Biochem. J. (1978) **170**, 497–486
Printed in Great Britain

The Formation of Lipid-Linked Sugars by Cell-Free Preparations of Lactating Rabbit Mammary Gland

By DAVID A. WHITE
Department of Biochemistry, University Hospital and Medical School, Clifton Boulevard, Nottingham NG7 2UH, U.K.

(*Received* 6 *July* 1977)

1. A lactating rabbit mammary-gland microsomal system catalysed the incorporation of mannose from GDP-[U-^{14}C]mannose into three endogenous acceptors, (i) polyprenyl phosphate mannose, (ii) lipid-linked oligosaccharide and (iii) protein. 2. Synthesis of polyprenyl phosphate mannose was stimulated by addition of dolichol phosphate to the incubation medium and was reversed by addition of GDP. The product had properties identical with those of authentic dolichol phosphate mannose. 3. The oligosaccharides derived from acid hydrolysis of the lipid-linked oligosaccharide fraction were of six, eight and nine to ten monosaccharide units, the octasaccharide being the major species formed. The oligosaccharide appeared to be attached to the lipid via a pyrophosphate bridge, since strong alkaline hydrolysis liberated an oligosaccharide phosphate. 4. Polyprenyl phosphate mannose served as a mannose donor to lipid-linked oligosaccharides and protein. When added as exogenous substrate it gave rise to a lipid-linked oligosaccharide of about six units. 5. Incorporation of radioactivity in protein was low, but polyacrylamide-gel electrophoresis of the protein fractions indicated that polypeptides of mol.wts. 115000, 75000 and 33000 were labelled.

The internal core region of the oligosaccharide chains of some glycoproteins is probably synthesized as a lipid-linked oligosaccharide (Behrens, 1974), and this oligosaccharide is then transferred to a growing polypeptide chain (Scheme 1).

Epithelial tissue of lactating rabbit mammary gland is continually synthesizing glycoproteins, both as constituents of milk and also to replace membrane glycoproteins lost during the secretory process. The present paper describes a microsomal preparation of lactating rabbit mammary gland that catalyses the formation of two lipid-linked sugar compounds that appear to be involved in the glycosylation of some proteins of the tissue. A preliminary report of this work has been presented (White, 1977).

Materials and Methods

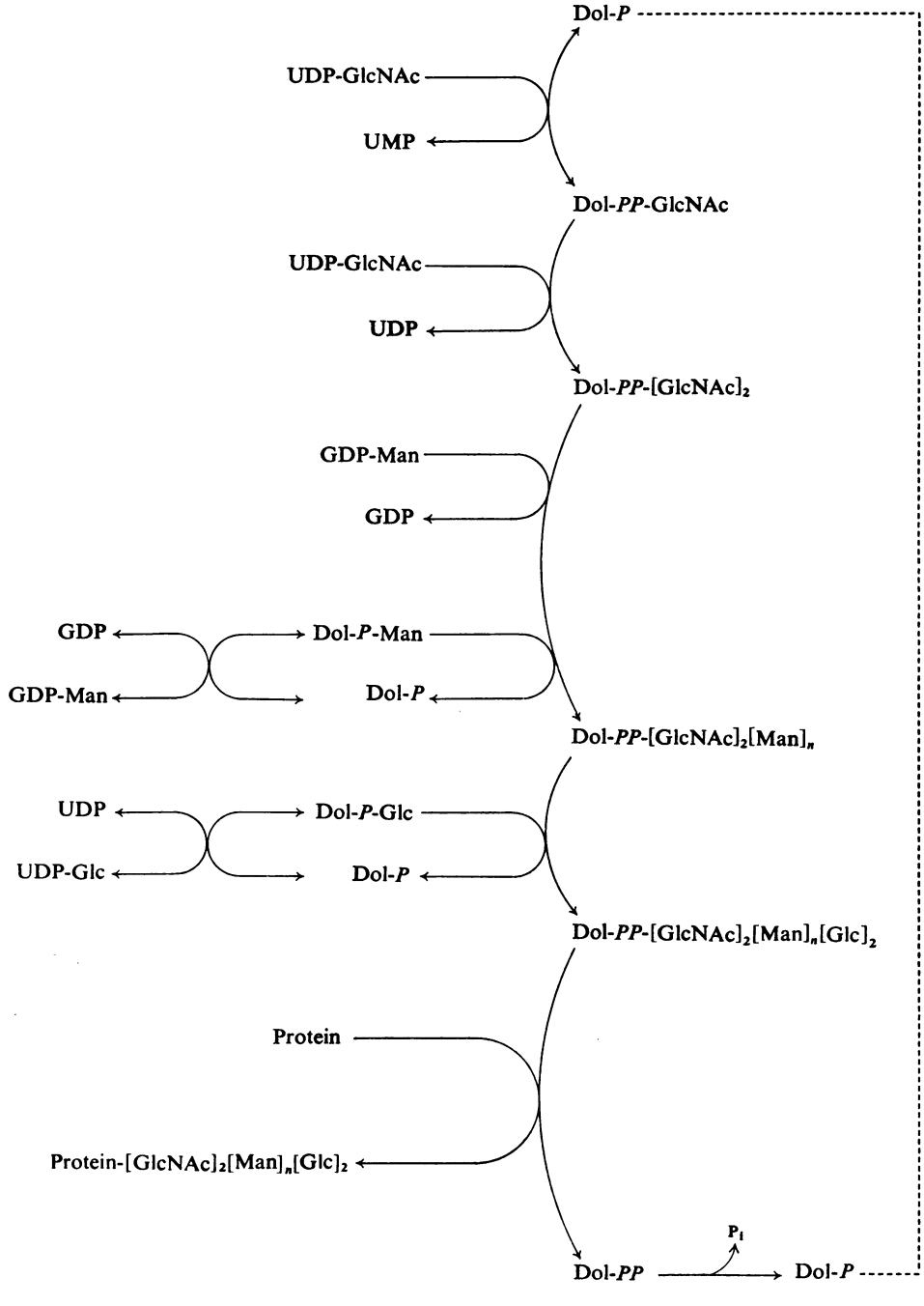
GDP, GMP and GDP-mannose were purchased from Sigma (London) Chemical Co., London S.W.6, U.K. GDP-[U-^{14}C]mannose was purchased from The Radiochemical Centre, Amersham, Bucks., U.K. All other chemicals and solvents were A.R. grade and purchased from commercial suppliers. I am indebted to Professor F. W. Hemming (Department of Biochemistry, University of Nottingham) for a gift of dolichol phosphate and to Dr. J. F. Kennedy (Department of Chemistry, University of Birmingham) for a gift of an amylose hydrolysate.

