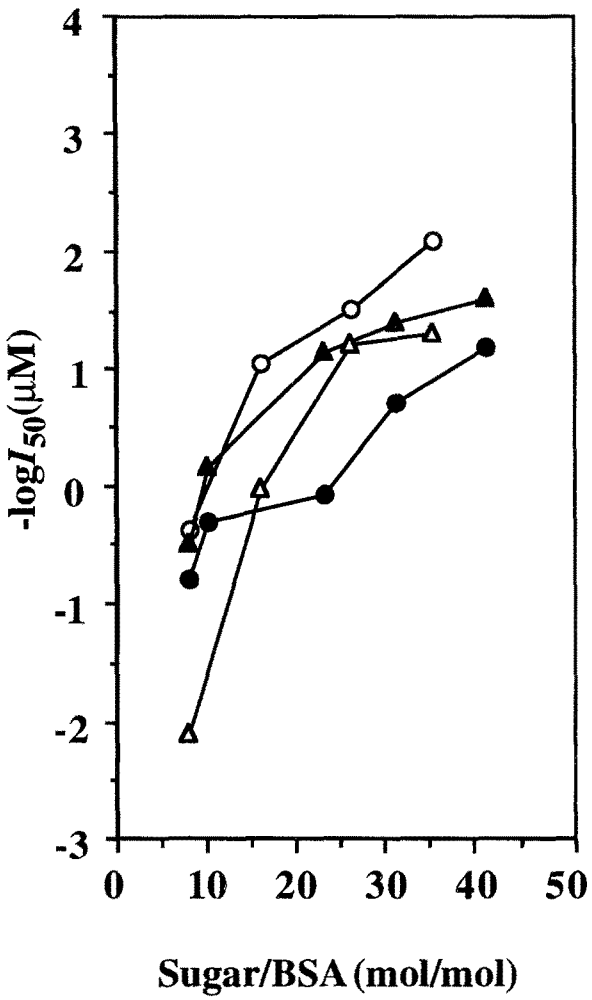


Figure 3. Inhibitory potency (I_{50}) of various Gal- or GalNAc-containing neoglycoproteins. Inhibition of ^{125}I-ASOR binding to rM-ASGP-BP on COS-1 cells (triangle) and HHL on HepG2 cells (circle) by neoglycoproteins [Gal-BSA(solid symbol) and GalNAc-BSA (open symbol)] was determined as described in 'Experimental procedures'. Concentration of a neoglycoprotein that causes 50% inhibition of ^{125}I-ASOR binding (I_{50}) was plotted against the number of sugar residues per mole of BSA.

showed the preference for GalNAc over Gal as expected of a mammalian hepatic lectin. Actually the affinities of rM-ASGP-BP and HHL towards all the Gal-terminated ligands were quite comparable (Table 1). On the other hand, the presence of an equatorial acetamido group at C-2 position of the sugar ring (i.e. GalNAc) enhanced the affinity for HHL, but considerably lowered the affinity for rM-ASGP-BP. This suggests that the C-2 contact region of the sugar-combining area of M-ASGP-BP is different from that of mammalian hepatic lectins. It is interesting to note that while all mammalian hepatic lectins have enhanced binding affinity towards GalNAc over Gal, the degree of this enhancement varied considerably depending on the species. At the monosaccharide level, RHL exhibited a 43-fold enhancement while rabbit lectin showed only 2.5-fold enhancement [20]. Projection of I_{50} values from Fig. 1(b) suggests that HHL may be quite similar to RHL.

As to the multivalent inhibitors, rM-ASGP-BP on COS-1 cells, HHL on Hep G2 cells as well as RHL on hepatocytes all manifested a strong cluster effect when small, flexible multivalent ligands were used (Table 1). Interestingly, the degree of affinity enhancement was quite comparable among the three lectins. For instance, the enhancement factor for a divalent ligand, D(LacAH)$_2$, was ~100 fold for all three lectins, and the trivalent YEE(LacAH)$_3$ manifested a 3–12 thousand-fold increase. With respect to neoglycoproteins, Fig. 3 shows that the affinity for all three lectins initially increased very rapidly with the increase in the number of sugar residues on BSA, and much more gradually beyond a sugar density of 20 mol mol^{-1}.

