Biochem. J. (1989) 262, 131–138 (Printed in Great Britain)

Neoglycolipids as probes of oligosaccharide recognition by recombinant and natural mannose-binding proteins of the rat and man

Robert A. CHILDS,* Kurt DRICKAMER,† Toshisuke KAWASAKI,‡ Steffen THIEL,§ Tsuguo MIZUOCHI*‖ and Ten FEIZI*¶

*Section of Glycoconjugate Research, M.R.C. Clinical Research Centre, Watford Road, Harrow, Middx. HA1 3UJ, U.K., †Department of Biochemistry and Molecular Biophysics, Columbia University, 630 West 168th Street, New York, NY 10032, U.S.A., ‡Department of Biological Chemistry, Faculty of Pharmaceutical Sciences, Kyoto University, Kyoto 606, Japan, and §M.R.C. Immunochemistry Unit, University of Oxford, South Parks Road, Oxford OX1 3QU, U.K.

Oligosaccharide recognition by three mammalian mannose-binding proteins was investigated by using as probes a series of structurally characterized neoglycolipids in t.l.c. binding assays. The neoglycolipids were derived from *N*-linked oligosaccharides of complex, high-mannose and hybrid types and from human milk oligosaccharides and simple di- and tri-saccharides. The three proteins, namely the recombinant carbohydrate-recognition domain of rat mannose-binding Protein A and the multi-subunit forms of rat and human serum mannose-binding proteins, were shown to have in common reactivity with oligosaccharide probes containing one or more non-reducing terminal *N*-acetylglucosamine residue(s). Substitution with galactose masks reactivity. The three proteins also bound to non-reducing terminal mannose residues in high-mannose-type oligosaccharides, non-reducing terminal fucose residues in the sequence Fucα1-4(Galβ1-3)GlcNAc and non-reducing terminal glucose residues in dextran oligomers; the recombinant binding domain gave consistently weaker binding. The relative reactivities with the various probes differ for each protein. Overall, the reaction patterns of the three mammalian proteins differ from that of the plant lectin concanavalin A, which showed preferential binding to the high-mannose type, weak binding to biantennary complex type and no binding to the fuco-oligosaccharide and simple oligosaccharide probes. As a group, the three mammalian proteins resemble bovine serum conglutinin and behave as lectins with rather broad sugar specificities directed at certain non-reducing terminal *N*-acetylglucosamine, mannose, glucose and fucose residues, but with subtle differences in fine specificities. These results illustrate the potential of neoglycolipids in studies of oligosaccharide recognition by natural and recombinant proteins of diverse biological systems.

INTRODUCTION

N-Glycosylation of proteins is the product of the concerted actions of an array of cellular glycosyltransferases and glycosidases (Kornfeld & Kornfeld, 1985). Understanding the roles of the resulting oligosaccharides, with a common trimannosyl-chitobiosyl core and a diversity of outer chains, remains one of the challenges of modern cell biology. Studies of oligosaccharide recognition using intact glycoproteins are frequently difficult to interpret for several reasons. First, a contribution of the protein moiety cannot always be excluded, and, secondly, the multiplicity of oligosaccharide structures typically associated with a single glycoprotein means that it may not be feasible to define precisely the oligosaccharide species recognized. As a new approach to studies of oligosaccharide recognition by cell-associated and secreted, natural and recombinant proteins, procedures are being developed whereby oligosaccharides derived from glycoproteins are analysed as immobilized probes after conjugation to lipid (neoglycolipids) and chromatography on silica-gel plates (Tang *et al.*, 1985; Stoll *et al.*, 1988). In the present study we have adopted such an approach using neoglycolipids constructed from the released *N*-linked oligosaccharides of several glycoproteins and other oligosaccharides to examine the reactivities of natural and recombinant mannose-binding proteins and to compare these with the reactivities of the plant lectin concanavalin A.

Mannose-binding proteins are water-soluble proteins of 200–600 kDa and composed of subunits of approx. 31 kDa. Each subunit contains three regions: an *N*-terminal sequence of 18–19 amino acid residues rich in cysteine residues, a collagen-like domain containing 18–20 repeats of Gly-Xaa-Yaa, and a *C*-terminal carbohydrate-recognition domain (Drickamer *et al.*, 1986; Oka *et al.*, 1987). In rat, two co-purifying forms of mannose-binding proteins have been identified in the liver, MBP-A and MBP-C (Drickamer *et al.*, 1986), and in serum, SMBP-I and SMBP-II (Oka *et al.*, 1988); in

Abbreviations used: HuMBP, human serum mannose-binding protein; SMBP-II, rat serum mannose-binding protein II; rMBP-A, recombinant carbohydrate-recognition domain of rat mannose-binding Protein A; Con A, concanavalin A.

‖ Present address: Division of Biomedical Polymer Science, Institute of Comprehensive Medical Science, Fujita-Gakuen Health University School of Medicine, Toyoake Aichi 470-11, Japan.

¶ To whom correspondence and reprint requests should be addressed.

man, mannose-binding proteins have been isolated from the liver (Wild *et al.*, 1983) and serum (Kawasaki *et al.*, 1983; Ezekowitz *et al.*, 1988). The three mannose-binding proteins that we have investigated here are (*a*) the recombinant carbohydrate-recognition domain of rat liver mannose-binding Protein A (designated rMBP-A), (*b*) the major naturally occurring rat serum MBP (SMBP-II), which is probably identical with MBP-A (Ikeda *et al.*, 1987), and (*c*) human serum MBP (HuMBP), which shows closer sequence similarity to rat MBP-C than to MBP-A (Ezekowitz *et al.*, 1988).

