Isolation and characterization of plasma-membrane glycoproteins from pig epidermis

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1. Non-desmosomal plasma membranes enriched in plasma-membrane marker enzymes and in metabolically labelled glycoproteins were isolated on a large scale from up to 500 g of pig ear skin slices. Sodium dodecyl sulphate/polyacrylamide-gel electrophoresis and periodic acid/Schiff staining revealed the presence of four major glycosylated components in the apparent molecular-weight range 150 000–80 000. 2. A large proportion of the marker enzymes, the D-[³H]glucosamine-labelled glycoproteins and the periodic acid/Schiff-stained glycoproteins were solubilized by 1% (w/v) sodium deoxycholate. However, several non-glycosylated proteins, in particular those with mol.wts. 81 000, 41 000 and 38 000 (possibly cytoskeletal components), were relatively resistant to solubilization. 3. The deoxycholate-solubilized membranes were fractionated by lectin affinity chromatography using both concanavalin A–Sephrose 4B and lentil lectin–Sephrose 4B. From 75 to 85% of the applied glycoprotein was recovered from the columns. From 30 to 40% of the recovered glycoprotein was specifically bound by the lectins and was eluted with 2% (w/v) α -methyl D-mannoside. The enrichment of labelled glycoproteins in the material bound by the lectins (2.5-fold) was similar with both lectins, although the yield was somewhat greater when lentil lectin was used. The glycoprotein-enriched fraction was also enriched in all the plasma-membrane marker enzymes, indicating their probable glycoprotein nature. 4. The glycoprotein-enriched fraction contained the four major periodic acid/Schiff-stained bands that were detected in the original plasma membrane. They had apparent mol.wts. 147 000, 130 500, 108 000 and 91 400. The higher-molecular-weight components contained relatively more D-[³H]glucosamine, indicating differences in the sugar composition or in the metabolic turnover of the individual glycoproteins in culture. The material bound by the lectins also contained a number of lower-molecular-weight Coomassie Brilliant Blue-stained components. These were weakly stained by periodic acid/Schiff reagent and were lightly labelled with D-[³H]glucosamine, indicating that they contained less carbohydrate than the four major glycoprotein bands. 5. Chloroform/methanol-extracted plasma membranes and isolated glycoproteins had a similar carbohydrate composition, containing sialic acid, hexosamine, fucose, xylose, mannose, galactose and glucose. Glucose was not enriched in the isolated glycoproteins, suggesting that it may be a contaminant. Xylose, however, was enriched in the isolated glycoproteins. It remains to be established whether this sugar, which is not usually found in plasma-membrane glycoproteins, is a genuine constituent of plasma-membrane glycoproteins in the epidermis.

Although the molecular basis of cell adhesion in the epidermis is not yet clear, it is likely that plasma-membrane glycoproteins are involved (Sker-

Abbreviation used: SDS, sodium dodecyl sulphate.

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row, 1978). Desmosomes, which play a major role in epidermal-cell adhesion, have been isolated after homogenization of cow snout epidermis in both citric acid, pH 2.6 (Skerrow & Matoltsy, 1974a) and NaOH, pH 9–11 (Drochmans *et al.*, 1978). Desmosomes isolated by the former procedure contained two glycoproteins of apparent mol.wts.

140000 and 120000 (Skerrow & Matoltsy, 1974b). The high-pH procedure yielded desmosome-tonofilament complexes in which glycoproteins in this molecular-weight range were minor components (Drochmans *et al.*, 1978).

We have isolated plasma-membrane-enriched vesicles from pig skin slices homogenized in near-neutral-pH buffers (Gray *et al.*, 1980). These membranes are thought to be derived from non-desmosomal (possibly microvillar) regions of the plasma membrane of cells in the living layers of the epidermis. SDS/polyacrylamide-gel electrophoresis using the system of Ugel *et al.* (1971) revealed weak periodic acid/Schiff-staining of glycoprotein components of apparent mol.wt. about 140000 and 120000–90000. Metabolic-labelling studies with both L-[³H]fucose and D-[³H]glucosamine indicated that these non-desmosomal plasma membranes had a more complex glycoprotein composition, containing major labelled components in the apparent mol.wt. range 150000–70000 (King *et al.*, 1980).