These effects of multivalency on affinity for the transfected COS-1 cells and Hep G2 cells may be explained partly by using a well-studied RHL system as reference. Detergent-solubilized, purified hepatic lectins (RHL, rabbit HL, and chicken HL) all appear to exist as stable hexamers (22, 24–26). The solubilized rM-ASGP-BP also appears to exist as oligomers composed of 6~8 subunits [11]. Such oligomers, having as many as 12 or more sugar-binding sites [27], are entities capable of expressing nM-range I_{50} values for highly multivalent ligands. On the rat and rabbit hepatocyte surface, the hexamers are also the likely minimal functional entity, although further aggregation of hexamers seems probable [28]. Although the status of oligomerization of rM-ASGP-BP on COS-1 cells is unknown, the fact that the cluster effects exhibited by these cells were similar to RHL on the isolated hepatocytes both with small as well as macromolecular multivalent ligands suggests that the rM-ASGP-BP subunits exist as aggregates in a similar fashion as RHL on the hepatic surface. However, lack of the cluster effect by tris-LacAHT for rM-ASGP-BP also suggests that the binding sites of the macrophage lectin are not as close together as that of hepatic lectins. For this reason we tested ligands with longer spatial separation of Gal residues [e.g. NAcYD (GGG-LacAH)$_2$] to see if the affinity for rM-ASGP-BP would improve. Results indicated that the elongation had little effect (Table 1).

All mammalian hepatic lectins are composed of two different polypeptides derived from separate genes [25, 29]. It has been amply documented [30, 31] that both subunits of RHL and HHL must be present for a tight binding of natural asialoglycoprotein ligands. As shown in Table 1, TAGP is bound by rat hepatocytes with ~ nM dissociation constant.

Inhibition data of component structures of TAGP using rat hepatocytes suggested that the affinity increased ~1000-fold by the addition of each Gal branch. Such an observation allowed us to propose that the three Gal residues of TAGP in its preferred conformation are complimentary to the configuration of the three combining sites of RHL on the rat hepatocyte surface, so that Gal residues are bound in a concerted manner generating the total binding force which is close to the product of the binding force at each site [32]. Moreover, photoaffinity labelling experiments with TAGP derivatives showed that the

two types of RHL subunits are arranged in a sterically specific and rigid manner on the rat hepatocyte surface [6]. As shown in Table 1, the degree of affinity enhancement manifested by TAGP for HHL is very large, ~10^5-fold, while rM-ASGP-BP on COS-1 cells had enhancement of 2000-fold, which is even less than that produced by the synthetic trivalent ligand, YEE(LacAH)$_3$. It is likely, therefore, that recognition of the unique configuration of Gal residues on TAGP is operative on Hep G2 cells as on rat hepatocytes, but is absent in rM-ASGP-BP on COS-1 cells. While proteins bearing this triantennary oligosaccharide must be preferred ligands for binding and endocytosis by mammalian hepatocytes, a random clustering of sugar residues as on the BSA neoglycoproteins may serve just as well for rM-ASGP-BP on COS-1 cells and M-ASGP-BP on the peritoneal macrophage. However, one cannot exclude the possibility that there exists in nature a unique oligosaccharide structure whose Gal residues are recognized with a much higher affinity by M-ASGP-BP. It is interesting to note that CHL, another lectin composed of one type of subunit, may also lack a specific ligand of unique oligosaccharide structure [23]. In the case of M-ASGP-BP, the elongation of the neck region [9], in addition to the homooligomeric subunit organization may be responsible for it to bind sugar residues flexibly.

Although the function of M-ASGP-BP on thioglycolate-elicited peritoneal macrophages is not well understood, one can speculate that, as in the case of mannan-binding protein which is a type of general defence molecule [33, 34], it probably interacts equally well or even better with Gal residues that are more widely separated than 15 represented by the triantennary structure. The relationship between the M-ASGP-BP binding specificity and the biological function of macrophages is an interesting question which remains to be further elucidated.

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