Purification and characterization of a differentiation-specific sialoglycoprotein of lactating-guinea-pig mammary tissue

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A large acidic glycoprotein, PAS-I, was purified from the fat-globule membrane of guinea-pig milk. Threonine and serine accounted for over 30 mol% of the amino acids, and galactose, *N*-acetylgalactosamine, *N*-acetylglucosamine, mannose and sialic acid were the principal sugars detected. On a molar basis, sialic acid accounted for over 60 % of the total sugar. Removal of sialic acid by treatment with neuraminidase revealed the presence of binding sites for peanut (*Arachis hypogaea*) agglutinin, a lectin specific for the sugar sequence β -D-Gal-(β 1 $\rightarrow$ 3)-D-GalNAc (the T antigen). The distribution of PAS-I-related epitopes, defined by five monoclonal antibodies, was determined in the mammary gland and in other guinea-pig tissues. PAS-I was maximally expressed on the apical surfaces of secretory cells in lactating mammary tissue and was either absent, or present in much lower amounts, in the glands of virgin or pregnant animals. PAS-I epitopes were not detected in liver, heart, spleen, pancreas, ovary, uterus, lung or intestine, either by immunofluorescence microscopy or by immunoblotting techniques. Several of the PAS-I-specific antibodies bound to mucins of high M_r in human fat-globule membrane, and similarities and differences between PAS-I and the human mucins are discussed. PAS-I and epitopes of this glycoprotein will be useful as indicators of differentiation in mammary cells and of markers of the apical surface of these cells during lactation.

INTRODUCTION

The temporal expression of certain glycoproteins on the surface of cells can serve to delineate stages in the differentiation and development of specific tissues and in the transformation or 'dedifferentiation' of cells into malignant phenotypes. Examples include stage-specific embryonic antigens (Johnson & Calarco, 1980; Gooi *et al.*, 1981; Feizi & Childs, 1985), fucosyl antigens in colon adenocarcinoma (Miyauchi *et al.*, 1982), and the expression of terminal β -D-Gal-(β 1 $\rightarrow$ 3)-D-GalNAc residues (the T antigen) on the surfaces of many tumour cells (Springer, 1984).

In previous studies a number of glycoproteins present on the surface of mammary cells during lactation have been purified and characterized (Basch *et al.*, 1976; Snow *et al.*, 1977; Imam *et al.*, 1981, 1982; Franke *et al.*, 1981; Greenwalt & Mather, 1985; Shimizu *et al.*, 1986). These proteins are secreted in association with the milk-fat-globule membrane, which is partly derived from the apical surface of secretory cells in mammary tissue [for reviews, see Anderson & Cawston (1975), Patton & Keenan (1975) and McPherson & Kitchen (1983)]. Since this membrane is secreted from terminally differentiated epithelial cells, the associated glycoproteins are potentially useful as developmental markers in the mammary gland [for a review, see Mather (1987)]. In this present paper we describe the isolation of a large acidic glycoprotein, PAS-I, from guinea-pig fat-globule

membrane and its biochemical characterization and distribution in various tissues.

EXPERIMENTAL

Materials

Galactose, CM-cellulose, Tos-Lys-CH₂Cl, Tos-Phe-CH₂Cl and neuraminidase (Type X, isolated from *Clostridium perfringens*) were obtained from Sigma Chemical Co. (St. Louis, MO, U.S.A.). Chaps was purchased from the Pierce Chemical Co. (Rockford, IL, U.S.A.) and peroxidase-conjugated peanut agglutinin (PNA) was from E-Y Laboratories (San Mateo, CA, U.S.A.). All other materials were from suppliers listed previously (Greenwalt & Mather, 1985).

Preparation of membrane fractions

Guinea-pig and human fat-globule membranes were prepared from milk as described by Johnson *et al.* (1985). Crude microsomal membrane fractions were prepared from lactating guinea-pig mammary gland, liver, lung, pancreas, kidney, heart, intestine, uterus and salivary gland. Tissues were homogenized for 20 s to 1 min with a Sorvall Omnimixer at speed setting 5, in 4 vol. (w/v) of ice-cold TBS [10-mM-Tris/HCl, pH 7.5, containing 0.9 % (w/v)-NaCl, 1.0-mM-6-aminohexanoic acid, aprotinin (0.11 units/ml), 1.0-mM-PMSF and saturating amounts of Tos-Lys-CH₂Cl and Tos-Phe-CH₂Cl]. The homo-

Abbreviations used: Chaps, 3-[(3-cholamidopropyl)dimethylammonio]propane-1-sulphonate; PAS, periodic acid/Schiff; PAS-I, periodic acid/Schiff-positive Band I; PMSF, phenylmethanesulphonyl fluoride; PNA, peanut (*Arachis hypogaea*) agglutinin; PAGE, polyacrylamide-gel electrophoresis; TBS, Tris-buffered saline (full composition and pH are given in the text); Tos-Lys-CH₂Cl ('TLCK'), *N*- α -tosyl-L-lysylchloromethane; Tos-Phe-CH₂Cl ('TPCK'), *N*-tosyl-L-phenylalanylchloromethane.

