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Binding specificity of D-mannose 6-phosphate receptor of rabbit alveolar macrophages*,†

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ABSTRACT

The existence of terminal D-mannosyl 6-phosphate groups (Man-6-*P*) was required (for an inhibitor) to exert a strong inhibitory potency against the binding of bovine serum albumin (BSA) conjugated with 17 molecules of penta-D-mannose 6-phosphate [$(M_5P)_{17}$-BSA] to the Man-6-*P* receptor in rabbit alveolar macrophages. In addition, the underlying oligosaccharide structures, such as linkage mode between the nonreducing sugar group and the penultimate sugar residue, and the length of sugar chain also affected the inhibitory potency in this system. In general, the oligosaccharides with an α-(1→2)-linked Man-6-*P* unit gave stronger inhibitory potencies than those with an α-(1→3)- or α-(1→6)-linked Man-6-*P* unit. Trisaccharides containing a terminal Man-6-*P* group were more potent inhibitors than disaccharides. A synthetic, branched, and divalent ligand, which does not have a penultimate sugar residue, gave about the same level of inhibitory potency as Man-6-*P* itself. The "cluster effect" was observed in this system, *i.e.*, as the number of Man-6-*P* units conjugated to BSA [$(Man\text{-}6\text{-}P)_{5.5,\ 8,\ and\ 46}$-BSA] increased, the stronger inhibitory potencies were observed with decreasing I_{50} values of 1.93, 1.36, and 0.0345 μM, respectively. Synthetic divalent oligosaccharides also showed higher inhibitory potencies than the corresponding monovalent oligosaccharides.

INTRODUCTION

Lysosomal enzymes are transported to lysosomes through the Golgi complex–endosome system or, alternatively, *via* cell-surface receptor after they are once secreted[1–3]. A D-mannose 6-phosphate (Man-6-*P*) unit on the high-mannose type oligosaccharide chains serves as the recognition marker in these processes. Two different kinds of receptors (cation-dependent and cation-independent) have been identified[4]. Both showed similar, but not identical, binding specificity toward phosphorylated oligosaccharides[5,6]. Recently, both types of receptors have been cloned[3], and the stoichiom-

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etry of ligand binding to the recognition domain of these receptors has been studied[7,8]. Moreover, the binding affinity of the Man-6-*P* receptor seems to increase when there are two Man-6-*P* units per oligosaccharide chain. The Man-6-*P* receptor appeared to recognize extended oligosaccharide structures of the phosphorylated oligosaccharides which were derived from lysosomal enzymes[9].

Macrophages have a large pool of lysosomal hydrolase activity, which is probably related to their unique physiological activities, such as inflammation, lysosomal-enzyme secretion, antigen processing, phagocytosis, and digestion of bacteria, as well as tumor cell lysis and tissue remodeling[10]. Therefore, these cells should provide an interesting system to study the Man-6-*P* receptor. Indeed, a Man-6-*P* receptor having similar features to those in fibroblast was identified by use of α-D-mannosidase of *Dictyostelium discoideum* as a ligand in rabbit alveolar macrophages[11].

Synthetic and semisynthetic ligands with well-defined structures having a variety of structural features with respect to branching pattern, chain length, position of phosphate groups, and linkage mode are now available to us. In the present paper, the binding affinity of these ligands to the cell-surface receptors of rabbit alveolar macrophages was studied by binding–inhibition experiments and the structure–function relationship of the binding was examined.

EXPERIMENTAL

Materials. — The high-molecular-weight phospho-D-mannan from *Hansenula hostii* (Y.2448) was a gift from Dr. Morey Slodki of the Northern Regional Research Center, USDA, Peoria, IL. Silicon oil (DC 550) was obtained from Accumetric (Elizabethtown, KY), mineral oil and D-mannose 6-phosphate from Sigma Chemical Co. (St. Louis, MO), and BSA (Bovuminar reagent, pure powder) from Armour Pharmaceutical Company (Kankakee, IL). $Na^{125}I$ (carrier-free) in 0.1M NaOH was obtained from Amersham Corp. (Arlington Hts., IL). All other reagents used were of analytical grade.

