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Biosynthesis of glycoproteins by membranes of *Acer pseudoplatanus*

Incorporation of mannose and *N*-acetylglucosamine

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Membrane preparations from *Acer pseudoplatanus* suspension cultures were demonstrated to incorporate radioactivity from GDP-[U-^{14}C]mannose and UDP-*N*-acetyl-[6-^{3}H]glucosamine into high-molecular-weight polymers characterized as glycoprotein. From 20 to 25% of the ^{14}C was incorporated as fucose with the remainder as mannose, whereas 90% of the ^{3}H was incorporated as *N*-acetylglucosamine with the remainder as *N*-acetylgalactosamine. Pronase digestion yielded radioactive glycopeptides that were separated into four fractions by gel-permeation chromatography and paper electrophoresis. The isolated glycopeptides differed in molecular weight and isotopes incorporated, as well as in amino-acid and monosaccharide composition. The membrane preparation also incorporated radioactivity from the added nucleotides into chloroform/methanol (2:1, v/v)- and chloroform/methanol/water (10:10:3, by vol.)-soluble lipids, and into an insoluble pellet.

Cultures of sycamore maple (*Acer pseudoplatanus*), when harvested in early exponential phase, yield membrane preparations that are rich in microsomal fraction ('microsomes') (Cella *et al.*, 1976). Such preparations should be suitable for investigating plant glycoprotein biosynthesis. In trial experiments, however, incorporation of mannose from GDP-[U-^{14}C]mannose into glycoproteins was insignificant (Smith *et al.*, 1976), although incorporation did take place into mannans and lipid-linked saccharides.

The formation of lipid-linked saccharides by other plant membranes has been well documented (Beevers & Mense, 1977; Delmer *et al.*, 1978; Ericson & Delmer, 1977, 1978; Forsee & Elbein, 1973, 1976; Forsee *et al.*, 1976; Lehle *et al.*, 1976). In membranes from *Phaseolus vulgaris* radioactivity from GDP-[U-^{14}C]mannose was demonstrated to be transferred to lipid-linked oligosaccharides (Ericson & Delmer, 1978) and to dolichyl phosphate mannose (Delmer *et al.*, 1978). Presumably, these lipid-linked saccharides are intermediates and are involved in a metabolic pathway similar to the mammalian pathway (Waechter & Lennarz, 1976; Parodi & Leloir, 1976) for the biosynthesis of the mannosyl-*NN'*-diacetylchitobiosyl asparagine linkage of glycoproteins. The role of the lipid-linked saccharides in *Acer* is therefore unclear, since no glycoproteins were found radiolabelled after incubation with GDP-[U-^{14}C]mannose (Smith *et al.*, 1976).

We have re-investigated the role of GDP-mannose as a precursor for protein-bound saccharides in *Acer* membranes. We have approached the isolation of labelled glycoproteins in a manner that relies on the solubilization of membrane glycoproteins before isolation, without use of organic-solvent extraction. This approach led directly to a solubilized fraction that could be characterized more easily.

Recent evidence suggests that the rough endoplasmic reticulum contains the enzymes necessary to mediate lipid-linked protein glycosylation in eukaryotic organisms (Czichi & Lennarz, 1977; Nagahashi & Beevers, 1968). Since this could be a critical factor, care was taken to ensure that the membranes used for our study contained this subcellular component.

Interconversion of sugar nucleotides follows well known biochemical pathways in both plants (Liao & Barber, 1971) and animals (Ginsburg, 1960). Thus metabolism of the added radiolabelled nucleotide before incorporation into high-molecular-weight material might be expected to occur. We have found that our membrane preparations do incorporate mannose into glycoproteins, and also retain the necessary discrimination to convert mannose into fucose and to insert it selectively into glycoproteins.

Abbreviations used: GlcNAc, *N*-acetylglucosamine; GalNAc, *N*-acetylgalactosamine; Rha, rhamnose.

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Materials and methods

Cell cultures

Cultures of *A. pseudoplatanus* were obtained from Dr. D. T. A. Lamport, Michigan State University, East Lansing, MI, U.S.A., and were cultured in B_5 medium (Gamborg *et al.*, 1968). For bulk preparation, cells were grown in 1800-ml Fernbach flasks at 22–28°C while being oscillated at 150 rev./min. Cells were harvested during early-exponential-phase growth (7 days after inoculation; doubling time, 5 days).

