Carbohydrate Research, 161 (1987) 133–143
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

NEOGLYCOLIPID MICRO-IMMUNOASSAYS APPLIED TO THE OLIGOSACCHARIDES OF HUMAN MILK GALACTOSYLTRANSFERASE DETECT BLOOD-GROUP RELATED ANTIGENS ON BOTH O- AND N-LINKED CHAINS

PING W. TANG AND TEN FEIZI*

Applied Immunochemistry Research Group, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ (Great Britain)

(Received July 9th, 1986; accepted for publication, September 24th, 1986)

ABSTRACT

Reduced O-linked chains and reducing N-linked chains were obtained from human milk galactosyltransferase by degradation with alkaline borohydride and hydrazinolysis, and then purified by ion-exchange chromatography. The reactivities of the conjugates of the oligosaccharides with L-α-phosphatidyl ethanolamine dipalmitoyl (PPEADP) towards monoclonal anti-Le^a and anti-SSEA-1 were then determined, either by antibody-binding assays after absorbing the neoglycolipids onto plastic wells, or by inhibition assays after incorporating the neoglycolipids into liposomes and testing them as inhibitors of antibody binding. The oligosaccharides were also immunostained with monoclonal anti-Le^a after h.p.t.l.c. and coupling to PPEADP. Antigenic activities were detected in the O-linked chains by all three assay systems, whereas, for the less abundant N-linked chains, reactivities were detected by the inhibition assays only. The results provide evidence for the expression of Le^a and SSEA-1 antigen activities on both the O- and N-linked chains of this enzyme glycoprotein.

INTRODUCTION

The carbohydrate chains of membrane-associated and secreted glycoproteins are antigenic structures. Observations that developmentally regulated and tumour-associated antigens recognised by numerous monoclonal antibodies are carbohydrate sequences of glycoproteins[1,2] led to the design of micro-immunoassay procedures[3] that could be applied widely in the analysis of antigens with limited amounts of oligosaccharides derived from glycoproteins. These procedures, which

*Author for correspondence.

0008-6215/87/$ 03.50

are suitable both for reduced and reducing oligosaccharides, involve conjugation of the oligosaccharides to the lipid L-α-phosphatidyl ethanolamine dipalmityol (PPEADP) to form neoglycolipids. A selective periodate-oxidation step is included prior to conjugation of the reduced oligosaccharides. The neoglycolipids can be absorbed onto insoluble matrices for assays of antibody binding or incorporated into liposomes for assays of inhibition of binding, thereby enabling the sugar epitopes to be assayed in a multivalent state. Due to the cooperative effects of multivalence, the antigenicities of the neoglycolipid liposomes are 200-300 times greater than those of the free oligosaccharides with certain antibodies[3,4]. The neoglycolipid micro-immunoassays have been applied to the reduced oligosaccharides[3] released from mucin-type glycoproteins by treatment with alkaline borohydride and to the reducing oligosaccharides[4] released from keratan sulphate by endo-β-D-galactosidase.

These antigen–antibody systems were well characterised, and the oligosaccharides used were purified and free from contaminating protein. We now report on the application of the neoglycolipid immunoassay procedures to carbohydrate chains released from galactosyltransferase of human milk[5]. This enzyme, which contains ~10% of carbohydrate, expresses blood group-related carbohydrate antigens, especially Le^a, and the onco-developmental antigen SSEA-1 recognised by monoclonal antibodies[6].

RESULTS

Analysis of the carbohydrate content of the oligosaccharide fractions. — The oligosaccharide contents of the O- and N-linked fractions were assessed by h.p.t.l.c., using material derived from 15 μg of galactosyltransferase. The O-linked fraction

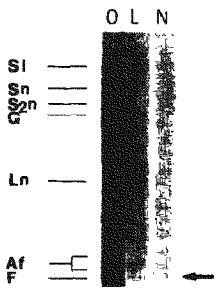

Fig. 1. H.p.t.l.c. and chromatogram-binding assays of the O-linked fraction from galatosyltransferase preparation 1. In lane O, the oligosaccharides were stained with orcinol (numerous faintly stained bands could be discerned with the naked eye but not in the photograph). In lanes L and N, their binding to the anti-Le^a antibody or to normal mouse serum (negative control), respectively, was tested after *in situ* derivatisation and coupling to PPEADP (see Experimental). The mark in lane L near the position of Ln is an artefact. Immunoreactivity was assessed after autoradiography for 16 h. Key: Sl, sialyl-lactose; Sn, sialyl-lacto-*N*-tetraose; S_2n, disialyl-lacto-*N*-tetraose; G, galactose; Ln, lacto-*N*-neotetraose; Af, asialofetuin N-linked oligosaccharides; and F, fetuin N-linked oligosaccharides. The arrow indicates the position of application of the sample.