Protein was determined by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

New Zealand White rabbits were obtained from the Joint Animal Breeding Unit, University of Nottingham School of Agriculture, Sutton Bonington, Leics., U.K.

Preparation of microsomal fractions

A 14-day-lactating rabbit was killed by cervical fracture and the mammary gland removed. This was well washed with homogenizing buffer to remove milk and dissected free of fat and connective tissue. The remaining alveolar tissue was chopped into small pieces with scissors and homogenized in potassium phosphate buffer (250mM), pH7.0, containing mercaptoethanol (5mM) and EDTA (1mM) to give a final 2:1 (w/v) homogenate. The homogenate was filtered through two thicknesses of nylon bolting cloth and centrifuged at 10000*g* for 20min. The pellet was discarded and the supernatant fraction was centrifuged at 100000*g* for 2h. The resulting pellet was retained as microsomal fraction I. Further centrifugation of the supernatant fraction at 100000*g* for 2h gave a pellet (microsomal fraction II), and centrifugation of the supernatant fraction from this run at 100000*g* for 12h provided another pellet (microsomal fraction III). Layers of fat floated to the surface of the centrifuge tubes, particularly in the



Scheme 1. *Pathway proposed by Behrens* (1974) *for glycosylation of some glycoproteins*
Abbreviation: Dol, dolichol.

early centrifugations, and these were removed after each run. Each of the microsomal fractions was resuspended in 100mM-Tris/maleate buffer, pH7.0, at a protein concentration of 80–100mg/ml. A microsomal fraction was also prepared by homogenizing the tissue in 0.3M-sucrose adjusted to pH7.0 with Tris base and following the above procedure to microsomal fraction I.

Chromatographic methods

Chromatography on paper, thin layer and DEAE-cellulose acetate was performed as described by Speake & White (1978).

Assay of the incorporation of [^{14}C]mannose from GDP-[U-^{14}C]mannose into endogenous acceptors of mammary-gland microsomal fraction

The standard assay mixture contained 5μM-GDP-[U-^{14}C]mannose (80000d.p.m.), 10mM-$MnCl_2$, 3mM-NaF, 2mM-MgATP, 2.5mM-EDTA, approx. 5.0mg of protein and 100mM-Tris/maleate buffer, pH7.0, in a final incubation volume of 200μl. The reaction was terminated by addition of chloroform/methanol (2:1, v/v; 4ml) and three fractions were obtained as described by White & Waechter (1975). Radioactivity in each fraction was determined as described by Speake & White (1978). When the effect of GDP was determined, the above system was incubated for 30s before addition of GDP (final concentration 200μM).

Large-scale preparation of [^{14}C]mannolipids and [^{14}C]protein

Bulk incubations containing the ingredients of the standard assay mixture in a final volume of 1.6ml were incubated at 37°C for 2.5min for isolation of mannolipid A or for 60min for mannolipid B and mannoprotein. Incubations were terminated with chloroform/methanol (2:1, v/v; 25ml) and extractions were carried out as described above by using appropriately scaled-up volumes of each solvent.