Ligand. — Penta-D-mannose 6-monophosphate (M_5P) was prepared from the high-molecular-weight phophomannan by the established method[12]. [α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→3)-α-D-Man*p*-(1→2)-D-Man]$_{17}$-BSA [$(M_5P)_{17}$-BSA] was prepared by the Gray's method[13]. (Man-6-*P*)$_n$-BSA (n = 5.5, 8, and 46) was prepared by a reductive amination method[14]. Other synthetic oligosaccharides and a glycopeptide containing Man-6-*P*, shown in Table III, were prepared by the methods of Srivastava and Hindsgaul[15–17], and Ichikawa and Lee[18], respectively. Sodium [6-(*N*-trifluoroacetylamino)hexyl α-D-mannopyranoside] 6-phosphate[19] (**22**) was prepared[20] from 6-(*N*-trifluoroacetylamino)hexyl α-D-mannopyranoside by phosphorylation with diphenyl chlorophosphate, followed by hydrogenolysis with PtO_2. Ligand concentration was measured by the phenol–H_2SO_4 method[21] with D-mannose as a standard sugar, and phosphate content of ligands by the method of Ames and Dubin[22].

Cells. — Alveolar macrophages were obtained from male rabbits (New Zealand White) by pulmonary lavage as described earlier[23]. The cells were routinely greater than

95% viable by the Trypan Blue exclusion, and by the measurement of intracellular and extracellular lactate dehydrogenase activities[24].

Iodination. — $(M_5P)_{17}$-BSA (10–20 μg) was iodinated with $Na^{125}I$ (37 MBq) by use of the Chloramine-T method[25].

Assay for [^{125}I-$(M_5P)_{17}$-BSA binding to alveolar macrophages. — Alveolar macrophages (1–5 × 10^6 cells/mL) and ^{125}I-$(M_5P)_{17}$-BSA (0.11–2.2nM) in the incubation medium (1 mL) containing 0.12M MaCl, 5mM KCl, 2mM $CaCl_2 \cdot 2H_2O$, 1mM $MgSO_4 \cdot 7H_2O$, 5mM D-glucose, 0.5% (w/v) BSA, 6 × 10^{-4}% (w/v) Phenol Red. 15mM sodium 1,4-piperazine-bis (ethanesulfonate), and 15mM *N,N*-bis (2-hydroxyethyl)-2-aminoethanesulfonic acid, adjusted to pH 6.7 with NaOH, were incubated for 3 h at 2° in capped polystyrene tubes (12 × 75 mm: Cat. no. 2058, Sarstedt, Princeton, NJ) which were rotated vertically (end over end) at 6 r.p.m. To test the Ca^{2+}-dependency of binding, a Ca^{2+}-free incubation medium with 10mM EDTA instead of 2mM $CaCl_2 \cdot 2H_2O$ was used. Assays were initiated by adding cells to an incubation mixture containing the ^{125}I-labeled ligand at 2°. To determine the amount of the cell-associated ^{125}I-$(M_5P)_{17}$-BSA, triplicate samples (100 μL) were removed from the incubation mixture, placed on 4:1 (v/v) silicon oil–mineral oil in a 0.4-mL polypropylene microcentrifuge tube (Cat. no. 710, Sarstedt, Princeton, NJ), and centrifuged for 1 min in an Eppendorf Model 5412 microcentrifuge. After centrifugation, the tubes were cut at the middle of the oil layer, and the tips containing the cell pellet were analyzed for radioactivity, which was counted with a Packard Minaxi counter. Nonspecific binding of ^{125}I-$(M_5P)_{17}$-BSA was determined by adding mM M_5P in the incubation mixture.

Inhibition assay. — Inhibitory potency of test ligands on the ^{125}I-$(M_5P)_{17}$-BSA binding to rabbit alveolar macrophages was assayed as follows. Cells (1 × 10^6 cells/mL) and ^{125}I-$(M_5P)_{17}$-BSA (0.18nM, 3 × 10^5 c.p.m./mL) were incubated in the presence of a test ligand at various concentrations for 3 h at 2° under the same conditions as described above. Assays were initiated by adding cells to incubation mixtures containing ^{125}I-$(M_5P)_{17}$-BSA and the test ligand at 2° in the incubation medium. The same methods as described above were used to determine the cell-associated ^{125}I-$(M_5P)_{17}$-BSA and the nonspecifically bound ^{125}I-$(M_5P)_{17}$-BSA. The concentration of a test ligand causing 50% inhibition of binding of ^{125}I-$(M_5P)_{17}$-BSA to the cell surface receptor (I_{50}) was obtained from the plot of percent inhibition against logarithm of the test-ligand concentration. Some results obtained from the assays were analyzed with a nonlinear-regression program LIGAND[26].