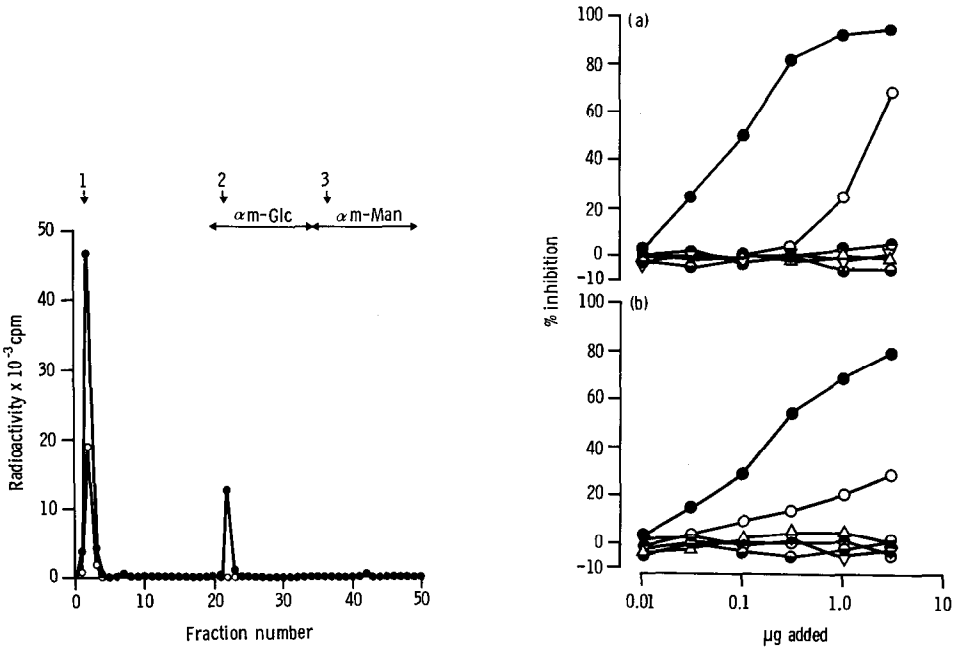

Fig. 2. Chromatography of ^{3}H-labeled oligosaccharides on a column of Con A–Sepharose: —○—, N-linked oligosaccharide fraction from galactosyltransferase preparation 1; —●—, products of partial acid hydrolysis of the N-linked fraction. Arrows indicate the position of elution of the references: 1, oligosaccharides from fetuin and unretained oligosaccharides from transferrin and ovalbumin; 2, oligosaccharides from transferrin eluted with 10mM methyl α-D-glucopyranoside; and 3, oligosaccharides from ovalbumin eluted with 500mM methyl α-D-mannopyranoside.

Fig. 3. Inhibition of binding of monoclonal antibodies (*a*) 115C2 and (*b*) anti-SSEA-1 to meconium glycoproteins by intact galactosyltransferase (preparation 2) and combined O- and N-linked oligosaccharide fractions derived therefrom: ●, intact galactosyltransferase; ◓, unconjugated oligosaccharides; ◒, unconjugated oligosaccharides in the presence of cholesterol; ○, lipid conjugates of the oligosaccharides in the presence of cholesterol (neoglycolipid liposomes); △, neoglycolipid liposomes of maltose; ▽, liposomes made with combined fractions derived from mock-oligosaccharide-release experiments using bovine serum albumin. Several points at less than zero inhibition represent slightly higher binding than in the positive (uninhibited) controls (see Fig. 4 also).