In order to clarify their glycoprotein composition, we have now isolated non-desmosomal epidermal plasma membranes on a large scale and have purified their constituent glycoproteins by lectin affinity chromatography (Lotan & Nicolson, 1979). The glycoproteins have been characterized with respect to their enzyme activity, their carbohydrate composition and their molecular-weight distribution after electrophoresis using the system of Laemmli (1970). We have found that this electrophoresis system gives better resolution of the high-molecular-weight glycoproteins than the system of Ugel *et al.* (1971), which we used previously.

Experimental

Materials

D-[6-³H]Glucosamine hydrochloride, substrates for enzyme assays and reagents for gel electrophoresis and tissue culture were all obtained as described previously (Gray *et al.*, 1980; King *et al.*, 1980). Sodium deoxycholate was from Koch-Light Laboratories Ltd., Colnbrook, Bucks., U.K. α -Methyl D-mannoside and phenylmethanesulphonyl fluoride were from Sigma. Concanavalin A-Sepharose 4B (nominally 10mg of lectin/ml of gel) and lentil lectin-Sepharose 4B (2mg lectin/ml of gel) were from Pharmacia Fine Chemicals. Standard sugars were from BDH and Sigma.

Plasma-membrane isolation

Glycoproteins were labelled by culturing skin slices in the presence of D-[³H]glucosamine for up to 42h (King *et al.*, 1980). Labelled skin slices were added to 100g portions of pig ear skin (0.2mm thick), which were homogenized in 600ml of 5mM-Tris/HCl (pH 7.5)/0.25M-sucrose and filtered

(King *et al.*, 1980). The filtrate was centrifuged at 376 *g*_{av.} for 15 min at 4°C and the supernatant was centrifuged at 113 700 *g*_{av.} for 30 min at 4°C. The pellet was washed and suspended in 5mM-Tris/HCl (pH 7.5)/0.25M-sucrose and the material from up to 500g of skin slices was pumped on to a Beckman Ti 14 zonal rotor loaded with 160ml each of 0.5M-sucrose, 1.0M-sucrose and 1.5M-sucrose, and filled with 2.0M-sucrose. All sucrose solutions contained 5mM-Tris/HCl, pH 7.5. The rotor was centrifuged at 45000 rev./min for 1h at 4°C, the fractions at the sucrose interfaces were pooled, diluted with water and centrifuged (113 700 *g*_{av.}, 1h) and were suspended in 5mM-Tris/HCl (pH 7.5)/0.25M-sucrose before assay of D-[³H]glucosamine-labelled glycoprotein, acid phosphatase, phosphodiesterase I, 2-naphthylamidase, 5'-nucleotidase and NADH:ferri-cyanide reductase as described previously (Gray *et al.*, 1980).

Solubilization of plasma membranes

The membranes banding at the 0.5M/1.0M-sucrose interface were washed three times in 10mM-Tris/HCl, pH 7.5, by centrifugation at 113 700 *g*_{av.}. Approx. 0.2mg of plasma-membrane protein was obtained per g wet weight of skin slices. The pellet was suspended in 1% (w/v) sodium deoxycholate/10mM-Tris/HCl (pH 7.5)/0.25mM-phenylmethanesulphonyl fluoride at a final concentration of 5–10mg of protein/ml, and after 30 min at 0–4°C was centrifuged at 113 700 *g*_{av.} for 1h at 4°C. The supernatant was removed and the pellet was suspended in 1% (w/v) sodium deoxycholate/10mM-Tris/HCl (pH 7.5)/0.25mM-phenylmethanesulphonyl fluoride.

Lectin affinity chromatography

Concanavalin A-Sepharose 4B and lentil lectin-Sepharose 4B (25ml of each) were washed as described by Zanetta & Gombos (1976), except that 1% (w/v) α -methyl D-mannoside was used instead of sucrose and 1% (w/v) sodium deoxycholate/10mM-Tris/HCl (pH 7.5)/0.25mM-phenylmethanesulphonyl fluoride was used instead of their SDS-containing buffer. Portions (4ml and 10ml respectively) of concanavalin A-Sepharose 4B and lentil lectin-Sepharose 4B in columns of 0.8cm internal diameter were washed with 1% (w/v) sodium deoxycholate/10mM-Tris/HCl (pH 7.5)/0.25mM-phenylmethanesulphonyl fluoride containing 2% (w/v) α -methyl D-mannoside at 5–10ml/h for 4h and then overnight with the same buffer containing no sugar. The deoxycholate-solubilized membrane material was applied to the columns, which were washed with 40–50ml of 1% (w/v) sodium deoxycholate/10mM-Tris/HCl (pH 7.5)/0.25mM-phenylmethanesulphonyl fluoride. Bound glycoproteins were eluted by using the same volume of buffer