RESULTS AND DISCUSSION

Time-course and equilibrium binding of ^{125}I-$(M_5P)_{17}$-BSA to rabbit alveolar macrophages at 2°. — The time course of binding of ^{125}I-$(M_5P)_{17}$-BSA to macrophages at 2° was determined with ^{125}I-$(M_5P)_{17}$-BSA concentrations ranging from 41pM to 2.4nM and for incubation periods up to 170 min (Fig. 1). The data showed that a 2-h incubation was sufficient to establish equilibrium for ^{125}I-$(M_5P)_{17}$-BSA binding to macrophages.

The 3-h incubation time was used to obtain an isotherm of ^{125}I-$(M_5P)_{17}$-BSA

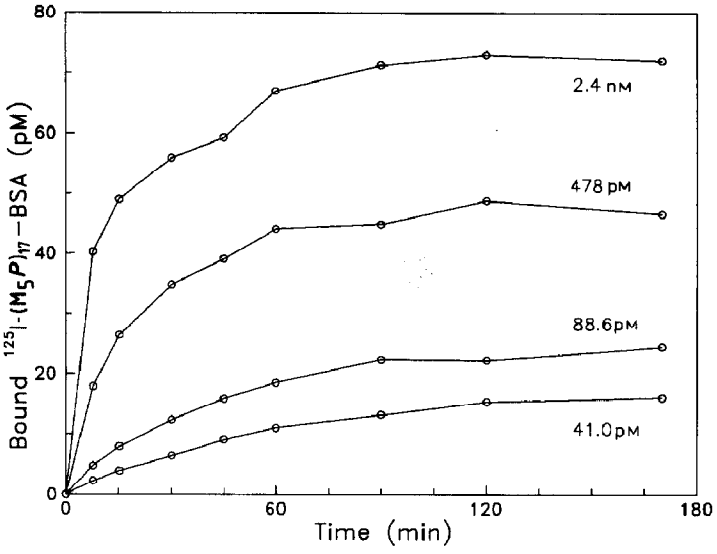


Fig. 1. Time-course of ^{125}I-$(M_5P)_{17}$-BSA binding to rabbit alveolar macrophages. Cells (10^6 cells/mL) were incubated in ^{125}I-$(M_5P)_{17}$-BSA in the concentrations ranging from 41pM to 2.4nM and for incubation periods up to 170 min, and at each time point the specific and nonspecific binding were determined as described in the Experimental section.

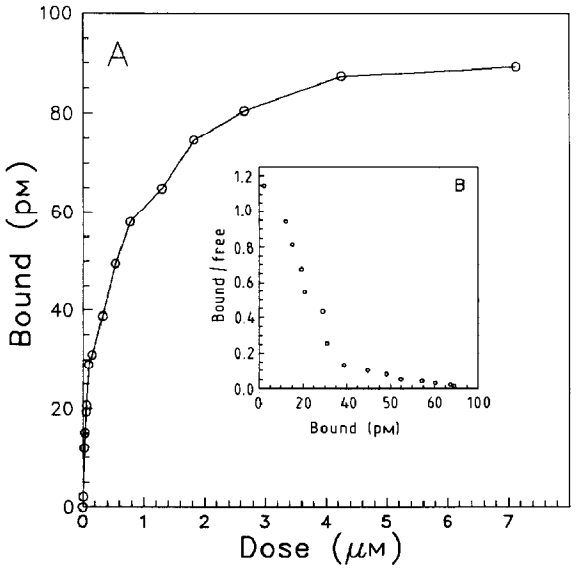


Fig. 2. Equilibrium binding of ^{125}I-(M_5P)-BSA to rabbit alveolar macrophages at 2→. The cells (10^6 Cells/mL) were incubated with ^{125}I-$(M_5P)_{17}$-BSA at the indicated concentrations at 2° for 3 h, and then the specific and nonspecific binding were determined as described in the Experimental section: (A) Dose *vs.* bound curve; (B) Scatchard plot.