(Fig. 1) gave four main orcinol-positive bands, three of which were in the region of lacto-*N*-neotetraose and the fourth was at the origin. Also, several faintly stained bands between sialyl-lactose and the origin could be discerned with the naked eye but not on the photograph. With the N-linked fractions, there were only faintly stained bands between the N-linked asialo-oligosaccharides of fetuin and the origin (not shown). Further evidence for the presence of N-linked oligosaccharides was obtained by chromatography of the borotritide-reduced oligosaccharides on Con A–Sepharose (Fig. 2). Before partial acid hydrolysis, all of the radioactivity (21,000 c.p.m.) attributable to the N-linked oligosaccharides was present in the unretained fraction (peak 1). After mild acid hydrolysis followed by borotritide reduction, the radioactivity was 68,000 c.p.m., of which 13,000 c.p.m. were bound and eluted

from the column with 10mM methyl α-D-glucopyranoside (peak 2) coincident with the position of elution of biantennary oligosaccharides from transferrin. No radioactive material was eluted with 500mM methyl α-D-mannopyranoside. Hence, for the N-linked oligosaccharides, (*a*) ~60% (corresponding to 13,000 out of 21,000 c.p.m. in the unhydrolysed oligosaccharides) is likely to be of the biantennary type with acid-labile outer residues, such as α-L-fucose (1→3/4)-linked to 2-acetamido-2-deoxy-D-glucose or sialic acid, which hinder binding to Con A–Sepharose[7]; (*b*) the remaining 40% (estimated as 8,000 c.p.m. is likely to be multi-antennary, or may have extended outer chains that hinder binding to the Con A–Sepharose; (*c*) there is an average of approximately two acid-labile monosaccharides per N-linked chain based on the assumption that, of the 55,000 c.p.m. in peak 1, obtained after labelling the acid-treated mixture, 47,000 c.p.m. (after subtraction of 8,000 c.p.m. attributable to unretained N-linked chains) may represent fucose and sialic acid that are not retained; and (*d*) there is no evidence for the presence of high-mannose oligosaccharides, in accordance with the results of metabolic labelling experiments with cultured HeLa cells[8].

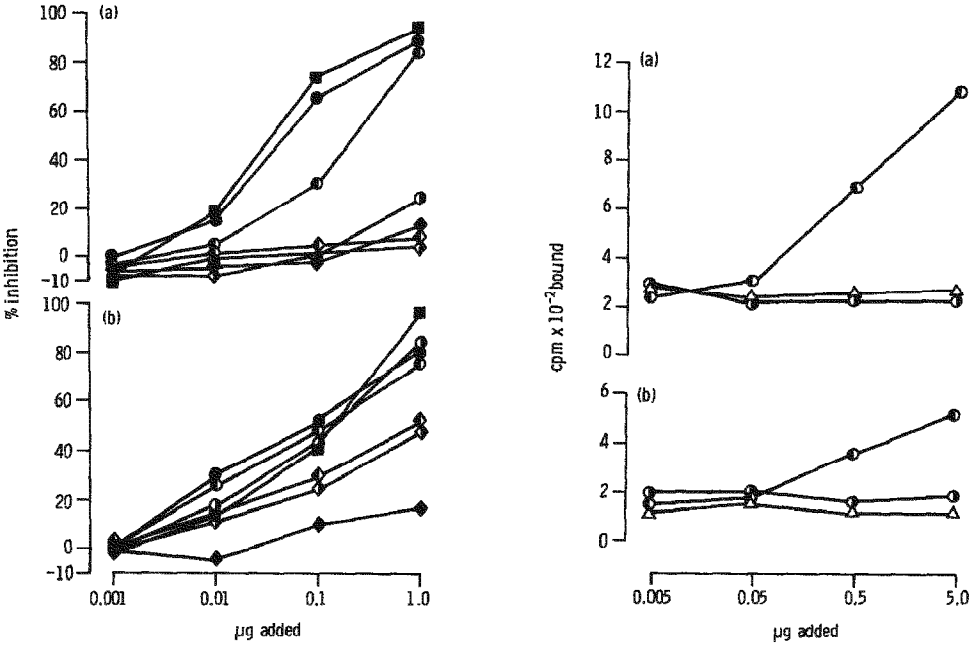

Fig. 4. Inhibition of binding of monoclonal antibodies (*a*) 115C2 and (*b*) anti-SSEA-1 to meconium glycoproteins: ■, meconium glycoproteins included for reference; ●, intact galactosyltransferase (preparation 1); ◐ and ◑, liposomes containing O- and N-linked oligosaccharides, respectively, derived from this preparation; ◆, deglycosylated galatosyltransferase; ◈ and ◇, liposomes containing O- and N-linked oligosaccharides, respectively, derived from the deglycosylated enzyme.

Fig. 5. Binding of antibody (*a*) 115C2 and (*b*) anti-SSEA-1 to the neoglycolipids coated onto wells of PVC plates: ◐ and ◑, neoglycolipids of O- and N-linked chains from galactosyltransferase preparation 1; △, neoglycolipids from maltose.