Incorporation of [^{14}C]mannose from mannolipid A into endogenous acceptors

(*i*) *With endogenous substrate.* Endogenous mannolipid A was prepared by incubating GDP-[U-^{14}C]-mannose (4μM; 1000000d.p.m.), MgATP (1.8mM), $MnCl_2$ (10mM), EDTA (2.5mM), NaF (3mM) and microsomal protein (70mg) in a final volume of 2ml at 37°C. After 2.5min, ice-cold unlabelled GDP-mannose (4μM; 22ml) was added and the mixture was centrifuged at 1000*g* for 25min at 4°C. The resulting pellet was resuspended in 100mM-Tris/maleate buffer, pH7.4, containing MgATP (1.8mM), $MnCl_2$ (10mM) and NaF (3mM) at a final volume of 3.5ml and incubated at 37°C. Samples (200μl) were removed at given times and extracted with chloroform/methanol (2:1, v/v; 4ml). Mannolipid and protein fractions were obtained as described above.

(*ii*) *With exogenous substrate.* Mannolipid A (30000c.p.m.), partially purified by DEAE-cellulose acetate chromatography, was sonicated in sodium deoxycholate (0.4%), at 45kHz in a Kerry sonicator (Kerry Ultrasonics, Hitchin, Herts., U.K.) and $MnCl_2$ (10mM) and microsomal protein (50mg) were added. After incubation at 37°C for 40min, chloroform/methanol (2:1, v/v; 6ml) was added with rapid mixing. Methanol (2ml) and NaCl (0.9%; 2.6ml) were added, mixed and centrifuged at 2000*g* for 10min to obtain two phases separated by a protein interphase. The upper phase was removed and the lower phase and pellet were washed with synthetic upper phase. After removing the lower phase the pellet was re-extracted with chloroform/methanol (2:1, v/v; 2ml) and then twice with chloroform/methanol/water (10:10:3, by vol.; 5ml). This fraction was analysed for lipid-linked oligosaccharide.

Partial characterization of mannolipid B

Mannolipid B was purified by DEAE-cellulose acetate chromatography and subjected to acid and alkaline hydrolysis (Speake & White, 1978). Paper electrophoresis of the products of alkaline hydrolysis was performed by the procedure of Waechter *et al.* (1975) in pyridine/acetate buffer, pH3.4 [pyridine/acetic acid/water, 1:10:189, by vol.]. The size of the oligosaccharide released by mild acid hydrolysis was determined by paper chromatography as described by Lucas *et al.* (1975) and Speake & White (1978).

Analysis of residual protein material

The residue left after extraction of the two mannolipids was solubilized by boiling for 3min in 50mM-sodium phosphate buffer, pH7.4, containing 1mM-dithiothreitol and 2% (w/v) sodium dodecyl sulphate. After centrifugation at 2000*g* for 10min to remove non-solubilized material, the supernatant fraction was applied to a column (21cm×3cm) of Sephadex G-75 loaded in 50mM-sodium phosphate, pH7.4, containing 1mM-dithiothreitol and 2% sodium dodecyl sulphate, and eluted with the same buffer. The column eluate was monitored for protein and radioactivity. Samples of the solubilized-protein peak were also examined by sodium dodecyl sulphate/polyacrylamide-gel electrophoresis on slab gels by using the discontinuous buffer system of Laemmli (1970) as described by Laskey *et al.* (1972). Parallel samples and reference proteins (β-galactosidase, bovine serum albumin, ovalbumin and lysozyme) were co-electrophoresed. The duplicate samples and reference gels

were stained for protein with Coomassie Blue (Betts & Mayer, 1975) and the lane containing the test sample was cut into slices (2mm) for assay of radioactivity.

Results

Incorporation of mannose from GDP-[U-^{14}C]mannose into endogenous acceptors of microsomal fractions of lactating rabbit mammary gland

The specific activities of the incorporation of radioactivity into chloroform/methanol(2:1, v/v)-soluble material for each of the microsomal fractions I, II and III from a typical fractionation were 42.02, 3.85 and 0.37c.p.m./min per mg of protein respectively. All subsequent experiments were performed with fraction I.