TABLE I

Dissociation of bound ^{125}I-$(M_5P)_{17}$-BSA from macrophages by M_5P[a]

Time (min)	*Bound ^{125}I-$(M_5P)_{17}$-BSA*	
	C.p.m.	*%*
0	3882	100
1.5	536	13.8
3	528	13.6
6	508	13.1
15	396	10.2
30	311	8.0

[a] Macrophages (1×10^6 cells/mL) and ^{125}I-$(M_5P)_{17}$-BSA (300pM) were incubated at 2° for 3 h. M_5P (final concentration 1 mM) was added to the mixture and, at indicated times, a sample (100 μL) was removed and cell-associated ^{125}I-ligand was determined.

TABLE II

Calcium-independency of ^{125}I-$(M_5P)_{17}$-BSA binding

Time	*Time course*[a]		*Equilibrium binding*[b]		
(h)	*Bound (pM)*		*Dose (nM)*	*Bound (pM)*	
	Ca (+)	*Ca (−)-EDTA*		*Ca (+)*	*CA (−)-EDTA*
0.5	12.3	12.4	0.11	102	110
1.0	26.8	26.3	0.23	181	189
			0.45	309	286
2.0	30.9	36.1	0.91	337	354
3.0	35.4	41.5	1.36	426	407
			2.27	499	477

[a] Macrophages (1×10^6 cells/mL) were incubated with ^{125}I-$(M_5P)_{17}$-BSA (0.1nM) in the presence or absence of Ca^{2+}. At indicated times, a sample (100 μL) was removed to determine the cell-associated ^{125}I-ligand, as described in the Experimental section. [b] Macrophages (5.5×10^6 cells/mL) were incubated with various concentrations of ^{125}I-$(M_5P)_{17}$-BSA (0.11–2.27nM) at 2° for 3 h in the presence or absence of Ca^{2+}. Cell-associated ^{125}I-$(M_5P)_{17}$-BSA was determined as indicated in the Experimental section.

binding to macrophages (Fig. 2). The data shown in Fig. 2 were analyzed by a nonlinear regression method using a computer program LIGAND, and one-site and two-site models were compared. The *F*-test of the analyses showed that the data fit a two-site model better, and gave the K_d values of 434 and 35.5pM for low- and high-affinity sites, respectively, and the receptor numbers of 5749 and 2601 sites/cell, respectively.

Dissociation of the cell surface-bound ligand by M_5P. — More than 85% of the ^{125}I-$(M_5P)_{17}$-BSA bound to the cell surface at 2° could be dissociated within 1.5 min by treating the cells with mM M_5P at 2° (Table I). However, a 30-min incubation in mM M_5P at 2° was routinely used to ensure maximal dissociation of surface-bound ligand.

Calcium-independent binding of ^{125}I-M_5P_{17}-BSA to rabbit alveolar macrophages. — In order to distinguish the individual contributions made by the "cation-dependent" and the "cation-independent" receptors to binding of ^{125}I-$(M_5P)_{17}$-BSA by the alveolar macrophages, the time-course and dose-dependency was examined in the presence and the absence of EDTA (Table II). Our results showed no difference in the binding under