Inhibition radioimmunoassays. — The Le^a and SSEA-1 activities of the combined O- and N-linked oligosaccharides were examined first in inhibition-of-binding assays using (*a*) the free oligosaccharides, (*b*) the free oligosaccharides sonicated in the presence of cholesterol, and (*c*) the neoglycolipids sonicated in the presence of cholesterol (Fig. 3). Inhibition of binding of 115C2 and anti-SSEA-1 antibodies was observed only in (*c*). Thus, conjugation to lipid prior to incorporation into cholesterol–PPEADP liposomes was required for antigenic activity.

Le^a and SSEA-1 activities were detected with neoglycolipids of both the O- and N-linked fractions when they were separately incorporated into liposomes and tested as inhibitors of the binding of antibodies 115C2 and anti-SSEA-1 (Fig. 4). Le^a activity was more strongly expressed by the O-linked liposomes (0.2 μg of enzyme-equivalent gave 50% inhibition) than by the N-linked liposomes (30% inhibition at 1.0 μg), whereas they expressed SSEA-1 activity to a comparable degree (0.10 and 0.14 μg, respectively, gave 50% inhibition). With anti-SSEA-1, the liposomes showed antigenic activities that were comparable with those of the original enzyme glycoprotein but, with 115C2 antibody, they were less active.

In order to assess the effectiveness of the chemical deglycosylation procedure[14], galactosyltransferase preparation 3, which had been subjected to the deglycosylation procedure, and the neoglycolipids derived from the residual O- and N-linked chains therefrom, were tested as inhibitors. The results indicated that the deglycosylation was extensive but incomplete. The inhibitory activities of the deglycosylated enzyme and of the neoglycolipids were substantially reduced with anti-SSEA-1 and virtually abolished with antibody 115C2 (Fig. 4).

Negative controls involved liposomes containing neoglycolipids derived from maltose and material derived from mock oligosaccharide-release experiments with bovine serum albumin. All were inactive as inhibitors of the two monoclonal antibodies (Fig. 3).

No inhibitory activity was detected with rabbit antibodies to bovine serum albumin, using the liposomes from bovine serum albumin at the highest level tested (corresponding to 10 μg of the original protein); at this level, the undegraded protein gave 70% inhibition of antibody binding (results not shown). Hence, any traces of peptide material in the liposome preparations derived from bovine serum albumin did not react with rabbit antibodies to this protein.

PVC-plate binding assays. — The reactivities of the lipid conjugates of the O- and N-linked fractions were also tested in direct binding assays after absorption onto the wells of PVC plates (Fig. 5). The O-linked fraction bound to 115C2 and SSEA-1 antibodies at antigen levels as low as 0.5 μg, but the N-linked chains did not bind at the highest level tested (5 μg).

Chromatogram binding assays. — In this assay (carried out with 115C2 antibody only), an immunoreactive band was detected in the O-linked fraction (Fig. 1), in the region of faintly orcinol-stained, slowly migrating components rather than in the main bands. No immunoreactive components could be detected in the N-linked fraction (not shown).

DISCUSSION

The neoglycolipid micro-immunoassay procedures recently described[3] could be applied readily in the antigenic analysis of the oligosaccharides of the galactosyltransferase glycoprotein which contains ~10% of carbohydrate. The O- and N-linked chains were sequentially released by treatment with alkaline borohydride and hydrazinolysis and freed from 99% of the original peptide material, and their antigenicities were assayed by antibody inhibition or binding assays after conjugation to PPEADP. The amounts (300–600 μg) of enzyme glycoprotein that yielded neoglycolipid material sufficient for numerous assays were by no means a minimum, and our exploratory experiments (inhibition radioimmunoassays and PVC-plate binding assays) have been performed using 30–50 μg of enzyme glycoprotein containing 3–5 μg of carbohydrate.

Several factors influence the antigenicities of the carbohydrate moieties of glycolipids[9–11]. Antigenicity is enhanced by multivalent (clustered) display and impaired by the presence of irrelevant saccharides. In addition, correct orientation of the carbohydrate chains on liposomes or insoluble matrices is important. These variables are difficult to control with mixtures of oligosaccharides of unknown compositions and proportions. In the present studies, the antigenicities detected varied with the three assay procedures, and more than one assay procedure was necessary for antigenic assignment. With the mixtures of oligosaccharides released from galactosyltransferase, the inhibition radioimmunoassays were the most informative, and they revealed stronger Le^a antigen activity in the O-linked fractions but equivalent SSEA-1 antigen activities in the O- and N-linked fractions. However, in the direct binding assay on PVC plates using the anti-Le^a and anti-SSEA-1 antibodies, and in the chromatogram binding assay using 115C2 antibody, antigenic activity could be detected only with the O-linked fraction. The lack of reactivity of the N-linked fraction in the chromatogram binding and PVC-plate binding assays probably reflects the small amounts of these chains and the relative excess of PPEADP in the reaction mixture which interfered less in the liposome-inhibition assay than in the direct binding assay. This possibility should be considered if antigens are not detected in all three assay systems.