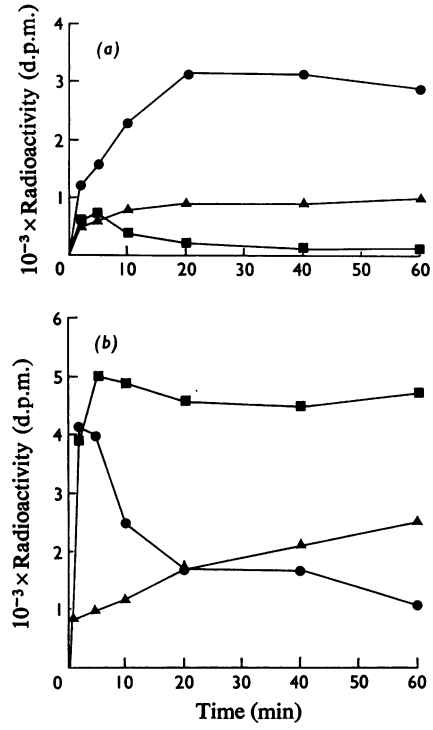


Fig. 1. *Time course for the incorporation of [^{14}C]mannose into endogenous acceptors of lactating rabbit mammary-gland microsomal fraction*

The incubation medium (200μl) contained GDP-[U-^{14}C]mannose (5μM, 80000d.p.m.), 10mM-$MnCl_2$, 3mM-NaF, 2mM-MgATP, 2.5mM-EDTA and approx. 5.0mg of microsomal protein in 100mM-Tris/maleate buffer, pH7.0. Reactions were started by addition of GDP-mannose. ●, Mannolipid A; ■, mannolipid B; ▲, protein. The microsomal fractions were prepared in (*a*) sucrose or (*b*) phosphate buffer.

The time-dependent incorporation of mannose into microsomal fractions depended on the homogenization buffer. With a microsomal fraction prepared in phosphate buffer (Fig. 1*b*) [^{14}C]mannose was rapidly incorporated into two lipid fractions, one (mannolipid A) soluble in chloroform/methanol (2:1, v/v) and the other (mannolipid B) soluble in chloroform/methanol/water (10:10:3, by vol.). Radioactivity in mannolipid A was maximal at 1–2min, after which time a rapid loss occurred. Incorporation into mannolipid B was maximal at approx. 10min and radioactivity was lost only slowly over the next hour. Incorporation of radioactivity into trichloroacetic acid-insoluble material increased slowly over the experimental period. The kinetics of incorporation by a microsomal fraction prepared in iso-osmotic sucrose (0.3M) differed markedly (Fig. 1*a*). Incorporation of radioactivity into mannolipid A was linear with time for about 15min, reaching a plateau in 20min at a value similar to that for the microsomal fraction prepared in phosphate, and falling only slightly during the following 40min. Activity in mannolipid B was very much lower than in microsomal fraction prepared in phosphate, and rapidly fell to zero. Incorporation of radioactivity into protein was also much less.

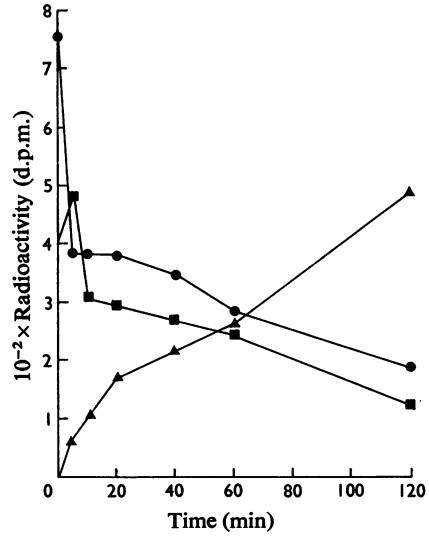


Fig. 2. *Incorporation of endogenous [^{14}C]mannolipid A into mannolipid B and protein*

^{14}C-labelled microsomal fraction was resuspended in 100mM-Tris/maleate buffer, pH7.4, containing 1.8mM-MgATP, 10mM-$MnCl_2$ and 3mM-NaF in a final volume of 3.5ml. Samples (200μl) were removed at the times indicated. ●, Mannolipid A; ■, mannolipid B; ▲, protein.

Incorporation of [^{14}C]mannose from endogenous [^{14}C]mannolipid A into mannolipid B and protein

Membrane-bound mannolipid A synthesized by incubation of GDP-mannose with a microsomal fraction for 2.5min was able to donate mannose to two endogenous acceptors (Fig. 2). Radioactivity was rapidly lost from the chloroform/methanol-soluble fraction, with a concomitant rise in radioactivity of the chloroform/methanol/water (10:10:3, by vol.)-soluble fraction. Radioactivity in this latter fraction was maximal after about 5min and was then rapidly lost. Incorporation of radioactivity into protein continued over 2h.

Characterization of mannolipids

(*a*) *Chloroform/methanol-soluble mannolipid A.* Mannolipid A co-chromatographed with authentic dolichyl phosphate mannose on DEAE-cellulose DE-52 (acetate), being bound to the column in chloroform/methanol (2:1, v/v) and eluted with either 10mM-ammonium acetate in methanol or chloroform/methanol/water (10:10:3, by vol.). It also co-chromatographed with dolichyl phosphate mannose on t.l.c. in acidic, neutral and basic solvents and liberated [^{14}C]mannose as the sole radioactive product of mild acid hydrolysis. Mannolipid A was stable to mild alkali, and its synthesis was stimulated by addition of exogenous dolichol phosphate to the standard incubation medium [e.g. − dolichol phosphate, 3059d.p.m.; + dolichol phosphate (10nM), 9931d.p.m., incubation for 1min] and reversed by addition of GDP (Fig. 3).