Membrane preparations

The buffer used for all reagents during membrane isolation was 50mM-Tricine {*N*-[2-hydroxy-1,1-bis(hydroxymethyl)ethyl]glycine} (pH 7.5)/15mM-$MgCl_2$/2mM-2-mercaptoethanol/0.4M-sucrose. Buffer used for cell homogenization contained, in addition, 1% poly(vinylpyrrolidone). All isolation procedures were carried out at 4°C. Cells suspended in 2vol. of buffer were homogenized in a Potter–Elvehjem tissue homogenizer and filtered through silk screen to remove intact cells. Membranes were concentrated by centrifugation as follows. The filtrate was cleared of large cellular debris by centrifugation at 400*g* for 10min in a Sorvall RC-2B centrifuge with a GSA rotor. Membranes were then sedimented at 28000*g* for 90min in the same centrifuge and rotor. The sedimented membranes were resuspended in buffer, layered over a pad of 1.46M-sucrose in buffer, and centrifuged at 68000*g* for 30min in a Spinco SW-41 rotor. Membranes that collected at the buffer/1.46M-sucrose interface were washed by resuspending in buffer and centrifuged as in the previous step.

The membrane preparation was partially characterized by subjecting it to centrifugation at 68000*g* for 4h on a five-step discontinuous density gradient of 42.5–38% (w/w) sucrose and examining the resulting bands by electron microscopy. Components of rough endoplasmic reticulum or polyribosomes were found in several bands including a mitochondrial band. Hence all incubation experiments were conducted with the whole membrane preparation (density 1.15 to 1.18).

Incubation of membranes with GDP-[U-^{14}C]-mannose and UDP-[6-^{3}H]GlcNAc

Typically 0.5ml of isolated membranes, along with 1μCi each of GDP-[U-^{14}C]mannose (sp. radioactivity 192Ci/mol) and UDP-[6-^{3}H]GlcNAc (sp. radioactivity 6600Ci/mol), was incubated for 30min at 22°C. After the incubation, 5ml of 50mM-Tricine (pH 7.5)/50mM-NaCl was added. The diluted membranes were solubilized by sonication for 10min. The insoluble particulate material remaining after sonication was removed by centrifugation at 48000*g* for 2–3h. The clear supernatant was applied to a Sephadex G-50 column (1.5cm × 90cm) and eluted with 50mM-Tricine (pH 7.5)/50mM-NaCl buffer. The high-molecular-weight components (V_0 peaks) were dialysed and used directly for digestion by Pronase.

Preparation of glycopeptides

High-molecular-weight fractions (Sephadex G-50 V_0 peaks) were digested with Pronase (Calbiochem) for 24h at 37°C (Spiro, 1965). A typical digest contained 4mg of protein, 600μg of Pronase, added in three time-spaced increments, and a drop of toluene as a preservative. Glycopeptides were separated on a Sephadex G-50 column (1.5cm × 90cm; 0.1M-acetic acid as eluent) into two fractions, A and B. Fraction B was desalted on a Sephadex G-15 column (1.5cm × 40cm; 0.1M-acetic acid as eluent).

Hydrazinolysis

Hydrazinolysis of glycopeptide samples was conducted as follows. Sample containing 13mg of hydrazine sulphate was dried under vacuum and dry hydrazine (100μl) was added. The tube was sealed under vacuum and incubated at 60°C for 18h. These conditions have been described as optimum for cleavage of peptide bonds without degradation of carbohydrate (Yosizawa *et al.*, 1966; Heath & Northcote, 1971).

Methods of analysis

Samples were routinely monitored for radioactivity in Triton X-100/toluene (2:1, v/v) with 0.4% 2,5-diphenyloxazole in a Beckman LS-200B liquid-scintillation counter. Proteins were assayed by the Lowry method (Lowry *et al.*, 1951), with bovine serum albumin as standard. Total carbohydrate was determined by the phenol/H_2SO_4 method (Dubois *et al.*, 1956).

Samples were hydrolysed for monosaccharide identification in 2M-HCl for 2h at 100°C in a sealed tube under vacuum. Samples for amino-acid analysis were hydrolysed in 6M-HCl for 24h at 100°C under similar conditions.