The amounts of residual peptide material in the oligosaccharide fractions were very small. However, in view of the potential of the neoglycolipid approach for establishing the saccharide nature of glycoprotein epitopes, several aspects of specificity for carbohydrate rather than peptide were investigated. It was found that conjugation to lipid was essential for detecting antigenic activity. As predicted, with an exclusively protein antigen (bovine serum albumin), there was no antigenic activity with the anti-protein antibodies when products obtained under the conditions used for preparing neoglycolipid liposomes of the O- and N-linked chains of galactosyltransferase were used as inhibitors of antibody binding.

Chemical deglycosylation procedures are often applied to glycoproteins in order to determine whether the antigens they express are associated with the

peptide or saccharide moieties. The inhibition of binding assay with neoglycolipid liposomes derived from deglycosylated galactosyltransferase was a convenient means of detecting residual oligosaccharide antigens. There was a loss of Le^a activity, but a considerable amount of residual SSEA-1 activity was detectable on both O- and N-linked fractions. On the other hand, the diminished antigenic activity provided additional evidence for carbohydrate specificity in the inhibition of binding assay.

The immunochemical studies with the O- and N-linked fractions (also supported by the chromatographic profiles of the N-linked chains on Con A–Sepharose) indicate that the fuco-oligosaccharide sequences with Le^a and SSEA-1 antigenicities, which are detectable by immunoblotting of the galactosyltransferase and can elicit an immune response in rabbits[6], are distributed on both the O- and N-linked chains of this enzyme glycoprotein. Whilst this study was under way, the occurrence of the SSEA-1 antigen sequence (3-fucosyl-*N*-acetyl-lactosamine) was detected[13] by chemical means among the *N*-linked oligosaccharides of human milk galactosyltransferase.

EXPERIMENTAL

Galactosyltransferase from human milk. — Human milk galactosyltransferase (provided by Dr. E. G. Berger) was isolated as described previously[5]. Three preparations of the enzyme were used: (1) was combined galactosyltransferase from donors of unknown blood-groups, (2) was from combined milk from blood-group A donors, and (3) was from a single donor (blood-group O non-secretor). For deglycosylation[14], an aliquot of galactosyltransferase preparation 3 was treated with anhydrous hydrogen fluoride for 1 h at 4°. The mixture was dialysed extensively against water and then lyophilised, and the residue was dissolved in isotonic saline.

Preparation of oligosaccharides. — Galactosyltransferase (600 μg each of preparations 1 and 3 or 300 μg of preparation 2) containing 5% of hexose, as determined by the phenol–sulphuric acid assay[15] using preparation 3, was treated[16] with 0.05M NaOH–MNaBH$_4$ (200 μL) at 50° for 24 h in order to release the O-glycosidically linked oligosaccharides. After acidification with acetic acid to pH 5.0, the mixture (250 μL) was applied to a column (0.4 × 1 cm) of Dowex AG50W-X8 (H^+) resin (200–400 mesh). Elution with water (1.2 mL) gave the O-glycosidically linked oligosaccharides (O-linked fraction). The fraction was concentrated to dryness at 60° *in vacuo* and methanol (200 μL) was thrice evaporated from the residue to remove boric acid. Elution with 3M ammonium hydroxide (1.2 mL) gave the peptides and glycopeptides with *N*-glycosidically linked oligosaccharides. The fraction was concentrated to dryness at 60° *in vacuo* and water (500 μL) was evaporated from the residue which was subjected to hydrazinolysis and *N*-acetylation[17]. The products (250 μL) were eluted from a column of Dowex AG50W-X8 (H^+) resin, as described above. The unretained fraction contained the N-linked oligosaccharides (N-linked fraction). The amounts of the O- and N-linked oligosaccharides released from

galactosyltransferase, which could not be measured on account of the small amounts available, are expressed in terms of the weight of glycoprotein used. Analysis of the dansyl amino acids[18] obtained from galactosyltransferase preparation 1, and from the O- and N-linked oligosaccharide fractions derived therefrom, showed that the latter fractions contained 0.4 and 0.6%, respectively, of the amino acids present in the enzyme preparation. Fractions corresponding to O- and N-linked oligosaccharides were also obtained from 100 μg of galactosyltransferase preparation 3 which had been deglycosylated.