containing 2% (w/v) α -methyl D-mannoside. The non-bound and bound fractions were each dialysed overnight against 5 litres of 1% (w/v) sodium deoxycholate/10 mM-Tris/HCl (pH 7.5)/0.25 mM-phenylmethanesulphonyl fluoride and were passed through 10 ml columns of Sephadex G-25 or G-50 to remove excess lectin. The recovery of D-[3 H]-glucosamine-labelled glycoproteins off these columns was always quantitative. Fractions were treated with 2 vol. of 95% ethanol at -20°C and were left overnight at -20°C before centrifugation at $37000g_{av}$. at 4°C for 1 h. The precipitates were dissolved in water and were dialysed overnight against a large excess of water. Ethanol precipitated 70 and 95% respectively of the D-[3 H]-glucosamine-labelled material in the non-bound and bound fractions.

Chloroform/methanol extraction

Washed plasma membranes (about 10 mg of protein) were dialysed against 10 mM-Tris/HCl, pH 7.5, collected by centrifugation, extracted twice with 6 ml of chloroform/methanol (2:1, by vol.) and suspended in methanol.

Chemical analyses

Samples (0.5–1.0 mg of protein) were hydrolysed in 0.05 M- H_2SO_4 at 80 – 85°C for 1 h to release sialic acids. The hydrolysates were passed through 2.5 ml columns of AG2 resin (X8, formate form) (Cook, 1976), which were washed with 50 ml of water before elution of sialic acids with 50 ml of 0.3 M-formic acid. The eluate was freeze-dried, dissolved in water and sialic acid was measured (as *N*-acetylneuraminic acid) by the method of Warren (1959). The mean recovery of a standard sample (100 μg) of *N*-acetylneuraminic acid during these procedures was 87%.

Hexosamines were released by hydrolysis of samples (0.5–1.0 mg of protein) in 4 M-HCl at 110°C under N_2 for 6 h. The hydrolysates were dried over $\text{P}_2\text{O}_5/\text{NaOH}$, dissolved in water and passed through 2.5 ml columns of Dowex 50 (X8, H^+ form) (Cook, 1976), which were washed with 20 ml of water. Hexosamines were eluted with 10 ml of 2 M-HCl, were dried as above, dissolved in water and assayed (as D-glucosamine) by the method of Davidson (1966). The mean recovery of a standard (100 μg) sample of D-glucosamine was 94%.

Hexoses were released by hydrolysis of samples (1.0–2.0 mg of protein) in methanolic 1 M-HCl at 90 – 95°C for 18 h under N_2 (Clamp, 1974). The hydrolysates were dried under N_2 , internal standard (mannitol) was added and samples were treated with 0.5 ml of trimethylsilylating reagent (chlorotrimethylsilane/hexamethyldisilazane/pyridine, 4:5:6, by vol.) for 30 min. Samples were dried, dissolved in hexane and were analysed by g.l.c. using

3% OV-1 on Gas-Chrom Q in a Pye-Unicam 204 gas chromatograph. The column oven was programmed to run for 6 min at 146°C , rising by $1^\circ\text{C}/\text{min}$ to 230°C . Injector and detector temperatures were 250°C and 300°C respectively and a flow rate of 60 ml/min of N_2 carrier gas was used. Standard sugars (fucose, xylose, mannose, galactose and glucose) were treated identically.

Protein was measured by the Hartree (1972) modification of the method of Lowry *et al.* (1951). Phospholipid phosphorus was measured by the method of Long & Yardley (1970).

Amino acid analysis was carried out by the Macromolecular Analysis Service of the University of Birmingham. Samples (0.25–0.5 mg of protein) were hydrolysed in sealed tubes in 6 M-HCl at 110°C for 24 h, and amino acids were measured with a Locarte amino acid analyser.