Sugars were identified by two methods. In the first method, sugars were chromatographed as their borate esters (Mopper, 1978), by using a high-pressure liquid-chromatography column (25cm × 0.3cm; Chromex DA-X8-11, Dionex Chemical Corporation). Identification and quantification were achieved by monitoring with cupric biscinchoninate (Mopper & Gindler, 1973). In the second method, sugars, along with an internal standard of 2-deoxyribose, were reduced with NaB^3H_4 to [^{3}H]alditols

and separated on the high-pressure liquid-chromatography column under the same conditions as for the aldoses, or by electrophoresis on paper strips in borate buffer (Takasaki & Kobata, 1974). Quantification was achieved by comparison of radioactivity in unknown sugars with the amount of radioactivity incorporated into the 2-deoxyribose. Since the specific radioactivity of the NaB^3H_4 was high, the contribution of ^3H from added sugar nucleotide was not significant. When the identity of incorporated radiolabelled sugar was determined, non-radioactive $NaBH_4$ was substituted in the above procedure.

Results

Incorporation of radioactivity and preliminary characterization of products

Membranes, when incubated for 30min with GDP-[U-^{14}C]mannose and UDP-[6-^{3}H]GlcNAc, incorporated ^3H and ^{14}C into soluble and insoluble components (Table 1, Fig. 1). A considerable portion of the incorporated radioactivity was found to be present in the Sephadex G-50 V_0 fraction. Radioactivity present in the pellet obtained after sonication of the membranes was extractable with both chloroform/methanol (2:1, v/v) and chloro-

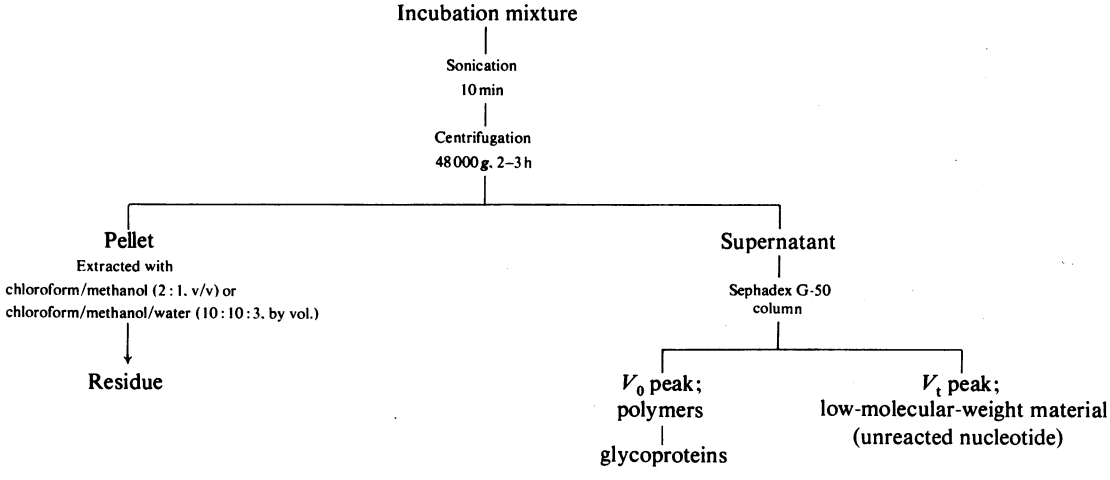

Fig. 1. *Isolation of reaction products from the standard incubation mixture*

Table 1. *Distribution of incorporated radioactivity among isolated fractions*

Membranes (0.5ml) were incubated for 30min with 1μCi each of GDP-[U-^{14}C]mannose (1.4×10^6c.p.m.) and UDP-[6-^{3}H]GlcNAc (4.9×10^5c.p.m.). Membranes were diluted, sonicated and centrifuged as described in the Materials and methods section. The supernatant was applied to the Sephadex G-50 column as described in the Materials and methods section. The Sephadex G-50 V_0 peak was collected and counted for radioactivity. The pellet from centrifugation of the sonicated membranes was extracted successively with three 2ml portions of chloroform/methanol (2:1, v/v), which were pooled and back-extracted with two 4ml portions of chloroform/methanol/water (3:47:48, by vol.) that were discarded. Next the residue was extracted with three 2ml portions of chloroform/methanol/water (10:10:3, by vol.). These extracts were also pooled. All extracts were evaporated to dryness before counting for radioactivity. The control incubation differed from the standard only in that the sugar nucleotides were added after sonication.