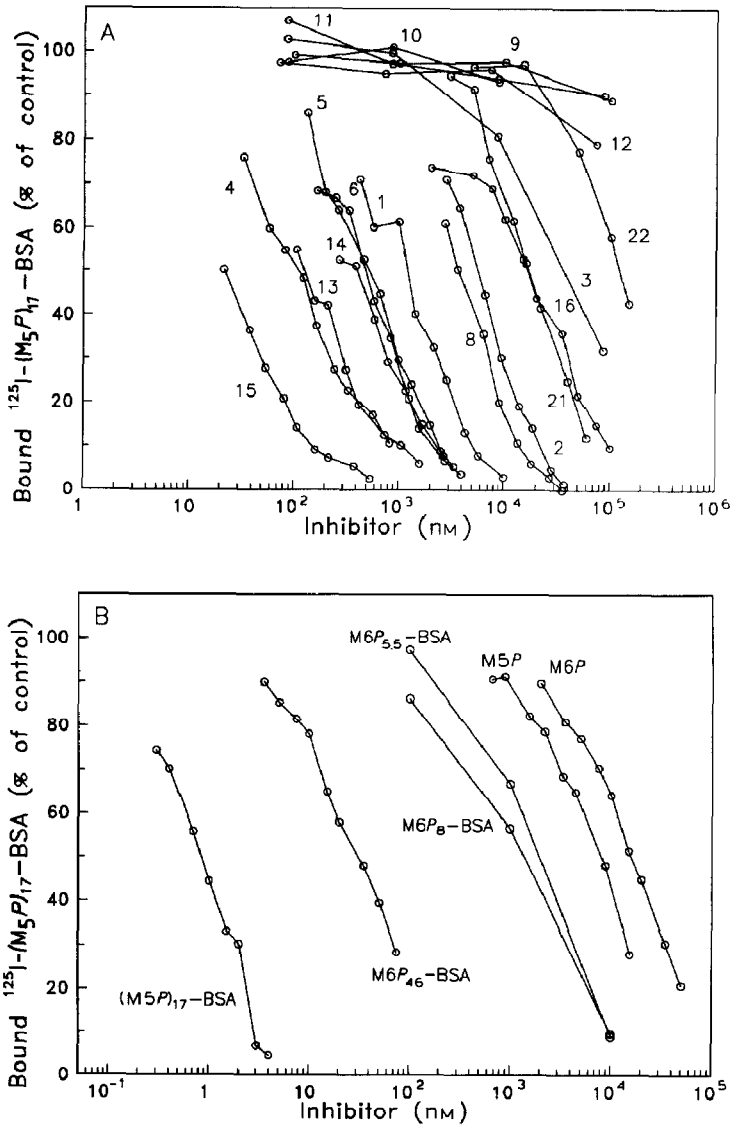


Fig. 3. Inhibitory potency of Man-6-*P*-carrying ligands on ^{125}I-$(M_5P)_{17}$-BSA binding to rabbit alveolar macrophages. The cells (10^6 cells/mL) and ^{125}I-$(M_5P)_{17}$-BSA (0.18nM) were incubated in the presence of each inhibitor at various concentrations for 3 h at 2°, and the specific and the nonspecific binding of radioactivity were determined as described in the Experimental section. The data were divided into Fig. A (**1–6, 8–16, 21,** and **22**) and Fig. B (**17–20**) (see Table *III* for the structures of these inhibitors).

these two conditions, suggesting that rabbit alveolar macrophages do not express the "cation-dependent" Man-6-*P* receptor on the cell surface. The results obtained in this study using $(M_5P)_{17}$-BSA was in agreement with that obtained from the equilibrium-binding study using lysosomal enzymes[11]. However, it was recently reported that the cation-dependent receptor does not have an absolute requirement for divalent cations in human tissues[27].

*Inhibitory potency of Man-6-*P*-bearing ligands on ^{125}I-$(M_5P)_{17}$-BSA binding to rabbit alveolar macrophages.* — The results of inhibition assays using $(M_5P)_{17}$-BSA as a labeled reference compound are summarized in Table III. The oligosaccharides possessing a Man-6-*P* residue at the penultimate position (**9–12**) gave barely detectable inhibitory effects, indicating that the presence of the terminal Man-6-*P* group is required for a strong inhibitory potency (Fig. 3A), or recognition of the Man-6-*P* unit requires at least its two equatorial hydroxyl groups to be free.