(*b*) *Chloroform/methanol/water* (10:10:3, *by vol.*)*-soluble mannolipid B.* (i) Chromatographic properties. Chromatography of mannolipid B on DEAE-cellulose acetate gave an elution profile very similar to that

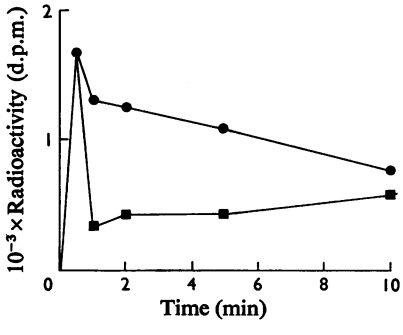


Fig. 3. *Effect of GDP on synthesis of mannolipid A* The incubation medium was as described in Fig. 1. GDP (final concn. 200μM) was added to incubations synthesizing mannolipid A, 30s after addition of GDP-[U-^{14}C]mannose. ●, Control; ■, + GDP.

Table 1. *Chromatographic properties of mannolipids A and B from incubations of microsomal fractions of lactating rabbit mammary gland with GDP-[U-^{14}C]mannose*
For details, see the text.

Solvent	Composition	R_F Manno-lipid A	R_F Manno-lipid B
A	Chloroform/methanol/water (65:25:4, by vol.)	0.6	0.17
B	Chloroform/methanol/aq. NH_3 (sp.gr. 0.88) (13:6:1, by vol.)	0.32	0.16
C	Chloroform/methanol/formic acid/water (140:30:70:1, by vol.)	0.79	0.12
D	Isobutyric acid/water/aq. NH_3 (sp.gr. 0.88) (57:39:4, by vol.)	0.95	0.75

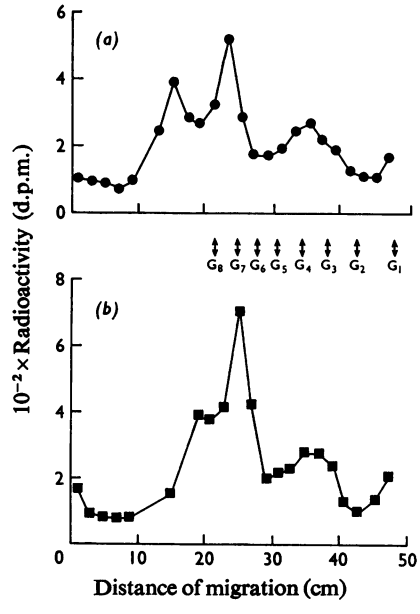


Fig. 4. *Paper chromatography of the oligosaccharides derived from mild acid hydrolysis of mannolipid B* Mannolipid B was hydrolysed with 1M-HCl at 100°C for 30min. The residue was dissolved in water and chromatography was performed on Whatman 3MM paper in isobutyric acid/water/aq. NH_3 (sp.gr. 0.88) (57:39:4, by vol.) for a period of 48h. The developed chromatogram was cut into segments (1cm) and the radioactivity in each segment was determined. The positions of saccharides derived from acid hydrolysis of amylose are shown as G_1, G_2, G_3 etc., where the subscript denotes the number of monosaccharide units in the oligosaccharide, i.e. G_1, glucose; G_2, maltose; G_3, maltotriose etc. Incubation times for synthesis of mannolipid B: (*a*) 60min; (*b*) 30min.

of the oligosaccharide isolated from mammary-gland explants, requiring 10mM-ammonium acetate in chloroform/methanol/water (10:10:3, by vol.) for elution (Speake & White, 1978). Mannolipid B also ran with a lower R_F than mannolipid A on t.l.c. in acidic, neutral and basic solvents and on t.l.c. on Avicel (Anachem, Luton, Beds., U.K.) in isobutyric acid/water/aq. NH_3 (sp.gr. 0.88) (57:39:4, by vol.) (Table 1).

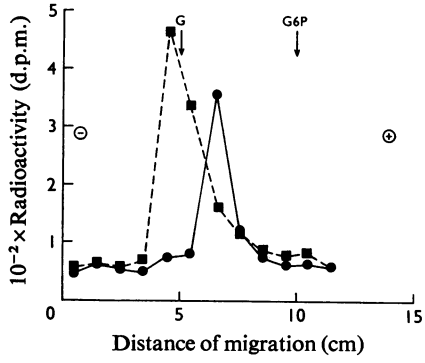


Fig. 5. *Electrophoresis of the strong-alkaline hydrolysate of mannolipid B before and after treatment with alkaline phosphatase*

Mannolipid B (9000d.p.m.) was hydrolysed with 10% (v/v) NH_3 (1ml) at 100°C for 3h. A sample was treated with calf intestinal alkaline phosphatase. The products of this treatment and of the original hydrolysate were electrophoresed at pH3.4 in pyridine/acetate buffer for 2h at 80V/cm. The paper was cut into 1cm strips and assayed for radioactivity. The positions of glucose 6-phosphate (G6P) and glucose (G) are shown.