	Radioactivity (c.p.m.)			
	Standard incubation		Control incubation	
Fraction	^3H	^{14}C	^3H	^{14}C
Sephadex G-50 V_0 peak	62000	133000	1500	10500
Chloroform/methanol (2:1)	29900	45500	650	2750
Chloroform/methanol/water (10:10:3)	69500	147000	332	1300
Insoluble residue	136000	104000	1650	1440

form/methanol/water (10:10:3, by vol.). Some of the incorporated radioactivity was insoluble and remained in the residue, which was not examined further. In control experiments in which the radiolabelled sugar nucleotides were added after sonication (Table 1), incorporation of radioactivity into all fractions was greatly decreased. Thus most of the incorporation took place during the 30min incubation period before sonication.

To exclude the possibility that the radioactivity in the Sephadex G-50 V_0 peak was in lipids tightly adsorbed to protein or dispersed in micelles, the supernatant obtained after sonication and centrifugation of the membranes was dialysed and then freeze-dried and extracted with both chloroform/methanol (2:1, v/v) and chloroform/methanol/water (10:10:3, by vol.). Small amounts of both the chloroform/methanol- and the chloroform/methanol/water-soluble counts were present in the freeze-dried supernatant. However, when this material was chromatographed on the Sephadex G-50 column and the V_0 peak was collected and extracted, no significant amount of radioactivity remained that was soluble in either solvent mixture used. Evidently the traces of lipids present were eluted from the Sephadex G-50 column at some point after the V_0 peak. Thus the radioactivity associated with the Sephadex G-50 V_0 peak is covalently attached to high-molecular-weight material. When the Sephadex G-50 V_0 peak was pooled and assayed it was found to contain 2.3 mg of protein, 0.26 mg of carbohydrate, 21600 c.p.m. of ^3H and 44500 c.p.m. of ^{14}C. These values are typical for this membrane preparation. The amount of radioactivity incorporated from membrane batch to batch is comparable when based on the protein content of the 0.5 ml portion of membranes used.

The Sephadex G-50 V_0 fraction was hydrolysed in 2M-HCl and chromatographed to determine if the incorporated radioactivity was still present as [^{14}C]mannose and [^3H]GlcNAc. These results are shown in Fig. 2. Glucose, galactose, mannose, fucose, rhamnose and xylose are separated with highly reproducible elution volumes on the anion-exchange column. The ^{14}C radioactivity was eluted from the column in three peaks. The largest peak was identified as mannose and contained 77% of the ^{14}C radioactivity. A second peak of ^{14}C eluted after the mannose and was identified as fucose. The fucose peak was estimated to contain 20% of the ^{14}C radioactivity. A third ^{14}C peak, which was eluted before the rhamnose standard, was estimated to contain 4% of the ^{14}C radioactivity. This peak was not identified, but was found also in mild acid hydrolysates of the GDP-[U-^{14}C]mannose (no fucose was found in these hydrolysates). The percentage of radioactivity incorporated as fucose into the Sephadex G-50 V_0 peak must be considered as a minimum, because the conditions chosen for hydrolysis of this material were more rigorous than those required for hydrolysis of glycosidic linkages involving fucose (Annison & Morgan, 1952), and some degradation undoubtedly occurred. Essentially all the ^3H was eluted from the high-pressure

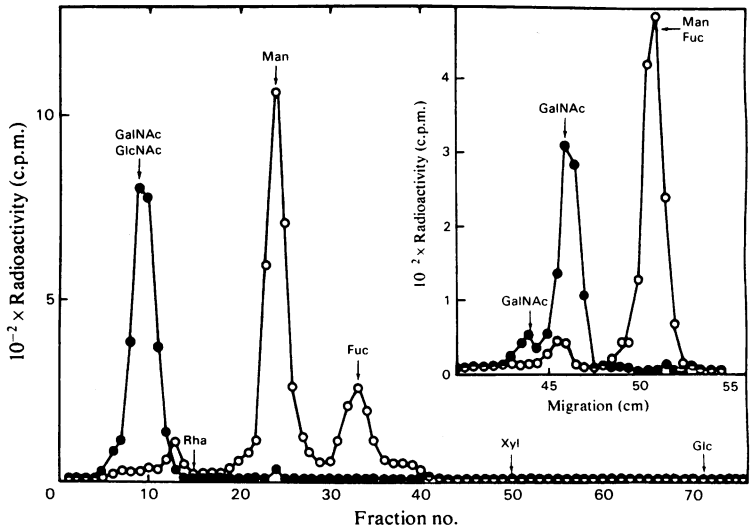

Fig. 2. *Identification of radiolabelled monosaccharides released from double-labelled high-molecular-weight products after hydrolysis with 2M-HCl at 100°C for 2h*