MATERIALS AND METHODS

Lectins

The rMBP-A (Drickamer *et al.*, 1986) was produced in *Escherichia coli* with the use of a bacterial expression vector (Drickamer, 1989). SMBP-II was prepared as described previously (Oka *et al.*, 1988), and HuMBP was prepared essentially as described by Kawasaki *et al.* (1983) but with a final ion-exchange step on a Mono Q (Pharmacia) column eluted with an NaCl gradient. For radiolabelling, rMBP-A (150 μg), SMBP-II (15 μg) and HuMBP (40 μg) were incubated with 1 mCi of ^{125}I-labelled Bolton & Hunter reagent (Amersham International, Little Chalfont, Bucks., U.K.) in accordance with the manufacturers' instructions. ^{125}I-labelled proteins were separated from unused reagent on a Sephadex G-25 column, PD10 (Pharmacia, Uppsala, Sweden), equilibrated in 0.5 M-NaCl in 20 mM-imidazole/HCl buffer, pH 7.8, 2 mM-EDTA and 0.1% (w/v) bovine serum albumin (solution A). Specific radioactivities of the radiolabelled lectins were rMBP-A, 1×10^6 c.p.m./μg, SMBP-II, 6×10^6 c.p.m./μg, and HuMBP, 7×10^6 c.p.m./μg, as measured in a Nuclear Enterprises 1600 γ-radiation counter. The radiolabelled proteins in approx. 3 ml of solution A were made 50 mM with respect to $CaCl_2$ and passed over a 1 ml (bed volume) column of either mannose–Sepharose 6B (Fornstedt & Porath, 1975) for rMBP-A or mannan–Sepharose 4B (Oka *et al.*, 1988) for SMBP-II and HuMBP. After a washing with 20 ml of solution A containing 50 mM-$CaCl_2$, radiolabelled lectins bound were eluted with solution A; 1 ml fractions were collected and the single peak of radioactivity was pooled and stored in the presence of 0.02% NaN_3 at 4 °C. Typical recoveries from the columns were rMBP-A, 12%, SMBP-II, 2%, and HuMBP, 25% of the radioactivities applied. Concanavalin A (Sigma Chemical Co., Poole, Dorset, U.K.) was radiolabelled with ^{125}I by the chloramine-T method (Greenwood *et al.*, 1963). Specific radioactivity was 4×10^6 c.p.m./μg.

Neoglycolipids

The following glycoproteins were used as sources of *N*-linked oligosaccharides: human IgG, mouse IgG, bovine RNAase B, ovalbumin grade V and human transferrin, purchased from Sigma Chemical Co. The release of *N*-linked oligosaccharides from glycoproteins by hydrazinolysis (Takasaki *et al.*, 1982) and treatments with glycosidases (Yamashita *et al.*, 1982; Mizuochi *et al.*, 1982), namely sialidase from *Arthrobacter ureafaciens* (Nakarai Chemicals Ltd., Kyoto, Japan) and β-galactosidase and β-*N*-acetylhexosaminidase from jack-bean meal (Seikagaku Kogyo Co., Tokyo, Japan), were as described previously. The preparation of neoglycolipids was essentially as described previously (Stoll *et al.*, 1988). From the glycoproteins 13 types of neoglycolipids, designated a to f and j to n in Table 1, were identified by t.l.c. and liquid secondary ion m.s. (Mizuochi *et al.*, 1989). These were a series of biantennary complex-type oligosaccharide derivatives designated bands a, c and e from asialo human and mouse IgG oligosaccharides, bands b, d and f from asialo human IgG oligosaccharides, band j from sialidase-treated transferrin oligosaccharides, and a series of high-mannose-type oligosaccharide derivatives, bands k, l, m and n from RNAase B. Bands j′ and j″ were obtained from sialidase-treated transferrin oligosaccharides by sequential digestions with β-galactosidase and β-*N*-acetylhexosaminidase respectively. In addition, a mixture of oligosaccharide derivatives were obtained from ovalbumin. Neoglycolipids were also prepared from oligosaccharides (gifs from Dr. A. Lundblad, Biocarb, Lund, Sweden) isolated from the urine of patients with GM_1 gangliosidoses, designated GM_1-A, GM_1-B and GM_1-C. These respectively contain the structures Galβ1-4GlcNAcβ1-2Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)-Manβ1-4GlcNAc, Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-4)Manα1-3(Galβ1-4GlcNAcβ1-2Manα1-6)Manβ1-4GlcNAc and Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-4)Manα1-3[Galβ1-4GlcNAcβ1-2(Galβ1-4GlcNAcβ1-6)Manα1-6]Manβ1-4GlcNAc. Neoglycolipids (Stoll *et al.*, 1988) from five human milk oligosaccharides, lacto-*N*-fucopentaoses I, II and III, lacto-*N*-difucohexaose I and lacto-*N*-neotetraose (gifts from Dr. W. M. Watkins), were also treated. These are designated P_1, P_2, P_3, H_1 and T as shown in Table 1. Neoglycolipids derived (Loveless *et al.*, 1989) from mannobiose, designated M_2, chitobiose, designated GN_2, and chitotriose, designated GN_3, were also tested, and from glucose dimer, designated G_2, and trimer, designated G_3 (obtained by acid hydrolysis of dextran T2000).