(ii) Mild acid hydrolysis. The aqueous phase obtained after mild hydrolysis contained more than 95% of the original radioactivity, and chromatography of this phase on Whatman 3MM paper in solvent D is shown in Fig. 4. The positions of saccharides derived from hydrolysis of amylose are given as references. Three radioactive peaks were seen on scanning the chromatogram for radioactivity, corresponding to oligosaccharides of chain length six, eight and nine to ten monosaccharide units, with that of eight units being the major peak. The same profile was seen for lipid-linked oligosaccharides extracted from 30, 60 and 120min incubations.

(iii) Alkaline hydrolysis. Alkaline hydrolysis of mannolipid B gave rise to a negatively charged product that migrated towards the anode on electrophoresis at pH3.5. Treatment of this product with calf intestinal alkaline phosphatase gave rise to a compound of lower electrophoretic mobility (Fig. 5), suggesting that the major product of alkaline hydrolysis is an oligosaccharide phosphate.

Formation of mannolipid B by using exogenous mannolipid A

The chloroform/methanol/water (10:10:3, by vol).-soluble material formed from bulk incubation of exogenous mannolipid A with a microsomal fraction

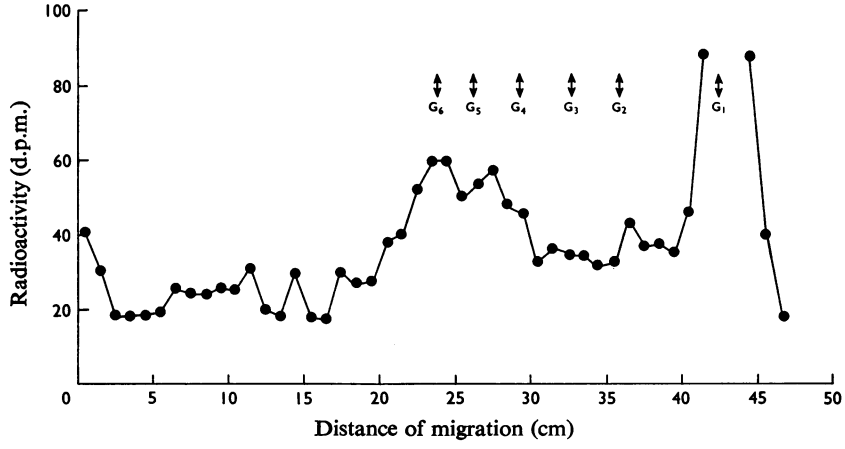


Fig. 6. *Determination of the size of the oligosaccharide of mannolipid B derived from incubation of exogenous mannolipid A with mammary microsomal fraction*

Mannolipid B derived from incubation of mannolipid A (30000d.p.m.) in sodium deoxycholate, with microsomal protein (50mg) in Tris/maleate buffer, pH7.4, containing 10mM-$MnCl_2$ was hydrolysed in 1M-HCl at 100°C for 30min. The water-soluble products were chromatographed on Whatman 3MM paper in solvent E (see the text) and the developed chromatogram was cut into strips (1cm) and assayed for radioactivity. The positions of saccharides derived from acid hydrolysis of amylose are shown as G_1, G_2 etc. (as in Fig. 4).

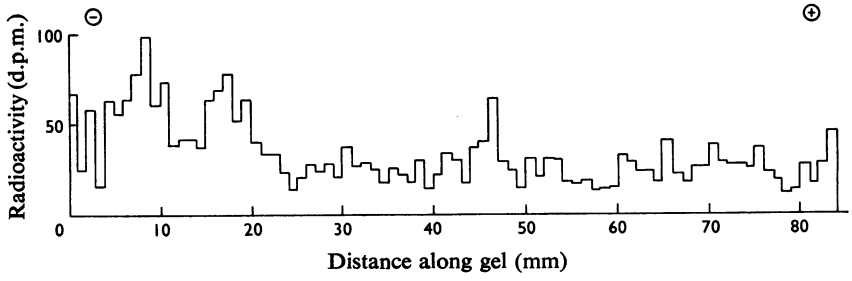


Fig. 7. *Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis of [^{14}C]mannoproteins on slab gels*
Proteins were solubilized by boiling in 80mM-Tris/HCl, pH6.8, containing 2% (v/v) sodium dodecyl sulphate, 100mM-dithiothreitol and 10% (v/v) glycerol and separated in a discontinuous buffer system.

ran with an R_F of 0.75 on Avicel in solvent E [isobutyric acid/water/aq. NH_3 (sp.gr. 0.88) (57:39:4, by vol.)] coincident with mannolipid B. The product was labile to mild acid, and paper chromatography of the water-soluble products derived from this treatment indicated an oligosaccharide of chain length six to eight monosaccharide units (Fig. 6). The large peak with an R_F corresponding to glucose is probably due to mannose derived from mannolipid A carried through in the extraction procedure.