Monosaccharides released were separated by high-pressure liquid chromatography as their borate esters and by high-voltage electrophoresis after reduction with $NaBH_4$ (insert); 0.25 ml fractions were collected. Symbols: O, ^{14}C radioactivity; ●, ^3H radioactivity.

liquid-chromatography column as one peak corresponding to GlcNAc or GalNAc. High-voltage electrophoresis of the reduced sugars (Fig. 2 insert) separated the ^3H into two peaks. A small amount of the added UDP-[6-^{3}H]GlcNAc (estimated to be 10%) had evidently epimerized to UDP-[6-^{3}H]-GalNAc before incorporation of the sugar into high-molecular-weight material.

Preparation of glycopeptides

The Sephadex G-50 V_0 peak obtained after sonication and column chromatography (Fig. 1) was considerably decreased in size after digestion with Pronase for 24h. When the digested material was re-chromatographed on a Sephadex G-50 column (1.5cm × 90cm, 0.1M-acetic acid as eluent) the ^3H and ^{14}C radioactivity was distributed in a manner that suggested the presence of two classes of glycopeptides (Fig. 3). The glycopeptides produced by the Pronase digestion differ both in molecular weight and in relative ratio of ^{14}C and ^3H incorporated. The higher-molecular-weight class (fraction A) contained primarily ^{14}C and only a small amount of ^3H. The lower-molecular-weight class (fraction B) contained significant amounts of both ^{14}C and ^3H radioactivity. The isotopic separation, as well as the relative size and shape of the glycopeptide peaks, was always observed between separate incubations and with membranes from different cell batches.

Characterization of glycopeptides

A partial resolution of isotopes occurred on the Sephadex G-50 column, suggesting that the glycopeptides of fraction B were heterogeneous. Attempts to resolve them by using gels of lower exclusion limit were not successful. On a Sephadex G-15 column the glycopeptides were eluted much closer to V_0 as a single peak, indicating that the radioactivity was associated with glycopeptides of considerable size. Sizing experiments with glycopeptides were conducted on a Sephadex G-50 column [1.5cm × 90cm; 50mM-Tricine (pH7.5)/50mM-NaCl as eluent] that had been standardized with the amylose series of oligosaccharides (G_4–G_{10}). Glycopeptides of fraction A were of such large size that a meaningful estimate of the molecular weight could not be obtained within the region of standardization of the Sephadex G-50 column. Chromatography of fraction B before and after the removal of amino acids by hydrazine is shown in Fig. 4. Chromatography of the untreated fraction B (Fig. 4*a*) on the standardized column produced a profile containing two peaks of ^3H activity and an uneven distribution of ^{14}C. After fraction B was reacted with hydrazine and re-chromatographed on the column (Fig. 4*b*) the degree of apparent heterogeneity was markedly decreased, as judged from the now more normal

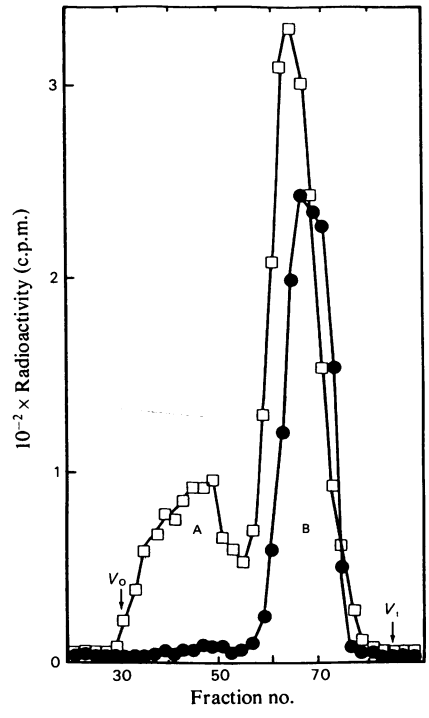

Fig. 3. *Gel filtration (Sephadex G-50 column, 1.5cm × 90cm, 0.1M-acetic acid) of glycopeptides produced after Pronase hydrolysis of the double-labelled high-molecular-weight fraction*