T.l.c. overlay experiments with radiolabelled lectins

Neoglycolipids were chromatographed on 10 cm aluminium-backed silica gel 60 high-performance t.l.c. plates (Merck; from BDH Chemicals, Poole, Dorset, U.K.) in chloroform/methanol/water (105:100:28, by vol.) (solvent system I) or (60:35:8, by vol.) (solvent system II). Plates were air-dried and dipped for 30 s in 0.1% (w/v) Plexigum P28 (Cornelius Chemical Co., Romford, Essex, U.K.) in n-hexane, air-dried and soaked for 2 h in 0.5 M-NaCl in 20 mM-imidazole/HCl buffer, pH 7.8 (solution B), containing 2% (w/v) bovine haemoglobin (BDH Chemicals). The plates were rinsed in solution B containing 50 mM-$CaCl_2$, drained, placed in a humidified chamber and overlaid for 2 h with 5×10^5 c.p.m. of ^{125}I-labelled lectins/ml of solution B containing 50 mM-$CaCl_2$ and 1% (w/v) haemoglobin. Plates were washed four times with solution B containing 50 mM-$CaCl_2$, dried and subjected to autoradiography at −70 °C with a Philips Ultra S intensifying screen and Kodak type S X-ray film.

With ^{125}I-Con A, overlay was performed with 0.15 M-NaCl in 20 mM-Tris/HCl buffer, pH 7.4, containing 1% (w/v) bovine serum albumin, 10 mM-$CaCl_2$ and 1 mM-$MnCl_2$.

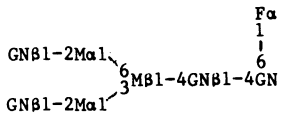
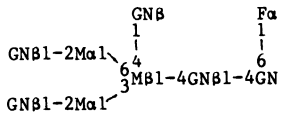
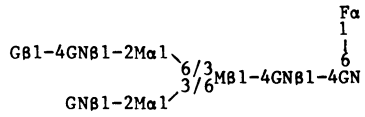
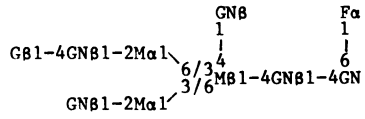
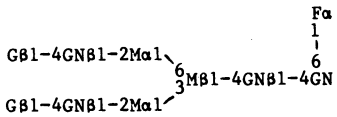
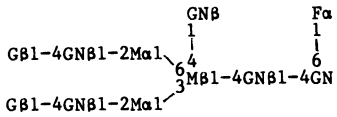
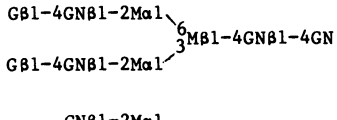
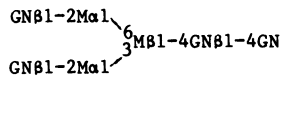
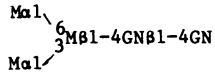
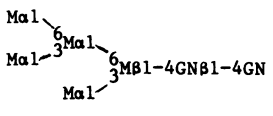
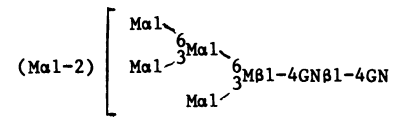
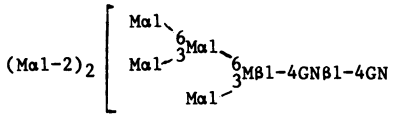
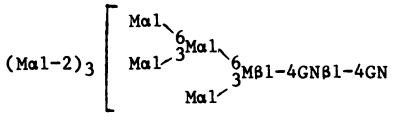
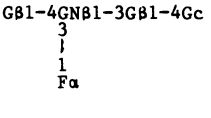
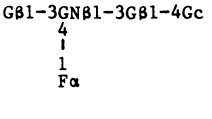
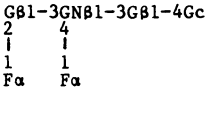
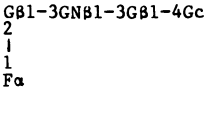
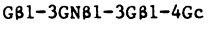
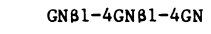
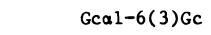
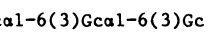
RESULTS

Reactivities of the rat proteins rMBP-A and SMBP-II

The reaction patterns of rMBP-A and SMBP-II with the neoglycolipids derived from the *N*-linked glyco-

Table 1. Structures of oligosaccharides investigated as neoglycolipids

Structures given for a to f and j to n are assignments for the major components in the neoglycolipid bands as described in the text. Abbreviations: F, fucose; G, galactose; GN, *N*-acetylglucosamine; Gc, glucose; M, mannose.