SDS/polyacrylamide-gel electrophoresis

This was performed on cylindrical 10% (w/v) acrylamide gels (1.2 ml of resolving gel, 0.2 ml of stacking gel), the system of Laemmli (1970) being used. Gels were prepared the day before use. Samples were solubilized with 2% (w/v) SDS/10 mM-2-mercaptoethanol at 100°C for 2 min and, where necessary, were clarified by centrifugation. Electrophoresis was carried out at 2–3 mA/gel and gels were stained for protein with Coomassie Brilliant Blue, for carbohydrate with the periodic acid/Schiff reagent or were sliced into 1 mm sections, which were dissolved as described previously (King *et al.*, 1980). Gels were scanned by using the densitometer accessory of a Pye-Unicam SP.8-100 spectrophotometer at 550 nm (protein) and 560 nm (carbohydrate).

Results and discussion

Isolation and solubilization of epidermal plasma membranes

Epidermal plasma membranes isolated on a large scale from pig ear skin slices were enriched in D-[3 H]-glucosamine-labelled glycoproteins and in the plasma-membrane marker enzymes acid phosphatase, phosphodiesterase I, 2-naphthylamidase and 5'-nucleotidase when compared with the crude particulate fraction (Table 1). They were depleted of NADH:ferricyanide reductase. The degree of enrichment was comparable with the values obtained when much smaller quantities of tissue were used (Gray *et al.*, 1980; King *et al.*, 1980).

SDS/polyacrylamide-gel electrophoresis using the buffer system of Laemmli (1970) revealed a broad major band of periodic acid/Schiff-stained glycoproteins in the apparent-mol.wt. range 150 000–80 000 (Fig. 1*d*). Within this range at least four different glycoprotein bands, labelled I to IV (Fig. 1),

Table 1. *Large-scale preparation of plasma membranes from pig epidermis*

The 113 700 g_{av} . pellet prepared from up to 500g of pig ear skin slices was fractionated by centrifugation on discontinuous sucrose gradients in a Beckman Ti 14 rotor. The fractions banding at each interface were pooled, diluted with water and collected by centrifugation. Radioactivity, protein and enzyme activity were measured in each fraction and the specific activity relative to the 113 700 g_{av} . pellet was calculated. The results are means $\pm$ S.E.M. The values in parentheses show the number of different preparations assayed. The plasma-membrane-enriched fraction banded at the 0.5 M/1.0 M-sucrose interface.

	Relative specific activity		
	0.5/1.0 M-sucrose	1.0/1.5 M-sucrose	1.5/2.0 M-sucrose
D-[3 H]Glucosamine (4)	2.2 ± 0.4	1.4 ± 0.2	0.6 ± 0.1
Acid phosphatase (4)	2.8 ± 0.7	2.1 ± 0.7	0.8 ± 0.3
Phosphodiesterase (4)	4.5 ± 0.7	3.0 ± 0.4	0.9 ± 0.2
2-Naphthylamidase (4)	2.4 ± 0.3	1.6 ± 0.2	0.7 ± 0.2
5'-Nucleotidase (1)	2.3	1.7	1.1
NADH : ferricyanide (3) reductase	0.5 ± 0.1	1.4 ± 0.2	1.0 ± 0.3

Fig. 1. *SDS/polyacrylamide-gel electrophoresis of epidermal plasma-membrane proteins and glycoproteins* Epidermal plasma membranes were solubilized with 1% (w/v) sodium deoxycholate as described in the Experimental section. Samples of the intact membranes (a and d), the material solubilized by deoxycholate (b and e) and the material not solubilized by deoxycholate (c and f) were treated with 2% (w/v) SDS/10 mM-2-mercaptoethanol and were analysed by electrophoresis on 10% acrylamide gels. A 50 μ g sample of protein was applied to gels (a)–(c), which were stained for protein and scanned at 550 nm. A 500 μ g portion of protein was applied to gels (d)–(f), which were stained for carbohydrate and scanned at 560 nm. The arrow indicates the direction of migration. BPB indicates the position of the Bromophenol Blue tracking dye.

could be detected. These components could not be distinguished when the gels were stained for protein with Coomassie Brilliant Blue (Fig. 1a), owing to the presence of considerable amounts of non-glycosylated protein in this region of the gel. In addition to these major glycoprotein bands, a number of lower-molecular-weight components were also weakly stained by the periodic acid/Schiff reaction (Fig. 1d).