Analysis of proteins labelled from GDP-[1-^{14}C]-mannose

The residue left after extraction of mannolipids A and B was solubilized in 100mM-sodium phosphate buffer, pH7.4, containing 2% (w/v) sodium dodecyl sulphate and subjected to gel chromatography on Sephadex G-75. Protein was eluted in two peaks, one at the void volume and a second that was just included into the column (results not shown). Most of the radioactivity was associated with the peak at the void volume and indicates that protein had been labelled during the incubation. Polyacrylamide-gel electrophoresis of the proteins eluted in the void volume indicated that at least three major polypeptides were labelled (Fig. 7). Much of the activity was in the high-molecular-weight region 115000 and 75000, with a third peak at mol.wt. of approx. 33000.

Discussion

The kinetics of incorporation of mannose from GDP-mannose into endogenous acceptors of mammary-gland microsomal fractions are similar to those reported for many other eukaryotic tissues [see reviews by Waechter & Lennarz (1976) and Hemming (1977)] and suggest that lipid-linked sugars are involved in glycosylation of proteins in this tissue. The incorporation of mannose into a polyprenyl phosphate mannose fraction is very rapid, and this fraction then loses radioactivity concomitant with an increase in radioactivity in a lipid-linked oligosaccharide, suggesting that the polyprenyl phosphate mannose is a mannose donor to the oligosaccharide-lipid. Incorporation of radioactivity into residual protein is much slower and probably reflects the very low amount of endogenous acceptor present in the tissue. If core sugars are attached to nascent polypeptide chains that are still bound to ribosomes in the mammary gland, as is suggested for hen oviduct (Kiely *et al.*, 1976), then only a very small fraction of acceptor protein would be in a situation appropriate for glycosylation in the microsomal system. The build-up of radioactivity on the oligosaccharide-lipid suggests that transfer of oligosaccharide to protein acceptor is the rate-limiting step. However, in the whole-cell system, where protein synthesis is continuing and a continual supply of protein acceptor is present, no such precursor–product relationship is seen (Speake & White, 1978). The different kinetic picture obtained with microsomal fractions prepared in sucrose is unexplained. It is similar to that seen with microsomal preparations of bovine adrenal medulla prepared in sucrose (White & Waechter, 1975). The poor incorporation of radioactivity into the lipid-linked oligosaccharide fraction possibly reflects an inhibition of the transfer of mannose from the polyprenyl phosphate mannose.

The properties of the chloroform/methanol-soluble product are consistent with those of a polyprenyl phosphate mannose; its synthesis was stimulated by dolichol phosphate and its chromatographic properties were identical with those of authentic dolichyl phosphate mannose. The chloroform/methanol/water (10:10:3, by vol.)-soluble product had properties consistent with those of a polyprenyl pyrophosphate-oligosaccharide. The size of the oligosaccharides released by mild acid were estimated by paper chromatography to be of six, eight and nine or ten monosaccharide units, with the major oligosaccharide of eight units. This compares with oligosaccharides derived from lipid-linked oligosacchar-

ides labelled from GDP-mannose in other cell-free systems [see Speake & White (1978) for a brief review]. Oligosaccharides isolated from the mammary-gland microsomal system are smaller than those derived from lipid-linked oligosaccharides extracted from mammary explants that had been incubated with radiolabelled sugars (Speake & White, 1978). The major oligosaccharides in this case were of 10–11 monosaccharide units. It seems likely that elongation of the oligosaccharide in the cell-free system is prevented by the lack of other nucleotide sugars, especially UDP-glucose. Leloir's group have shown that glucose from UDP-glucose is incorporated into dolichyl phosphate glucose and a lipid-linked oligosaccharide in rat liver, brain and kidney, human lymphocytes and pig thyroid (Behrens & Leloir, 1970; Behrens *et al.*, 1971; Parodi *et al.*, 1973); since then, Spiro *et al.* (1976) have isolated an oligosaccharide-lipid from bovine thyroid slices incubated with [U-^{14}C]glucose and detected [^{14}C]glucose on acid hydrolysis of the oligosaccharide. Herscovics *et al.* (1977) also detected glucose by g.l.c. of the sugars obtained from hydrolysis of an oligosaccharide-lipid fraction extracted from calf pancreas microsomal fraction, and Robbins *et al.* (1977) demonstrated that addition of UDP-glucose to cell-free preparations of fibroblasts synthesizing oligosaccharide lipids from GDP-mannose and UDP-*N*-acetylglucosamine increased the oligosaccharide chain length by two units. Thus it appears that glucose is a component of the lipid-linked oligosaccharide in many tissues and the absence of UDP-glucose in the mammary microsomal system may explain the difference between the whole cell and cell-free systems.