Designation	Structure
a	
b	
c	
d	
e	
f	
j	
j′	
j″	
k	
l	
m	
n	
P_3	
P_2	
H_1	
P_1	
T	
M_2	
GN_2	
GN_3	
G_2	
G_3	

protein oligosaccharides and the penta- and hexasaccharides from human milk showed considerable similarities. Both proteins reacted strongly with the biantennary complex-type oligosaccharide probes with two non-reducing terminal *N*-acetylglucosamine residues with or without a core-region fucose residue, band a (Fig. 1) and band j′ (Fig. 2) respectively. Reactivities with the biantennary analogue with a single non-reducing terminal *N*-acetylglucosamine residue (band c) were weaker with SMBP-II and barely detectable with rMBP-A. Both proteins bound to bands b and d, suggesting that they react with the small amount of biantennary oligosaccharide analogues containing a bisecting *N*-acetylglucosamine residue. This requires further investiga-

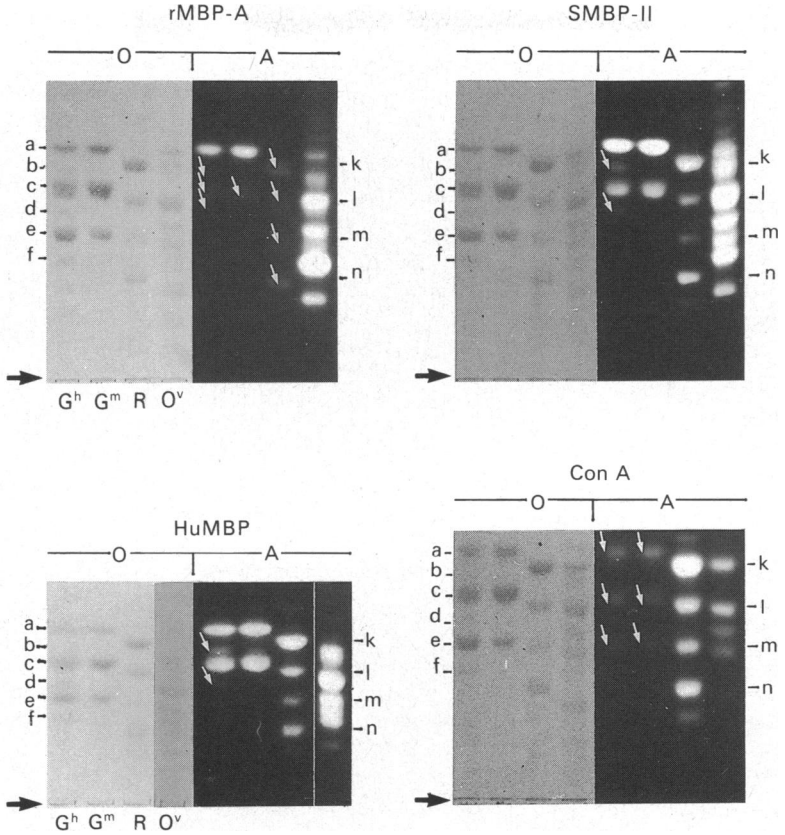


Fig. 1. Reaction patterns of ^{125}I-labelled mannose-binding proteins and Con A with neoglycolipids derived from glycoproteins

Neoglycolipids (2.5 μg of carbohydrate per lane) derived from asialo-oligosaccharides of human IgG (G^h), and mouse IgG (G^m) and from RNAase B (R) and ovalbumin (O^v) were chromatographed in solvent system I. Reactivities with ^{125}I-labelled rMBP-A, SMBP-II, HuMBP and Con A were assessed by autoradiography (panels A, white bands) as described in the Materials and methods section; the same lanes were then stained with orcinol reagent (panels O, dark bands) to detect the neoglycolipid bands designated a, c and e (in lanes G^h and G^m), b, d and f (lane G^h) and k to n (lane R), whose structures are given in Table 1. Autoradiography was for 14 days (rMBP-A), 18 h (SMBP-II), 8 h (HuMBP) and 2 days (Con A). Black arrows indicate positions of application; white arrows weakly reactive bands.

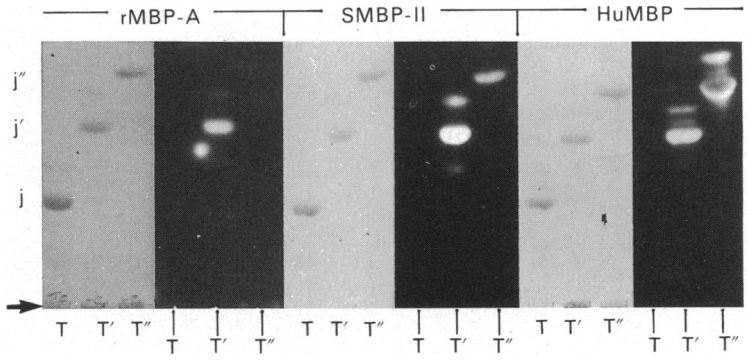


Fig. 2. Reaction patterns of ^{125}I-labelled mannose-binding proteins with neoglycolipids derived from asialotransferrin oligosaccharides

Neoglycolipids prepared from asialo-oligosaccharides of transferrin (lane T, band j), asialo-oligosaccharides treated with β-galactosidase (lane T′, band j′) and asialo-oligosaccharides treated with β-galactosidase and β-*N*-acetylhexosaminidase (lane T″, band j″) were chromatographed in solvent system I. Reactivities with ^{125}I-labelled rMBP-A, SMBP-II and HuMBP were assessed by autoradiography (right-hand panels, white bands); the same lanes were then stained with orcinol reagent to detect the neoglycolipid bands (left-hand panels, dark bands). Autoradiography was for 6 days (rMBP-A), 18 h (SMBP-II) and 8 h (HuMBP). Black arrow indicates position of application. The fast-migrating minor components in lanes T′ and T″ were not identified.