Treatment with 1% (w/v) sodium deoxycholate/10 mM-Tris/HCl (pH 7.5)/0.25 mM-phenylmethane-sulphonyl fluoride solubilized $65 \pm 4\%$ of the total membrane protein, $72 \pm 3\%$ of the D-[^3H]-glucosamine-labelled glycoprotein and between 65 and 88% of the plasma-membrane marker enzymes. Comparison of the deoxycholate-soluble (Fig. 1e) and the deoxycholate-insoluble (Fig. 1f) fractions by SDS/polyacrylamide-gel electrophoresis indicated that the major glycoproteins were fully extracted from the membrane. However, a number of non-glycosylated proteins, notably those of mol.wt. about 81000, 41000 and 38000, were more resistant to extraction and were enriched in the deoxycholate-insoluble fraction (Fig. 1c). These deoxycholate-insoluble proteins may be components of a membrane-associated cytoskeletal system (Mescher *et al.*, 1981).

Lectin affinity chromatography

The overall recovery of protein from concanavalin A-Sepharose 4B was higher than that of D-[^3H]-glucosamine-labelled glycoproteins (Table 2), indicating some irreversible adsorption of glycoproteins to the lectin (Allan *et al.*, 1972). The material bound by concanavalin A was enriched about 2.5-fold in D-[^3H]-glucosamine-labelled glycoprotein. This glycoprotein-enriched fraction was enriched up to 6-fold in all the plasma-membrane marker enzymes (results not shown), indicating the probable glycoprotein nature of these enzymes. Similar values for the recovery and enrichment of labelled glycoproteins were obtained when chromatography was carried out at room temperature, in

the presence of 0.1 M-KCl and when the solubilized membrane material was incubated with the concanavalin A-Sepharose for 18 h before elution (results not shown). No additional glycoprotein was bound when the fraction that was not retained was passed through a second concanavalin A-Sepharose 4B column.

The total recovery and the proportion of D-[^3H]-glucosamine-labelled glycoprotein bound by lentil lectin-Sepharose 4B were slightly greater than when concanavalin A-Sepharose 4B was used (Table 2). However, the enrichment (2.5-fold) of D-[^3H]-glucosamine-labelled glycoproteins in the bound fraction was similar to that obtained with concanavalin A. The difference between the two lectins was not as pronounced as that found with lymphocyte plasma membranes (Allan *et al.*, 1972; Hayman & Crumpton, 1972).

Characterization of isolated plasma-membrane glycoproteins

SDS/polyacrylamide-gel electrophoresis indicated that the material bound by both lectin columns (Figs. 2b and 2e) contained the major periodic acid/Schiff-stained glycoprotein bands detected in the original plasma membrane (Fig. 1). Bands I, II and III were now clearly stained by Coomassie Brilliant Blue (Figs. 2a and 2d), indicating the removal of most of the non-glycosylated proteins in this molecular-weight range. The four major glycoprotein bands had apparent mol.wts. of 147000 ± 2700 , 130500 ± 1200 , 108000 ± 2500 and 91400 ± 1800 (mean of seven determinations $\pm$ S.E.M.). The profile of D-[^3H]-glucosamine labelling (Figs. 2c and 2f) did not correspond precisely with the intensity of periodic acid/Schiff staining of the glycoproteins. In general the higher-molecular-weight components were more heavily labelled. This may reflect differences in the sugar composition of the individual glycoprotein components or differences in their relative turnover rates in culture (Hudson & Johnson, 1977).

The material bound by the lectins contained a

Table 2. Lectin affinity chromatography of epidermal plasma-membrane glycoproteins solubilized with deoxycholate

The deoxycholate-solubilized plasma membranes were passed through columns of concanavalin A-Sepharose 4B and lentil lectin-Sepharose 4B, which were washed and eluted with α -methyl D-mannoside as described in the Experimental section. The total amount of D-[^3H]-glucosamine and protein recovered from the column is expressed as a percentage of that applied. The amounts bound and eluted with α -methyl D-mannoside are expressed as a percentage of the total recovered from the column. The results are means $\pm$ S.E.M. Values in parentheses show the number of column runs.