In a mock-oligosaccharide-release experiment, fractions corresponding to O- and N-linked fractions were obtained by using bovine serum albumin (fraction V, Miles Scientific). N-Linked oligosaccharides obtained using the above procedures from fetuin (type IV, Sigma), asialofetuin (obtained by hydrolysis of fetuin: 12.5mM H_2SO_4, 80°, 1 h), ovalbumin (grade V, Sigma), and human transferrin (Sigma) were used as reference compounds. Additional reference compounds were galactose, maltose, sialyl-lactose [α-NeuAc-(2→3)-β-Gal-(1→4)-β-Glc] (Sigma), lacto-*N*-neotetraose [β-Gal-(1→4)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc] (gift from Dr. Winifred M. Watkins), sialyl-lacto-*N*-tetraose [α-NeuAc-(2→3)-β-Gal-(1→3)-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc], disialyl-lacto-*N*-tetraose {α-NeuAc-(2→3)-β-Gal-(1→3)-[α-NeuAc-(2→6)]-β-GlcNAc-(1→3)-β-Gal-(1→4)-Glc} (gifts from Drs. G. Strecker and J. C. Michalski).

T.l.c. — Oligosaccharides were resolved by h.p.t.l.c. on Silica Gel 60 (Merck, 5641), using 1-butanol–acetone–water (6:5:4) and detection with 0.2% orcinol in 2M ethanolic H_2SO_4.

Chromatography on concanavalin A (Con A)–agarose. — N-Linked oligosaccharides were chromatographed (2.1-mL fractions) on a column of Con A–agarose (8 mg of Con A/mL of agarose, Sigma) equilibrated in a solution containing 10mM Tris-HCl (pH 8.0), 150mM NaCl, mM $CaCl_2$, mM $MgCl_2$, and 0.2% of NaN_3 (Con A buffer) as described previously[7,19]. One aliquot of the N-linked fraction derived from 10 μg of galactosyltransferase preparation 1 was labelled by reduction[4] with NaB^3H_4. Another aliquot was treated[20] with 0.02M H_2SO_4 (100°, 150 min) in order to release the acid-labile residues, and the products were reduced with NaB^3H_4. The ^3H-labelled products (in 200 μL of Con A buffer) were eluted from the Con A–agarose column with Con A buffer containing 10mM methyl α-D-glucopyranoside and 500mM methyl α-D-mannopyranoside. As reference compounds, N-linked fractions obtained from 10 μg each of fetuin, ovalbumin, or transferrin were similarly labelled and chromatographed. The radioactivity of each fraction was determined by scintillation counting, and the elution profiles were obtained after subtraction of radioactive counts (63,000 c.p.m. in the excluded volume) in a labelling experiment performed in the absence of oligosaccharides. Under these conditions, the radioactive counts attributable to the reference N-linked oligosaccharides chromatographed in accordance with their known structures as follows. With fetuin[21], all of the counts (345,000 c.p.m.) were in the excluded

volume, as expected of triantennary complex chains[7,22]; with transferrin[23] (total counts, 231,000 c.p.m.), 14% was in the excluded volume and 86% was eluted with 10mM methyl α-D-glucopyranoside, as expected for triantennary and biantennary complex chains[7,21]; and with ovalbumin[24] (total counts, 307,000 c.p.m.), 15% was in the excluded volume and 85% was eluted with 500mM methyl α-D-manno-pyranoside, as expected for triantennary and high-manno-oligosaccharides, respectively[7,21].

Neoglycolipids. — For the antibody-binding assays on PVC plates and inhibition-of-binding assays, the reduced O-linked oligosaccharide fractions released from galactosyltransferase (100–600 μg) were dried at 60° *in vacuo* and oxidised[3,25] with sodium periodate (10–60 μg as appropriate) in 40mM imidazole hydrochloride (pH 6.5, 100 μL) at 0° for 45 min. To the resulting mixtures was added chloroform–methanol (1:1; 240–480 μL, as appropriate) containing PPEADP (240–480 μg) and $NaCNBH_3$ (100–200 μg), and the mixtures were incubated[3] at 50° for 16 h. The reducing oligosaccharides released by hydrazinolysis of galactosyltransferase (100–600 μg) were coupled to PPEADP as above without periodate oxidation*. The entire reaction mixtures were used for antibody binding on PVC plates and inhibition-of-binding assays.