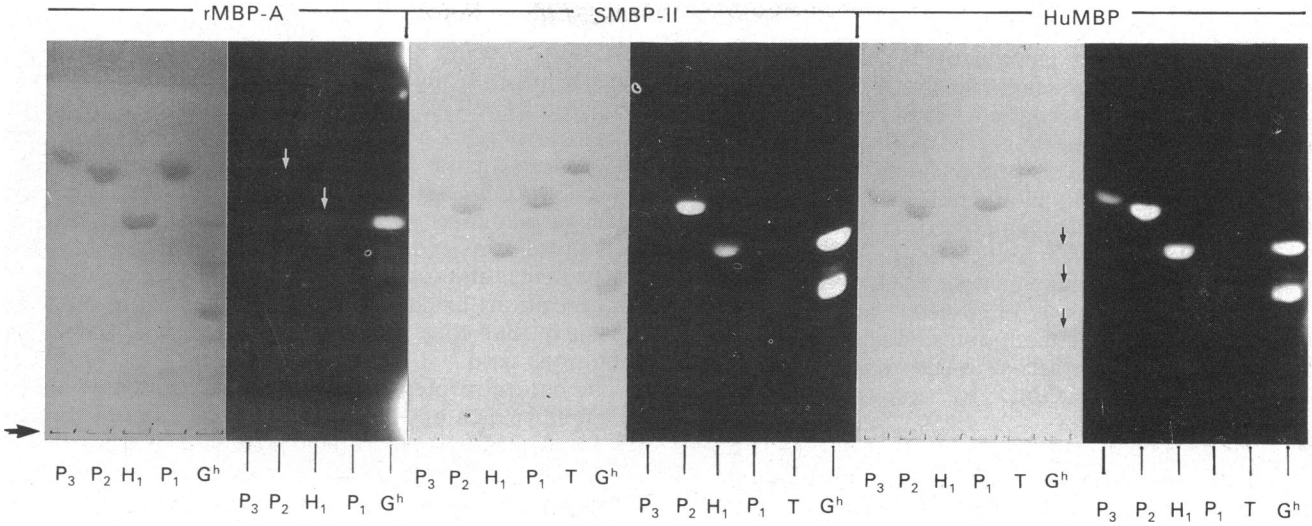


Fig. 3. Reaction patterns of ^{125}I-labelled mannose-banding proteins with neoglycolipids derived from milk oligosaccharides

Neoglycolipids prepared from lacto-*N*-fucopentaoses I, II and III (P_1, P_2 and P_3 respectively), lacto-*N*-difucohexaose I (H_1) and lacto-*N*-neotetraose (T), and, for reference, oligosaccharides of human IgG (G^h), were chromatographed in solvent system I. Reactivities with ^{125}I-labelled rMBP-A, SMBP-II and HuMBP were assessed by autoradiography (right-hand panels, white bands); the same lanes were then stained with orcinol to detect the neoglycolipid bands (left-hand panels, dark bands). Autoradiography was for 6 days (rMBP-A), 11 days (SMBP-II) and 3 days (HuMBP). Large black arrow indicates position of application; white arrows indicate bands weakly reactive with rMBP-A; small black arrows indicate positions of orcinol-stained bands a, c and e from human IgG (G^h).

tion with purified oligosaccharides. On the biantennary structures both proteins require the *N*-acetylglucosamine residues to be in a terminal position, for no binding was detected to the digalactosyl oligosaccharide probes with or without a core-region fucose, band e (Fig. 1) and j (Fig. 2) respectively, or to the analogue having a bisecting *N*-acetylglucosamine, band f.

When tested with neoglycolipids derived from bi-, tri- and tetra-antennary oligosaccharides from urine, GM_1-A, GM_1-B and GM_1-C, all of which have galactose residues at the non-reducing end of their outer chains and a single *N*-acetylglucosamine at the reducing end, the two lectins bound to the β-galactosidase-treated but not to the untreated derivatives (results not shown).

The two proteins appeared to have relatively lower affinities for the glycoprotein oligosaccharide probes with non-reducing terminal mannose rather than *N*-acetylglucosamine residues. SMBP-II bound moderately well to the high-mannose-type structures, bands k to n from RNAase (Fig. 1), and the trimannosyl core structure, band j″ (Fig. 2), whereas with rMBP-A binding was extremely weak, bands k to n, or undetectable, band j″. Taken together, the results indicate a preferential reaction with the biantennary oligosaccharides having two unsubstituted *N*-acetylglucosamine residues. The two proteins gave multiple strong bands of reactivity with the oligosaccharide probes derived from ovalbumin; this is in accordance with the known abundance of oligosaccharides of hybrid type with non-reducing terminal *N*-acetylglucosamine residues in addition to high-mannose-type chains in this glycoprotein (Tai *et al.*, 1977; Nomoto & Inoue, 1983).

In binding experiments with the milk oligosaccharide probes, reactivities with both proteins were detected only with the lacto-*N*-fucopentaose II and difucohexaose I probes (designated P_2 and H_1 in Fig. 3). With rMBP-A the binding was very weak. With both proteins the binding was less strong than with the biantennary *N*-acetylglucosamine-terminating oligosaccharides (bands a and c) from IgG (G^h).