	Recovery off column as % of material applied		Bound fraction as % of total recovered	
	D-[^3H]-Glucosamine	Protein	D-[^3H]-Glucosamine	Protein
Concanavalin A (9)	74 ± 8	93 ± 6	31 ± 2	13 ± 2
Lentil lectin (8)	86 ± 7	103 ± 9	38 ± 3	16 ± 3

Fig. 2. SDS/polyacrylamide-gel electrophoresis of epidermal plasma-membrane glycoproteins isolated by lectin affinity chromatography

Deoxycholate-solubilized plasma membranes (labelled metabolically with D-[^3H]glucosamine) were fractionated by lectin affinity chromatography as described in the Experimental section. The material that was bound and then eluted from concanavalin A (a-c) and lentil lectin (d-f) was dialysed, passed through Sephadex G-50, precipitated with ethanol and was solubilized with 2% (w/v) SDS/10mm-2-mercaptoethanol. A 50 μg portion of protein was analysed by electrophoresis on 10%-polyacrylamide gels, which were stained for protein and scanned at 550 nm (a and d). A 500 μg sample of protein was applied to gels (b and e), which were stained for carbohydrate and scanned at 560 nm. The latter gels were then frozen and sliced into approx. 1 mm sections, which were dissolved in H_2O_2 before measurement of their radioactivity (c and f). The arrow indicates the direction of migration. BPB indicates the position of the Bromophenol Blue tracking dye.

number of other protein components in addition to the four major glycoprotein bands (Figs. 2*a* and 2*d*). These were weakly stained by the periodic acid/Schiff reaction (Figs. 2*b* and 2*e*) and were only labelled to a small extent with D-[³H]glucosamine (Figs. 2*c* and 2*f*), indicating that they contained a smaller proportion of carbohydrate than the major glycoprotein components. A protein band corresponding to a mol.wt. of about 27 000 was present in the material bound by concanavalin A–Sepharose 4B (Fig. 2*a*), indicating leakage of lectin from the column. However this band was not entirely due to concanavalin A, since a protein band of similar molecular weight was present in decreased amounts in the material bound by lentil lectin–Sepharose 4B (Fig. 2*d*). Also this band was weakly stained by periodic acid/Schiff reagent, whereas concanavalin A contains no carbohydrate (Olson & Liener, 1967).

No significant periodic acid/Schiff-stained components were detected when the material that was not bound by the lectins was examined by SDS/polyacrylamide-gel electrophoresis (results not shown). Although this fraction contained a large proportion of the total D-[³H]glucosamine-labelled material recovered from the lectin columns (Table 2), electrophoresis indicated that the major labelled glycoprotein bands were absent. Much of the D-[³H]glucosamine-labelled material in these fractions remained at the top of the gel.

The present analyses suggest that non-desmosomal plasma membranes have a more complex glycoprotein composition than have desmosomal membranes. Desmosomes isolated at pH 2.6 contained only two major glycoprotein components, of apparent mol.wts. 140 000 and 120 000 (Skerrow & Matoltsy, 1974*b*). This may reflect genuine differences in the glycoprotein composition of different regions of the epidermal-cell plasma membrane.

Alternatively some glycoproteins may be selectively extracted from desmosomes during their isolation at low pH.

In common with other tissues (Hughes, 1976), plasma membranes isolated from pig epidermis contained protein-bound sialic acid, hexosamines, fucose, mannose and galactose (Table 3). These sugars were all enriched approx. 2-fold in plasma-membrane glycoproteins isolated with both immobilized lectins (Table 3). Preliminary analyses by g.l.c. suggest that *N*-acetylglucosamine is the main hexosamine. This would indicate that much of the carbohydrate is *N*-glycosidically linked to asparagine residues (Kornfeld & Kornfeld, 1976), which would agree with our previous finding that tunicamycin, an inhibitor of *N*-glycosylation, considerably decreased the incorporation of D-[³H]glucosamine into epidermal plasma-membrane glycoproteins (King & Tabiwo, 1981). Drochmans *et al.* (1978) found that desmosomes isolated at pH 9–11 contained glucosamine, but only traces of galactosamine.

The delipidated plasma membranes also contained significant amounts of glucose and xylose (Table 3). The former sugar was not enriched in the isolated glycoproteins, suggesting that it is probably a contaminant. Glucose was also consistently found in desmosomes isolated from cow snout epidermis (Drochmans *et al.*, 1978). Although often found in plasma-membrane preparations, glucose is usually considered to be a contaminant when present in isolated glycoproteins (Brown *et al.*, 1981). However, the origin of exogenous glucose is not clear in the present case. One possible contaminant is glycogen, which is present in significant amounts in the skin (Adachi, 1961). Xylose is found in plant glycoproteins (Yasuda *et al.*, 1970) and has been described in trypanosome plasma membranes

Table 3. *Carbohydrate composition of epidermal plasma-membrane glycoproteins*

Epidermal plasma membranes were washed, dialysed and extracted with chloroform/methanol (2:1, by vol.), whereas isolated glycoproteins were precipitated with ethanol, dissolved in water and dialysed before sugar analysis as described in the Experimental section. Values are means $\pm$ S.E.M. for three different preparations (four for sialic acid).