For the chromatogram binding assays, O-linked oligosaccharides from 15 μg of galactosyltransferase (preparation 1) were subjected to h.p.t.l.c., oxidised with periodate *in situ*, and coupled to PPEADP as described previously[3]. N-Linked oligosaccharides from 15 μg of galactosyltransferase were chromatographed and coupled to PPEADP without periodate oxidation.

Immunological assays. — The specificity of mouse hybridoma antibody 115C2 (referred to as anti-Le^a, a gift from Drs. J. Hilkins and J. Hilgers) involves[26] the Le^a-related sequence β-Gal-(1→3)-[α-Fuc-(1→4)]-β-GlcNAc-(1→3)-β-Gal-(1→4)-[α-Fuc-(1→3)]-Glc/GlcNAc. The specificity of anti-SSEA-1 (a gift from Dr. D. Solter) involves[27] the 3-fucosyl-*N*-acetyl-lactosamine sequence, β-Gal-(1→4)-[α-Fuc-(1→3)]-GlcNAc. Both antibodies were used as ascites fluids. Rabbit antibodies to bovine serum albumin were purchased from Miles Scientific. Rabbit antibodies to mouse immunoglobulins and swine antibodies to rabbit immunoglobulins were purchased from Dako immunoglobulins.

For the solid-phase radio-binding assays, solutions of neoglycolipids from galactosyltransferase preparation 1 (10 μg of enzyme equivalent) were dried under N_2, dissolved in aqueous 40% methanol, and serially diluted. Aliquots (50 μL) were used for coating onto the wells of 96-well round-bottom flexible polyvinyl chloride (PVC) microtitre plates (Linbro, Flow Laboratories), and their binding to 1:100 dilutions of antibodies 115C2 and anti-SSEA-1 (or normal mouse serum, 1:100, as a negative control) was assayed[3] using ^{125}I-labelled mouse immunoglobulins.

*The conditions for maximal coupling of reduced and non-reduced oligosaccharides are currently being optimized.

For the inhibition radioimmunoassays, liposomes were prepared by mixing oligosaccharides conjugated to PPEADP with cholesterol (ratio of cholesterol to hexose in the original glycoprotein, 4:1). The liposomes were used[3,26,27] as inhibitors of the binding of antibodies 115C2 (1:150,000 dilution) and anti-SSEA-1 (1:2,000 dilution) to human meconium glycoproteins immobilised on PVC plates (0.15 μg per well). Lecithin was omitted as carrier lipid since, subsequent to the original report[3], it was found not to be required for antigenicity of the PPEADP-containing neoglycolipid liposomes. In one experiment with galactosyltransferase preparation 2, the periodate-oxidised O-linked and N-linked fractions were combined prior to conjugation to PPEADP and incorporation into liposomes. In another experiment, liposomes with neoglycolipid material derived from deglycosylated galactosyltransferase were prepared using reagent proportions and conditions identical to those with the native enzyme. As negative controls, liposomes prepared from the neoglycolipid of maltose and from the products of mock-oligosaccharide release experiments using bovine serum albumin were used. In the control, the fractions corresponding to periodate-oxidised O- and N-linked fractions were combined. Inhibition radioimmunoassays with antiserum to bovine serum albumin were performed by an adaptation of the above method using immobilised bovine serum albumin (1 μg per well), antiserum (1:70 dilution), and ^{125}I-labelled antibodies to rabbit immunoglobulins (10^5 c.p.m.).

Chromatogram binding assays were performed[3] using the O- and N-linked fractions from galactosyltransferase preparation 1 which had been subjected to h.p.t.l.c. and then conjugated to PPEADP, as described above, and overlaid with antibody 115C2 (1:100 dilution) or normal mouse serum (1:100 dilution) followed by ^{125}I-labelled rabbit anti-mouse immunoglobulins.

ACKNOWLEDGMENTS

We thank Dr. E. G. Berger for providing native and deglycosylated galactosyltransferase and for stimulating these studies, Drs. J. Hilkins, J. Hilgers, and D. Solter for the monoclonal antibodies, Drs. J. C. Michalsky, G. Strecker, and W. M. Watkins for the reference oligosaccharides, and Drs. R. A. Childs and E. F. Hounsell for helpful criticism.