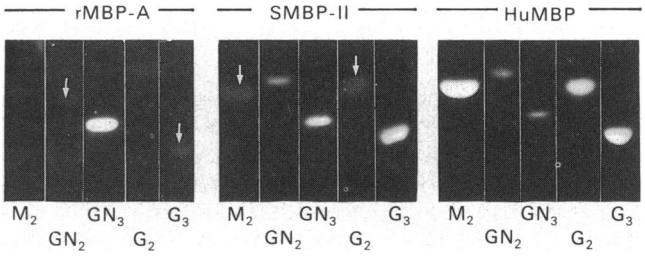


Fig. 4. Reaction patterns of ^{125}I-labelled mannose-binding proteins with neoglycolipids derived from di- and trisaccharides

Neoglycolipids prepared from mannobiose (M_2), chitobiose (GN_2), chitotriose (GN_3) and glucose dimer (G_2) and trimer (G_3) were chromatographed in solvent system II. Reactivities with ^{125}I-labelled rMBP-A, SMBP-II and HuMBP were assessed by autoradiography (white bands). Autoradiography was for 6 days (rMBP-A), 20 h (SMBP-II) and 4 h (HuMBP). Chromatography was upward; white arrows indicate weakly reactive bands.

With the five simple oligosaccharide probes (Fig. 4) there was a difference in the reaction patterns of the two carbohydrate-binding proteins. With rMBP-A, a relatively strong reaction was observed only with the chitotriosyl probe, GN_3, and trace reactivities with the chitobiosyl and glucotriosyl probes, GN_2 and G_3. In contrast, SMBP-II bound to all five probes in the order $G_3 > GN_3 > GN_2 > G_2 = M_2$.

Reactivities of the human protein HuMBP

Overall, the reaction pattern of HuMBP with the oligosaccharide probes resembled that of SMBP-II, but some differences were revealed. The human protein bound relatively more strongly than SMBP-II to the biantennary oligosaccharide probe having only one non-reducing terminal *N*-acetylglucosamine, band c (Fig. 1); binding to the lacto-*N*-fucopentaose III (P_3) was detected in addition to the reactivities with lacto-*N*-fucopentaose II (P_2) and difucohexaose I (H_1) probes (Fig. 3). Also, among the simple oligosaccharide probes the intensity of reaction with mannobiose was stronger than with SMBP-II, the hierarchy in the reactivity being $M_2 > G_3 = G_2 > GN_3 = GN_2$.

Reactivities of Con A

In contrast with the three mammalian proteins, the plant lectin Con A bound relatively weakly to the biantennary oligosaccharide bands a, c and e and showed a stronger reactivity with the high-mannose-type oligosaccharide probes k to n from RNAase B (Fig. 1), in accordance with previous knowledge (Ogata *et al.*, 1975; Baenziger & Fiete, 1979). With the ovalbumin oligosaccharide probes, reactivity was restricted to the faster-migrating bands, two of which correspond to the high-mannose-type bands k and l from ribonuclease. No binding was observed with the probes derived from the milk oligosaccharides or from the five simple oligosaccharides (results not shown).

DISCUSSION

The salient conclusions from these studies can be summarized as follows. First, with the *N*-linked biantennary complex-type oligosaccharides investigated, the three proteins rMBP-A, SMBP-II and HuMBP show preferential reactivities with those containing two peripheral *N*-acetylglucosamine residues. The presence of a single terminal galactose residue markedly decreases binding with the two rat proteins, and when two galactose residues are present binding is abolished with all three proteins. Secondly, the binding patterns with the trimannosyl core structure and the series of high-mannose-type structures indicate that the three proteins recognize mannose residues, and, as with *N*-acetylglucosamine, exposed non-reducing terminal residues are required. Affinity for accessible mannose residues on *N*-linked chains was weak with rMBP-A, stronger with SMBP-II and strongest with HuMBP. The hierarchy of reactivities of these proteins with mannose residues was also apparent in the binding to the mannobiose probe. These properties contrasted with those of the plant lectin Con A, which showed preferential binding to the high-mannose-type structures and no binding to neoglycolipid probes derived from the five simple oligosaccharides and the fuco-oligosaccharides recognized by the MBPs. Thirdly, from the reactivities of the three proteins with the lacto-*N*-fucopentaose II and lacto-*N*-difucohexaose I probes [and of HuMBP with lacto-*N*-fucopentaose III], it is predicted that any oligosaccharide chain with a peripheral Fucα1-4(Galβ1-3)GlcNAc [and Fucα1-3(Galβ1-4)GlcNAc sequence with HuMBP] is a potential target for these proteins. Neither the chitobiose sequence nor the Fucα1-6GlcNAc sequence in the core region of complex-type oligosaccharides is a recognition structure for these proteins. Fourthly, the three proteins also show affinities for non-reducing terminal glucose residues. Thus the *N*-linked oligosaccharide precursor sequences $Glc_{1-3}Man_9GlcNAc_2$ in their dolichol-linked form or after transfer to protein may be among candidate endogenous ligands for these proteins. Experimental evidence (Mori *et al.*, 1988) has, so far, only been obtained for high-mannose-type oligosaccharides of rat hepatic glycoproteins as endogenous ligands for mannose-binding proteins, although this might simply mean that the concentrations or half-lives of other ligands in the liver are such that they could not be detected under the conditions used.