In addition, the following results confirmed the earlier report that the receptor recognition extends beyond the Man-6-*P* unit[9]. Firstly, the oligosaccharides having an α-D-(1→2)-linkage between the terminal Man-6-*P* group and the penultimate α-D-mannosyl residue (**1,4–6**) gave stronger inhibitory potencies than those having an α-D-(1→3)- or -(1→6)-linkage at the corresponding position (**2,3,** and **7**) (Fig. 3A). Although the favorable binding of a α-D-Man *p*-6-*P* group (1→2)-linked to the penultimate α-D-mannosyl residue was shown with naturally-occurring oligosaccharides of lysosomal enzymes[7], our studies using synthetic and semisynthetic ligands of simpler structures led to a clearer demonstration of this effect. Secondly, the trisaccharides possessing a terminal Man-6-*P* group (**4–6**) were more inhibitory than the corresponding disaccharides (**1–3**) and also Man-6-*P* (Fig. 3A). Thirdly, a synthetic divalent ligand containing only Man-6-*P* groups (in the form of aminohexyl mannoside 6-phosphate) (**16**) showed only about the same level of inhibitory potency as the monovalent ligand Man-6-*P* (Figs. 3A and B). It should be emphasized here that the divalent ligand, which does not have a proper terminal disaccharide sequence, failed to give a strong inhibitory effect. This provided a clearer demonstration of the importance of the sugar residue internal to the Man-6-*P* group in the receptor–ligand interaction. Fourthly, the linkage positions of the penultimate α-D-mannosyl residue on the adjacent D-mannosyl group showed some differences (**4** and **6**), although a (1→6)-linkage of this residue (**6**) appeared to be not as detrimental as for the Man-6-*P* group (**3**). Finally, the effect of the aglycon on Man-6-*P* is somewhat perplexing. When $-(CH_2)_8CO_2Me$ was the aglycon, the inhibitory potency was not too different from that of Man-6-*P* itself. However, when the aglycon was $-(CH_2)_6-NHCOCF_3$, the potency was much less than that of Man-6-*P*. It is possible that the longer aliphatic chain may be contributing to a lower critical micelle concentration, and thus seemingly to a higher inhibitory potency.

We also clearly demonstrated the "cluster effect" in the Man-6-*P* receptor system of rabbit alveolar macrophages. The branched divalent ligands with two terminal Man-6-*P* groups at both ends (**15**) gave much stronger inhibitory effects (6–20 time higher) than the constituent monovalent ligands (**5** and **6**) or its partially dephosphorylated analogs (**13** and **14**), as shown in Fig. 3A and Table III. Even with Man-6-*P*, which

TABLE III

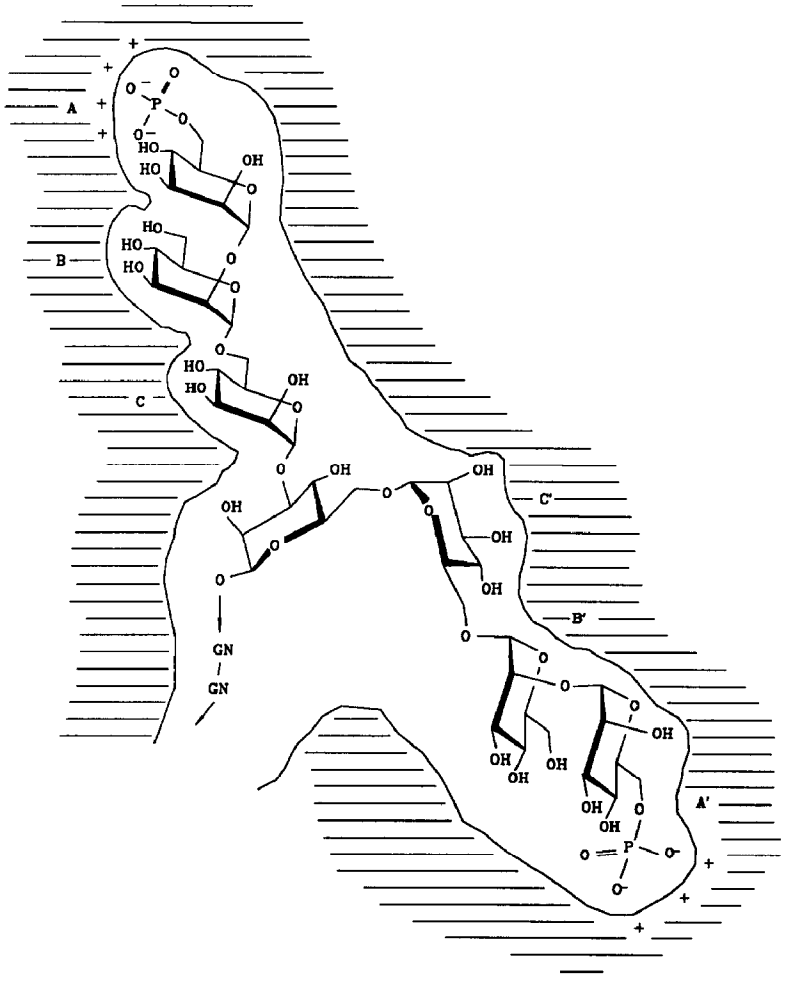
Inhibitory potencies of Man-6-*P* bearing ligands on ^{125}I-$(M_5P)_{17}$-BSA binding to cells