Polyprenyl phosphate mannose served as a mannose donor to the lipid-linked oligosaccharide as both endogenous and exogenous substrate. In the latter case the major lipid-linked oligosaccharide was of six monosaccharide units (Fig. 6) and again suggests a lack of substrate for elongation.

Although radioactivity from GDP-mannose was incorporated into protein in the microsomal system, analysis of this fraction proved very difficult, since the radioactivity incorporated per mg of protein was low and consequently low radioactivity counts were obtained on polyacrylamide gels. However, the pattern of radioactivity suggested that polypeptides of approx. 115000, 75000 and 33000 mol.wt. are labelled. It has not been shown conclusively that this radioactivity is being incorporated via the lipid-linked intermediates, but, if it is, then either the system is able to transfer incomplete oligosaccharide chains to protein or there is a very small amount of endogenous lipid-linked oligosaccharide that requires the addition of only a small amount of mannose before transfer to protein. The low rate of transfer of oligosaccharide to protein may reflect an inability of the microsomal system to transfer incomplete oligosaccharide chains.

It is concluded that lactating rabbit mammary gland utilizes a lipid-linked sugar system for glycosylation of some of its glycoproteins. The identity of these glycoproteins is unclear; possible candidates are membrane-bound glycoproteins involved in secretion or secreted glycoproteins such as α-lactalbumin. Rat α-lactalbumin contains *N*-acetylglucosamine, mannose, galactose, fucose and sialic acid (Brown *et al.*, 1977), which suggests that a lipid-linked oligosaccharide might be involved in its glycosylation. However, casein, the major glycoprotein synthesized by lactating rabbit mammary gland, does not appear to be glycosylated by this pathway (Speake & White, 1978).

I thank the Medical Research Council for financial support of this work and Miss Susan Hudspeth for excellent technical assistance. Dr. T. M. A. Wilson of this Department is also thanked for advice on polyacrylamide-gel electrophoresis.

References

Behrens, N. H. (1974) in *Biology and Chemistry of Eucaryotic Cell Surfaces* (Lee, E. Y. C. & Smith, E. E., eds.), pp. 159–180, Academic Press, New York

Behrens, N. H. & Leloir, L. F. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **66**, 153–159

Behrens, N. H., Parodi, A. J. & Leloir, L. F. (1971) *Proc. Natl. Acad. Sci. U.S.A.* **68**, 2857–2860

Betts, S. A. & Mayer, R. J. (1975) *Biochem. J.* **151**, 263–270

Brown, R. C., Fish, W. W., Hudson, B. G. & Ebner, K. E. (1977) *Biochim. Biophys. Acta* **491**, 82–92

Hemming, F. W. (1977) *Biochem. Soc. Trans.* **5**, 1223–1231

Herscovics, A., Golovtchenko, A. M., Warren, C. D., Bugge, B. & Jeanloz, R. W. (1977) *J. Biol. Chem.* **252**, 224–234

Kiely, M. L., McKnight, G. S. & Schimke, R. T. (1976) *J. Biol. Chem.* **251**, 5490–5495

Laemmli, U. K. (1970) *Nature (London)* **227**, 680–685

Laskey, R. A., Gurdon, J. B. & Crawford, L. V. (1972) *Proc. Natl. Acad. Sci. U.S.A.* **69**, 3665–3669

Lowry, O. H., Rosebrough, N. J., Farr, A. R. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275

Lucas, J. J., Waechter, C. J. & Lennarz, W. J. (1975) *J. Biol. Chem.* **250**, 1992–2002

Parodi, A. J., Staneloni, R., Cantarella, A. I., Leloir, L. F., Behrens, N. H., Carminatti, H. & Levi, J. A. (1973) *Carbohydr. Res.* **26**, 393–400

Robbins, P. W., Krag, S. S. & Lu, T. (1977) *J. Biol. Chem.* **252**, 1780–1785

Speake, B. K. & White, D. A. (1978) *Biochem. J.* **170**, 273–283

Spiro, M. J., Spiro, R. G. & Bhoyroo, V. D. (1976) *J. Biol. Chem.* **251**, 6400–6408

Waechter, C. J. & Lennarz, W. J. (1976) *Annu. Rev. Biochem.* **45**, 95–113

Waechter, C. J., Lucas, J. J. & Lennarz, W. J. (1975) *J. Biol. Chem.* **250**, 1992–2002

White, D. A. (1977) *Biochem. Soc. Trans.* **5**, 1022–1023

White, D. A. & Waechter, C. J. (1975) *Biochem. J.* **146**, 645–651