The natural protein, SMBP-II, is composed of about 20 subunits each of 31 kDa, and on the basis of the 25-residue *N*-terminal amino acid sequence of the purified protein it is thought to be identical with rat MBP-A. Therefore the significance of the subtle differences observed in binding patterns of rMBP-A and SMBP-II (see Table 2) is not yet clear. There are several possibilities. First, the differences may be a result of the multivalence of SMBP-II and higher association constants arising from multiple clustered binding sites. Secondly, other regions of the subunit, for example the *N*-terminal and collagen-like domains, may influence the folding and hence the reactivities of the *C*-terminal carbohydrate-recognition domain. Thirdly, MBP-A may not be completely identical with SMBP-II (in spite of the fact that they have a common *N*-terminal amino acid sequence, as reported by Ikeda *et al.*, 1987). Fourthly, SMBP-II may contain a mixture of structurally related subunits, some containing the rMBP-A binding domain and others with different carbohydrate-recognition properties.

Our results indicate that the three mannose-binding proteins recognize residues that are located terminally on *N*-linked oligosaccharides; this is in agreement with conclusions reached by Mori *et al.* (1988) with mannose-binding proteins of rat and rabbit. Maynard & Baenziger (1982), using glycoproteins and glycopeptides as inhibitors of the binding of rat hepatocyte mannose/*N*-acetylglucosamine-specific lectin to ^{125}I-labelled agalacto-orosomucoid, concluded that this lectin is similar in specificity to Con A, and that it is able to bind to the core sugars of *N*-linked oligosaccharides in the presence of more peripheral substituents such as *N*-acetylneuraminic acid and galactose. They assigned the term 'core-specific lectin' to this protein. In view of the microheterogeneity of oligosaccharides on glycoproteins, it is possible the inhibitions observed with the hepatocyte lectin with sialylated and galactosylated glycoproteins and glycopeptides reflect the presence of variable amounts of incomplete oligosaccharide chains in these preparations. Also, this lectin is probably not the same as rMBP-A or SMBP-II used in the present study, but is believed to be the same as MBP-C from rat liver described by Drickamer *et al.* (1986), also designated L-MBP or SMBP-I (Oka *et al.*, 1988). L-MBP is physicochemically and immunochemically distinct from SMBP-II, although both forms have a similar subunit size (32 kDa/31 kDa) and collagen-like domains. Expression of the recombinant carbohydrate-recognition domain of MBP-C should allow a clear comparison of the binding specificity of this protein with the rMBP-A, and overcome the difficulties in separating the two lectins from natural sources.

Although the neoglycolipid overlay technique as used in the present study is semi-quantitative, it does afford a

Table 2. Reaction scores ('foot-prints') of four mammalian carbohydrate-binding proteins, rMBP-A, SMBP-II, HuMBP and bovine serum conglutinin, and of the plant lectin Con A, with three groups of neoglycolipids selected to show the differences in reaction patterns

The designations of the neoglycolipids are those in Table 1. Reaction scores –, (+), +, ++ and +++ (nil to strong) refer to relative intensities with the neoglycolipid bands within each group.

Neoglycolipid	Reaction score				
	rMBP-A	SMBP-II	HuMBP	Conglutinin*	Con A
a	+++	+++	+++	+++	+
b	(+)	+	+	–	–
k	+	++	+++	+	+++
l	(+)	+	++	+	++
m	(+)	+	+	++	++
n	+	++	++	+++	+++
P_3	–	–	+	–	–
P_2	(+)	++	+++	++	–
H_1	(+)	+	+++	++	–
P_1	–	–	–	–	–
T	–	–	–	–	–
M_2	–	(+)	+++	++	–
GN_2	(+)	+	+	+++	–
GN_3	+++	++	+	+++	–
G_2	–	(+)	+++	++	–
G_3	(+)	+++	+++	–	–

* Result with conglutinin taken from Loveless *et al.* (1989), Mizuochi *et al.* (1989) and R. W. Loveless & T. Feizi (unpublished work).

comparison of the characteristic 'foot-prints' of the carbohydrate-binding proteins with a panel of oligosaccharides, and provides an extremely sensitive and convenient way of distinguishing and comparing binding specificities. The comparison drawn up in Table 2 to include bovine serum conglutinin, similarly investigated recently as well as rMBP-A, SMBP-II, HuMBP and Con A, clearly shows that the binding specificities of four mammalian proteins are all related but not identical. The differences in fine specificities may be relevant to the differences in carbohydrate-mediated biological activities of these proteins. Thus conglutinin (Lachmann, 1967), but not rMBP-A (W. M. Loveless & T. Feizi, unpublished work) or SMBP-II (Kawasaki *et al.*, 1985), binds to erythrocytes coated with iC3b, and SMBP-II and HuMBP, but not conglutinin, activate complement through the classical pathway (Ikeda *et al.*, 1987).