Ligands		*Number of Man-6-P-unit/mol*	I_{50} (μM)	
Compound No.	*Structure*[a]		*Exp. 1*	*Exp. 2*
Monosaccharide				
	M6*P*	1	10.5	13.1
Di-, tri-, and penta-saccharides containing a terminal nonreducing M6P group				
1	M6*P*→2M→R	1	1.2	1.2
2	M6*P*→3M→R	1	5.7	4.9
3	M6*P*→6M→R	1	38.7	62.3
4	M6*P*→2M→2M→R	1	0.11	0.18
5	M6*P*→2M→3M→R	1	0.53	0.49
6	M6*P*→2M→6M→R	1	0.49	0.42
7	M6*P*→3M→3M→3M→2-D-Man	1	8.2	5.9
Di- and tri-saccharides containing M6P units on penultimate M residues				
8	M6*P*→2M6*P*→R	2	3.9	3.4
9	M→2M6*P*→R	1		>100
10	M→2M6*P*→2M→R	1		>100
11	M→2M6*P*→3M→R	1		>100
12	M→2M6*P*→6M→R	1		>100
Branched oligosaccharides containing terminal, nonreducing M6P groups				
13	M6*P*→2M ↓ M→2M→3(6)M→R	1	0.12	0.40[b]
14	M→2M ↓ M6*P*→2M→3(6)M→R	1	0.40	0.87[b]
15	M6*P*→2M ↓ M6*P*→2M→3(6)M→R	2	0.021	0.058[b]
16	M6*P*AH→Ala | M6*P*AH→Ala-Asp-Tyr	2	16.1	20.2[b]
BSA-derived neoglycoprotein				
17	$(M_5P)_{17}$-BSA	17	0.00085	–
18	$(M6P)_{5.5}$-BSA	5.5	1.9	–
19	$(M6P)_8$-BSA	8	1.4	–
20	$(M6P)_{46}$-BSA	46	0.035	–
Simple aliphatic glycosides of M6P				
21	M6P-R	1	18	–
22	M6PAHCOCF$_3$	1	131	–

[a] Abbreviations: M = α-D-Man*p*-(1→; M6*P* = α-D-Man*p* 6-phosphate (1→; R = $O(CH_2)_8CO_2Me$; AH = 6-aminohexyl. [b] In these experiments, 5-times more radioactively-labelled reference ligands were used.

only partially fulfills the binding requirement, the effect of "clustering" was quite evident, as shown in (Man-6-*P*)$_n$-BSA. As the number (5.5, 8, and 46) of Man-6-*P* units coupled to BSA increased, the inhibitory potencies increased, as evidenced by the decreasing I_{50} values of 1.93, 1.36, and 0.0345μM, respectively (Table III and Fig. 3B). This phenomenon is similar to the phenomena observed for many other vertebrate lectins, such as the D-Man–L-Fuc receptor in rabbit alveolar macrophages, the D-GlcNAc-receptor of chicken hepatocytes, and the D-Gal-receptor of rat hepatocytes[28].

Kornfeld and assoc.[2,7] showed that the cation-independent Man-6-*P* receptor molecule has two Man-6-*P* binding sites, and that the underlying oligosaccharide structure played a role in the binding. It was also suggested that a proper distance between two Man-6-*P* units is probably required. Our results extend these suggestions to form the following model to aid in our understanding of the binding (other models which can accommodate these data are also possible). Each binding site is composed of



Scheme 1. Model of contiguous recognition subsites on Man-6-*P* receptor.

possibly three contiguous recognition subsites, A–B–C (Scheme 1); subsite A binds to a Man-6-*P* group, subsite B recognizes a (1→2)-linked α-D-mannosyl residue, and subsite C binds to an α-D-mannosyl residue. Of these, subsite A plays the most important role, because the Man-6-*P* receptor did not bind an unphosphorylated oligomannoside. Comparison of binding of **4**, **5**, and **6** showed that the stereochemistry at subsite C is relevant. The fact that the diphosphorylated branched structure **15** was a better ligand than the monophosphorylated **13** and **14** can be explained by considering two such sites in the case of a cation-independent type receptor[7].