The fraction contained 4mg of protein, 0.45mg of carbohydrate, 34700c.p.m. of ^3H and 102900c.p.m. of ^{14}C. Hydrolysis with Pronase was allowed to proceed for 24h at 37°C. Samples (200μl) of alternate fractions were assayed for radioactivity. Symbols: □, ^{14}C radioactivity, ●, ^3H radioactivity. Fractions 30–50 constitute glycopeptide peak A and fractions 59–75 constitute glycopeptide peak B; 2ml fractions were collected.

bell-shaped profile. Hydrazinolysis apparently decreased the size of the larger glycopeptides by removal of amino acids that were not hydrolysed by Pronase. Hydrazinolysis is known to remove the terminal asparagine residue in *N*-glycosidic linkages as well as the acetate groups of hexosamines, leaving the oligosaccharide intact. Some heterogeneity was still present after hydrazinolysis, as judged from the column profile, as the ^{14}C peak had two shoulders and was eluted from the column before ^3H. The molecular weight of the oligosaccharide (Fig. 4*c*) was estimated as 2300, corresponding to a degree of polymerization of about 14 hexose units. The molecular weight for the ^{14}C peak before hydrazinolysis was estimated as 4400 (Fig. 4*a*).

Fractions A and B differ greatly in the $^{14}C/^3H$

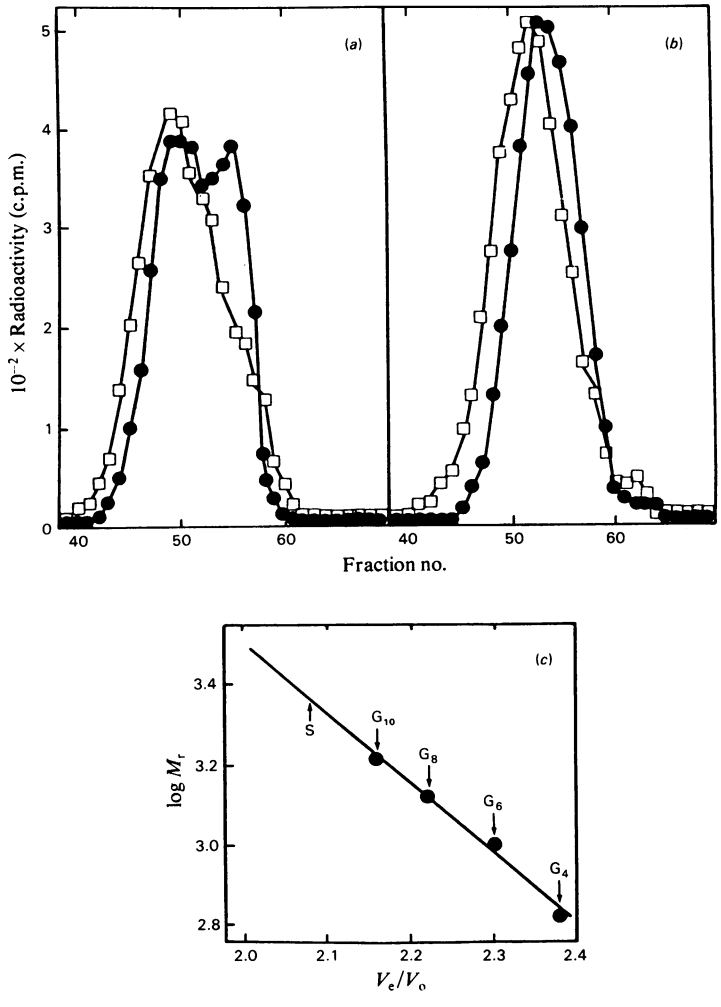

Fig. 4. *Effect of hydrazinolysis on the apparent molecular weight of glycopeptide fraction B*