REFERENCES

1 T. Feizi, H. C. Gooi, R. A. Childs, J. K. Picard, K. Uemura, L. M. Loomes, S. J. Thorpe, and E. F. Hounsell, *Biochem. Soc. Trans.*, 12 (1984) 591–596.
2 T. Feizi, *Nature (London)*, 314 (1985) 53–57.
3 P. W. Tang, H. C. Gooi, M. Hardy, Y. C. Lee, and T. Feizi, *Biochem. Biophys. Res. Commun.*, 132 (1985) 474–480.
4 P. W. Tang, P. Scudder, H. Mehmet, E. F. Hounsell, and T. Feizi, *Eur. J. Biochem.*, 160 (1986) 537–545.
5 A. C. Gerber, I. Kodrowski, S. R. Wyss, and E. G. Berger, *Eur. J. Biochem.*, 93 (1979) 453–460.

6 R. A. CHILDS, E. G. BERGER, S. J. THORPE, E. AEGERTER, AND T. FEIZI, *Biochem. J.*, 238 (1986) 605–611.
7 R. D. CUMMINGS AND S. KORNFELD, *J. Biol. Chem.*, 257 (1982) 11235–11240.
8 G. J. A. M. STROUS AND E. G. BERGER, *J. Biol. Chem.*, 257 (1982) 7623–7628.
9 D. M. MARCUS AND G. A. SCHWARTING, *Adv. Immunol.*, 23 (1976) 203–240.
10 T. FEIZI, R. A. CHILDS, S. HAKOMORI, AND M. E. POWELL, *Biochem. J.*, 173 (1978) 245–254.
11 R. KANNAGI, R. STROUP, N. A. COCHRAN, D. L. URDAL, W. W. YOUNG, JR., AND S. HAKOMORI, *Cancer Res.*, 43 (1983) 4997–5005.
12 G. R. GRAY, *Arch. Biochem. Biophys.*, 163 (1974) 426–428.
13 T. ENDO, J. AMANO, E. BERGER, AND A. KOBATA, *Carbohydr. Res.*, 150 (1986) 241–263.
14 A. J. MORT AND D. T. LAMPORT, *Anal. Biochem.*, 82 (1977) 289–309.
15 M. DUBOIS, K. A. GILLES, J. K. HAMILTON, P. A. REBERS, AND F. SMITH, *Anal. Chem.*, 28 (1956) 350–356.
16 R. N. IYER AND D. M. CARLSON, *Arch. Biochem. Biophys.*, 142 (1971) 101–105.
17 S. TAKASAKI, T. MIZUOCHI, AND A. KOBATA, *Methods Enzymol.*, 83 (1982) 263–268.
18 L. N. MACKEY AND T. A. BECK, *J. Chromatogr.*, 240 (1980) 455–461.
19 R. A. CHILDS, M. GREGORIOU, P. SCUDDER, S. J. THORPE, A. R. REES, AND T. FEIZI, *EMBO J.*, 3 (1984) 2227–2233.
20 H. C. GOOI, E. F. HOUNSELL, J. K. PICARD, A. LOWE, D. VOAK, E. LENNOX, AND T. FEIZI, *J. Biol. Chem.*, 260 (1985) 13218–13224.
21 J. U. BAENZIGER AND D. FIETE, *J. Biol. Chem.*, 254 (1979) 789–795.
22 J. U. BAENZIGER AND D. FIETE, *J. Biol. Chem.*, 254 (1979) 2400–2407.
23 G. A. JAMIESON, M. JETT, AND S. L. DE BERNARDO, *J. Biol. Chem.*, 246 (1971) 3686–3693.
24 T. TAI, K. YAMASHITA, M. OGATA-ARAKAWA, N. KOIDE, T. MURAMATSU, S. IWASHITA, Y. INOUE, AND A. KOBATA, *J. Biol. Chem.*, 250 (1975) 8569–8575.
25 R. H. RAJA, R. D. LEBOEUF, G. W. STONE, AND P. H. WEIGEL, *Anal. Biochem.*, 139 (1984) 168–177.
26 H. C. GOOI, N. J. JONES, E. F. HOUNSELL, P. SCUDDER, J. HILKENS, J. HILGERS, AND T. FEIZI, *Biochem. Biophys. Res. Commun.*, 131 (1985) 543–550.
27 H. C. GOOI, T. FEIZI, A. KAPADIA, B. B. KNOWLES, D. SOLTER, AND M. J. EVANS, *Nature (London)*, 292 (1981) 156–158.