	Sugar (μ g/mg of protein)		
	Glycoproteins isolated using:		
	Delipidated plasma membrane	Concanavalin A–Sepharose 4B	Lentil lectin–Sepharose 4B
Sialic acid	8.2 $\pm$ 1.7	16.9 $\pm$ 3.1	16.2 $\pm$ 2.2
Hexosamine	28.9 $\pm$ 0.4	59.3 $\pm$ 3.9	59.9 $\pm$ 1.4
Fucose	3.3 $\pm$ 1.0	6.7 $\pm$ 1.0	6.3 $\pm$ 1.6
Xylose	18.7 $\pm$ 4.5	45.0 $\pm$ 9.0	33.3 $\pm$ 6.6
Mannose	19.7 $\pm$ 2.6	38.0 $\pm$ 5.8	37.3 $\pm$ 3.8
Galactose	23.0 $\pm$ 4.2	38.7 $\pm$ 1.9	40.0 $\pm$ 7.5
Glucose	17.0 $\pm$ 2.6	16.7 $\pm$ 1.9	10.7 $\pm$ 2.6

Table 4. *Amino acid composition of epidermal plasma-membrane glycoproteins*

Samples of delipidated plasma membranes and of isolated glycoproteins were subjected to amino acid analysis as described in the Experimental section. Each value is the mean for three different preparations. The s.e.m. was about 5% or less of the mean value in all cases.

	Composition (residues/100 residue)		
	Delipidated plasma membranes	Glycoproteins isolated using:	
		Concanavalin A-Sephrose 4B	Lentil lectin-Sephrose 4B
Asp	9.0	9.9	10.2
Thr	5.3	6.7	6.8
Ser	7.1	8.5	7.8
Glu	10.8	9.8	10.1
Pro	7.0	6.3	6.5
Gly	7.6	7.4	7.8
Ala	8.0	7.0	7.1
CyS	0.6	0.7	0.8
Val	6.2	6.9	7.2
Met	2.0	1.4	1.6
Ile	4.8	4.7	4.6
Leu	8.8	9.1	9.3
Tyr	3.0	3.3	3.3
Phe	4.0	4.4	4.6
His	2.5	2.7	2.5
Lys	7.2	5.5	5.6
Arg	5.2	4.6	4.8

(Hunt & Ellar, 1974), but is not a normal component of animal plasma-membrane glycoproteins. Nevertheless, xylose was found to be enriched in glycoproteins isolated from epidermal plasma membranes (Table 3). Xylose occurs as the sugar linking glycosaminoglycan chains to the core protein in proteoglycans (Rodén & Horowitz, 1978), and the presence of this sugar might reflect the presence of membrane-bound proteoglycans in the epidermis. However, the levels of xylose, relative to hexosamine, are higher than would be expected if it were derived only from proteoglycans. Determination of whether xylose is a genuine constituent of epidermal plasma-membrane glycoproteins will require the further fractionation and characterization of the individual components.

Comparison of the amino acid composition of the plasma membranes with that of their glycoprotein components (Table 4) revealed some small but reproducible differences. Glycoproteins isolated by using both immobilized lectins were enriched in threonine, valine, leucine, tyrosine and phenylalanine, which are all relatively hydrophobic amino acids (Nozaki & Tanford, 1971). This, together with their depletion in the charged residues glutamate, lysine and arginine, suggests that hydrophobic interactions may be important in the anchorage of these molecules in the plasma membrane. As might be expected, the isolated glycoproteins were enriched in aspartate (or asparagine) and serine, which are

involved in the linkage of carbohydrates to protein (Kornfeld & Kornfeld, 1976).

The isolated plasma membranes were depleted of membrane lipids. They contained $7 \pm 2 \mu\text{g}$ of phosphorus/mg of protein compared with $26 \pm 1.2 \mu\text{g}$ of phospholipid phosphorus/mg of protein in the original plasma membranes.

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