Molecular weights were estimated by gel-permeation chromatography [Sephadex G-50 column, 1.5 cm × 90 cm, 0.05 M-Tricine (pH 7.5)/0.05 M-NaCl]. The elution profile in (*a*) was obtained before hydrazinolysis (molecular weight estimated at ^{14}C peak = 4400). The elution profile in (*b*) corresponds to glycopeptide fraction B after hydrazinolysis. Symbols: □, ^{14}C radioactivity; ●, ^{3}H radioactivity. (*c*) shows the standardization of the gel column with malto-oligosaccharides (G_4–G_{10}). S denotes the position of the sample after hydrazinolysis (mol.wt. 2300); 2 ml fractions were collected.

ratio and in their apparent size. Thus it seemed of immediate interest to determine the distribution of ^{14}C radioactivity between fucose and mannose in each peak. Fractions A and B were hydrolysed with acid as described, and the hydrolysates were applied to the high-pressure liquid-chromatography column. In fraction A, 29% of the ^{14}C was found in mannose and the remainder (71%) in fucose, whereas in fraction B, 50–60% of the ^{14}C was in mannose and 40–50% was found in fucose. Thus it would appear that the ^{14}C isotope introduced to the membranes as GDP-[U-^{14}C]mannose was partially metabolized and incorporated into glycoproteins as either fucose or mannose. Fraction B could be resolved further into three subfractions by electrophoresis on paper with a pH 2.0 acetic acid/formic acid buffer (Fig. 5*b*). Fraction A migrated as a single peak (Fig. 5*a*). After electrophoresis, four subfractions (IA, IB, IIB and IIIB) were recovered for analysis of the carbohydrate and amino-acid compositions. Table 2 gives the composition of each glycopeptide expressed as mol% of each constituent. It can be seen that each subfraction differs in both amino-acid and sugar compositions.

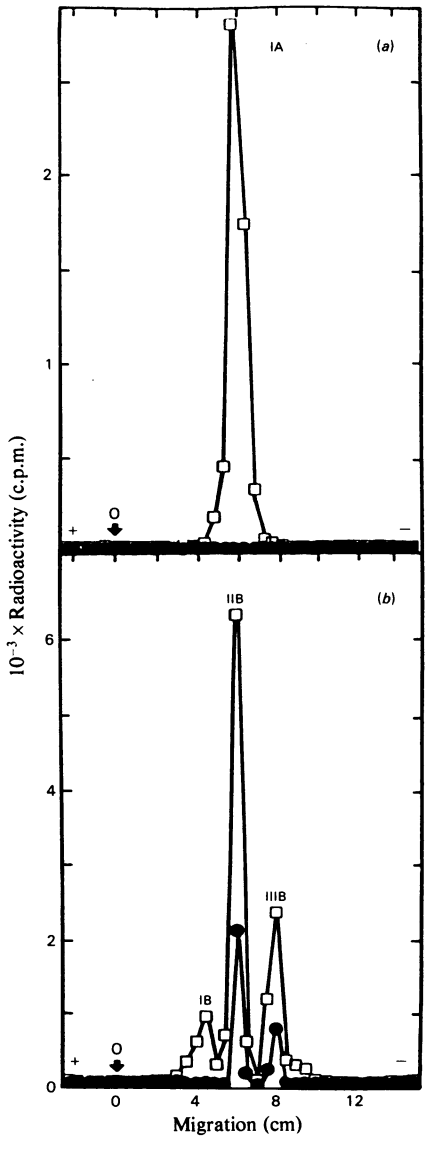

Fig. 5. *Paper electrophoresis of glycopeptide fractions A and B*

Glycopeptide fractions were applied to pre-washed strips (3 cm × 18 cm) of Whatman no. 1 paper and electrophoresed for 2 h at 15.6 V/cm with 1 M-acetic acid/0.7 M-formic acid (pH 2.0) as buffer. O, indicates the point of sample application. Symbols: □, ^{14}C radioactivity; ●, ^{3}H radioactivity.

Discussion

A primary objective of this investigation was to determine whether *Acer* membranes were capable of incorporating mannose from GDP-mannose into glycoprotein. The evidence presented in this study fully supports the role of GDP-mannose as a precursor for both protein-bound mannose and protein-bound fucose. Susceptibility of the Sephadex G-50 V_0 peak to hydrolysis by Pronase indicated that virtually all the radiolabelled sugars present in this fraction are covalently attached to glycoprotein. They cannot be in lipids or other low-molecular-weight products tightly adsorbed to protein because they could not be extracted with organic solvents or removed by dialysis. Further, it is unlikely that a glycosidase contaminating the Pronase could have yielded the distinctly different and characteristic molecular-weight species described in Figs. 3 and 4. Glycosidic enzymes produce a broad range of oligosaccharides, including mono- and di-saccharides. These oligosaccharides would have been detected easily on the Sephadex G-15 column.