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REFERENCES

1 K. von Figura and A. Hasilik, *Annu. Rev. Biochem.*, 55 (1986) 167–193.
2 S. Kornfeld, Faseb J., 1 (1987) 462–468.
3 N. M. Dahms, P. Lobel, and S. Kornfeld, *J. Biol. Chem.*, 264 (1989) 12 115–12 118.
4 B. Hoflack and S. Kornfeld, *J. Biol. Chem.*, 260 (1983) 12 008–12 014.
5 A. Varaki and S. Kornfeld, *J. Biol. Chem.*, 255 (1980) 10 847–10 858.
6 M. Natowicz, J. U. Baenziger, and W. Sly, *J. Biol. Chem.*, 257 (1982) 4412–4420.
7 P. Y. Tong, W. Gregory, and S. Kornfeld, *J. Biol. Chem.*, 264 (1989) 7962–7969.
8 P. Y. Tong and S. Kornfeld, *J. Biol. Chem.*, 264 (1989) 7970–7975.
9 M. Natowicz, D. W. Halletth, C. Frier, M. Chi, P. H. Schlesinger, and J. U. Baenziger, *J. Cell. Biol.*, 96 (1983) 915–919.
10 C. F. Nathan, H. W. Murray, and Z. A. Cohn, *N. Engl. J. Med.*, 303 (1980) 622–626.
11 V. L. Shepherd, H. H. Freeze, A. L. Miller, and P. D. Stahl, *J. Biol. Chem.*, 259 (1984) 2257–2261.
12 R. K. Bretthauer, G. J. Kaczorowski, and M. J. Weise, *Biochemistry*, 12 (1973) 1251–1256.
13 G. R. Gray, *Methods Enzymol.*, 50 (1978) 155–160.
14 Y. C. Lee and R. T. Lee, *Biochemistry*, 19 (1980) 156–163.
15 O. P. Srivastava and O. Hindsgaul, *Can. J. Chem.*, 64 (1986) 2324–2330.
16 O. P. Srivastava and O. Hindsgaul, *Carbohydr. Res.*, 155 (1987) 57–72; O. P. Srivastava and O. Hindsgaul, *ibid.*, 161 (1987) 195–210; O. P. Srivastava and O. Hindsgaul, 161 (1987) 324–329.
17 O. P. Srivastava and O. Hindsgaul, *J. Org. Chem.*, 52 (1987) 2869–2875.
18 Y. Ichikawa and Y. C. Lee, *Carbohydr. Res.*, 198 (1990) 235–246.
19 Y. Ichikawa and Y. C. Lee, unpublished results.
20 K. L. Matta, M. S. Chowdhary, R. K. Jain, and A. Abbas, *Carbohydr. Res.*, 150 (1986) c1–c4.
21 J. F. McKelvy and Y. C. Lee, *Arch. Biochem. Biophys.*, 132 (1968) 99–110.
22 B. N. Ames and D. T. Dubin, *J. Biol. Chem.*, 235 (1960) 769–775.
23 C. A. Hoppe and Y. C. Lee, *J. Biol. Chem.*, 257 (1982) 12 831–12 834.
24 T. Berg, D. Bowman, and D. P. O. Seglen, *Exp. Cell. Res.*, 72 (1972) 571–574.
25 M. J. Krantz, N. A. Holtzman, C. Stowell, and Y. C. Lee, *Biochemistry*, 15 (1976) 3963–3968.
26 P. J. Munson and D. Rodbard, *Anal. Biochem.*, 107 (1980) 220–239.
27 U. Junghans, A. Waheed, and K. von Figura, *FEBS. Lett.*, 237 (1988) 81–84.
28 Y. C. Lee and R. T. Lee, in M. Horowitz (Ed.), *The Glyoconjugates*, Vol. IV, Part B, Academic Press, New York, 1982 pp. 57–83.