In their lack of binding to terminal galactose residues, and their recognition of non-reducing terminal *N*-acetylglucosamine and mannose residues on *N*-linked oligosaccharides and the fucose residue on lacto-*N*-fucopentaose II, the three mannose-binding proteins investigated here and bovine conglutinin (Loveless *et al.*, 1989; and Table 2) resemble the membrane-associated mannose/fucose receptor of rat alveolar macrophages (Stahl *et al.*, 1978; Shepherd *et al.*, 1981) and the sinusoidal cells of rat liver (Maynard & Baenziger, 1982; Mori *et al.*, 1983). As a group, these various proteins may be considered as having combining sites with rather broad sugar specificities. However, the existence of multiple closely associated combining sites has not yet been ruled out. This question would lend itself to investigation by use of the combined techniques of recombinant protein engineering and neoglycolipid overlay. In fact, this is an ideal approach to establish carbohydrate-binding specificities and detect the minimal protein structure required for carbohydrate binding, to investigate proteins not known to bind carbohydrate but containing amino acid sequences suggestive of such activity (Drickamer, 1988), and to investigate diverse cellular proteins not yet suspected of having carbohydrate-recognition domains. As discussed earlier (Thorpe *et al.*, 1988; Feizi, 1989), a great advantage of this approach is that it will allow endogenous oligosaccharides that are available only in limited quantities to be tested. The oligosaccharides of glycoproteins and those of proteoglycans and glycolipids on cell surfaces, viruses, extracellular matrices and cellular secretions have the potential to encode a large amount of information (Feizi, 1985). The neoglycolipid approach promises to increase our understanding of the roles of these oligosaccharides as recognition markers in biological systems.

T. M. was supported by the Cancer Research Campaign. We are grateful to Dr. Elizabeth F. Hounsell for discussion and to Maureen Moriarty for the preparation of the manuscript.

REFERENCES

Baenziger, J. U. & Fiete, D. (1979) J. Biol. Chem. **254**, 789–795
Drickamer, K. (1988) J. Biol. Chem. **263**, 9557–9560
Drickamer, K. (1989) Biochem. Soc. Trans. **17**, 13–15
Drickamer, K., Dordal, M. S. & Reynolds, L. (1986) J. Biol. Chem. **261**, 6878–6887
Ezekowitz, R. A. B., Day, L. E. & Herman, G. A. (1988) J. Exp. Med. **167**, 1034–1046
Feizi, T. (1985) Nature (London) **314**, 53–57
Feizi, T. (1989) Ciba Found. Symp. **145**, 62–79

Fornstedt, N. & Porath, J. (1975) FEBS Lett. **57**, 187–191
Greenwood, F. C., Hunter, W. M. & Glover, J. S. (1963) Biochem. J. **89**, 114–123
Ikeda, K., Sannoh, T., Kawasaki, N., Kawasaki, T. & Yamashina, I. (1987) J. Biol. Chem. **262**, 7451–7454
Kawasaki, N., Kawasaki, T. & Yamashina, I. (1983) J. Biochem. (Tokyo) **94**, 937–947
Kawasaki, N., Kawasaki, T. & Yamashina, I. (1985) J. Biochem. (Tokyo) **98**, 1309–1320
Kornfeld, R. & Kornfeld, S. (1985) Annu. Rev. Biochem. **54**, 631–664
Lachmann, P. J. (1967) Adv. Immunol. **6**, 479–527
Loveless, W. R., Childs, R. A., Stoll, M., Mizuochi, T., Olroyd, R. & Lachmann, P. J. (1989) Biochem. J. **258**, 109–113
Maynard, Y. & Baenziger, J. U. (1982) J. Biol. Chem. **257**, 3788–3794
Mizuochi, T., Taniguchi, T., Shimizu, A. & Kobata, A. (1982) J. Immunol. **129**, 2016–2020
Mizuochi, T., Loveless, R. W., Lawson, A. M., Chai, W., Lachmann, P. J., Childs, R. A., Thiel, S. & Feizi, T. (1989) J. Biol. Chem., in the press
Mori, K., Kawasaki, T. & Yamashina, I. (1983) Arch. Biochem. Biophys. **222**, 542–552
Mori, K., Kawasaki, T. & Yamashina, I. (1988) Arch. Biochem. Biophys. **264**, 647–656
Nomoto, H. & Inoue, Y. (1983) Eur. J. Biochem. **135**, 243–250
Ogata, S., Muramatsu, T. & Kobata, A. (1975) J. Biochem. **78**, 687–696
Oka, S., Itoh, N., Kawasaki, T. & Yamashina, I. (1987) J. Biochem. (Tokyo) **101**, 135–144
Oka, S., Ikeda, K., Kawasaki, T. & Yamashina, I. (1988) Arch. Biochem. Biophys. **260**, 257–266
Shepherd, V. L., Lee, Y. C., Schlesinger, P. H. & Stahl, P. D. (1981) Proc. Natl. Acad. Sci. U.S.A. **78**, 1019–1022
Stahl, P. D., Rodman, J. S., Miller, M. J. & Schlesinger, P. H. (1978) Proc. Natl. Acad. Sci. U.S.A. **75**, 1399–1403
Stoll, M. S., Mizuochi, T., Childs, R. A. & Feizi, T. (1988) Biochem. J. **256**, 661–664
Tai, T., Yamashita, K., Ito, S. & Kobata, A. (1977) J. Biol. Chem. **252**, 6687–6694
Takasaki, S., Mizuochi, T. & Kobata, A. (1982) Methods Enzymol. **83**, 263–268
Tang, P. W., Gooi, H. C., Hardy, M., Lee, Y. C. & Feizi, T. (1985) Biochem. Biophys. Res. Commun. **132**, 474–480
Thorpe, S. J., Bellairs, R. & Feizi, T. (1988) Development **102**, 193–210
Wild, J., Robinson, D. & Winchester, B. (1983) Biochem. J. **210**, 167–174
Yamashita, K., Mizuochi, T. & Kobata, A. (1982) Methods Enzymol. **83**, 105–126

Received 2 November 1988/2 March 1989; accepted 14 March 1989