A comparison of our results with those of Smith *et al.* (1976) reveals differences and some similarities. They report no incorporated radioactivity from GDP-[U-^{14}C]mannose into protein; we found 9.5% of the added ^{14}C from the nucleotide and 12.5% of the ^{3}H from UDP-[6-^{3}H]GlcNAc was transferred to protein. Both groups observed incorporation of ^{14}C into an insoluble pellet and into chloroform/methanol (2:1)- or chloroform/methanol/water (10:10:3)-soluble lipids. Possible reasons for the different results with the membrane preparations include differences in the activities of the preparations and in relative amounts of rough endoplasmic reticulum. The membranes as isolated by Smith *et al.* (1976) had a density of around 1.13, whereas in our hands they ranged in density from 1.15 to 1.18, and thus possibly included more rough endoplasmic reticulum, as well as plasma membrane, which contains mannosyltransferase activity (Klaus & Kindle, 1979). Plasma membranes are not clearly separated from mitochondria by isopycnic gradient centrifugation.

Four glycopeptides (IA, IB, IIB and IIIB) were isolated as a result of Pronase digestion of the Sephadex G-50 V_0 peak, gel chromatography and paper electrophoresis (Table 2). Fractions IA, IB and IIB have fairly simple amino-acid compositions, six in fraction IB and seven in fractions IA and IIB. All fractions contain substantial amounts of aspartic acid, serine and threonine, any one of which could participate in linkage of the oligosaccharide to the protein. The large amounts of arabinose and galactose isolated from fractions IA and IB should be noted. Two types of arabinogalactans have been reported as being in cell walls and in the culture medium from *Acer* cells (Keegstra *et al.*, 1973). One type is associated with the pectic polymers and is found linked to a rhamnogalacturonan. The second type is linked to a hydroxyproline-rich glycoprotein that is also found in the cytoplasm (Lamport, 1970;

Table 2. *Monosaccharide and amino-acid composition in mol% of Acer glycopeptides radiolabelled by GDP-[U-^{14}C]mannose and UDP-[6-^{3}H]GlcNAc*

Values of monosaccharides shown were corrected for small amounts of carbohydrate extracted from a paper control. Amino acids were analysed by ion-exchange chromatography with a Dionex amino-acid analyser.

	Content (residues/molecule)			
Glycopeptide fraction ...	IA	IB	IIB	IIIB
Amino acid				
Asp	1.0	10.0	2.9	15.0
Thr	1.0	—	1.0	6.0
Ser	1.7	9.8	1.6	7.9
Glu	1.4	20.0	2.6	9.3
Gly	1.7	9.2	3.0	13.0
Ala	2.3	—	2.7	10.0
Val	—	—	—	2.8
Met	—	3.0	—	0.9
Ile	—	—	—	2.2
Leu	—	—	—	2.3
His	1.0	—	0.9	1.9
Lys	—	2.3	—	—
Monosaccharide				
GlcNAc	2.3	0.7	4.7	7.5
Rha	2.5	—	1.1	—
Man	2.7	5.2	30.0	7.2
Fuc	3.8	7.2	11.0	4.5
Ara	44.0	11.0	16.0	2.5
Gal	30.0	9.8	17.0	3.5
Xyl	0.9	1.3	0.5	1.6
Glc	4.8	9.8	5.7	1.7

Heath & Northcote, 1971). The monosaccharide components of fractions IA and IB suggest a precursor relationship to these macromolecules.

Cell membranes from other plants have been reported to contain complex hemicellulose-like molecules (Bowles & Northcote, 1972; Green & Northcote, 1978; Jilka *et al.*, 1972; Nordin *et al.*, 1975; Northcote, 1979) that are excreted to the exterior of the cell surface. The *Acer* glycoproteins, based on their monosaccharide composition reported in the present study, could be classed as hemicellulose also. The large oligosaccharide side chains contribute to an uncertain classification.

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