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genates were filtered through four layers of cheesecloth and centrifuged for 15 min at 2500 g. The 'post-nuclear' supernatant was then re-centrifuged for 90 min at 100 000 g, and the final sediment (designated 'crude microsomes') was resuspended and stored in TBS at -90 °C.

In some experiments membrane proteins were desialylated by treatment with neuraminidase (0.01 unit/mg of membrane protein) for 1 h at 37 °C in 50 mM-sodium acetate buffer, pH 5.1. The membrane suspensions were then re-adjusted to pH 7.4 with an equal volume of 50 mM-Tris/HCl buffer, pH 8.9.

Electrophoretic and blotting techniques

Fractions were analysed by SDS/PAGE and by preparative electrofocusing essentially as described by Mather *et al.* (1980).

Immunoblotting techniques were performed using peroxidase-conjugated goat anti-(mouse IgG) antibody as detecting agent and the Bio-Rad Immuno-Blot assay kit according to the manufacturer's instructions. Immunoreactive proteins were also detected by autoradiographic techniques essentially as described by Kaetzel *et al.* (1984).

Glycoproteins binding the lectin, PNA, were identified in membrane fractions, before and after treatment with neuraminidase. Proteins were separated by SDS/PAGE, electrophoretically transferred to nitrocellulose paper, and PNA-binding proteins were detected with peroxidase-conjugated PNA.

Purification of PAS-I

Guinea-pig fat-globule membrane (1–3 mg of membrane protein/ml) was solubilized with 1% (v/v) Triton X-100 as described by Mather *et al.* (1980), and the mixtures were centrifuged at 100 000 g for 1 h. The supernatant fractions, containing approx. 35% of the total protein, were diluted with an equal volume of 0.1 M-sodium acetate buffer, pH 3.5 or 4.5, and applied to a column (18 cm × 1.6 cm) of CM-cellulose that had been previously equilibrated with 50 mM-sodium acetate buffer, pH 3.5 or 4.5, containing 0.5% (v/v) Triton X-100. The cation-exchanger was then rinsed with at least 2 column volumes of the same buffer. Because PAS-I is extremely acidic, the glycoprotein was eluted in the void volume of the column. At pH 3.5, all other fat-globule-membrane proteins remained firmly bound to the ion-exchanger. At pH 4.5 results were more variable, and eluates were sometimes contaminated with other proteins. In most experiments, therefore, acetate solutions at pH 3.5 were used. The eluates were collected, dialysed overnight against 50 mM-Tris/HCl buffer, pH 7.4, at 4 °C and then concentrated either by pressure filtration using a Diaflo ultrafiltration unit (Amicon Corp., Lexington, MA, U.S.A.) fitted with a PM-10 membrane, or by adsorption and elution from DEAE-cellulose with 10 mM-Tris/HCl buffer, pH 7.5, containing 0.4% (w/v) Chaps and 3 M-NaCl. The Chaps detergent and NaCl were then removed by extensive dialysis against deionized water at 4 °C.

Control samples containing all components except solubilized membrane protein were also processed by the above procedures. These controls were used to monitor the possible contamination of purified preparations with sugars, especially glucose (see Table 2 below).

Monoclonal antibodies and immunofluorescence microscopy

Immunolocalization studies using five monoclonal antibodies to PAS-I were carried out with both frozen and paraffin-embedded tissue. Details of antibody preparation, tissue processing and immunofluorescence staining were as described by Johnson & Mather (1985).

Analytical techniques

Purified PAS-I preparations were hydrolysed in 6 M-HCl under N₂ for 3 days at 110 °C. Amino acids in the hydrolysates were analysed with a Joel model JLC-6AH amino acid analyser. Neutral sugars were analysed, as alditol acetate derivatives, by g.l.c. (Sawardeker *et al.*, 1965) coupled with m.s., and sialic acid was measured by the method of Aminoff (1961). Protein was analysed by the method of Lowry *et al.* (1951) and in the presence of Triton X-100, either by the modification described by Peterson (1977) or with the bicinchoninic acid reagent (Smith *et al.*, 1985). In each case bovine serum albumin was used as standard.

RESULTS

Preliminary characterization of membrane-bound PAS-I

PAS-I is readily detected with the PAS reagent after separation of guinea-pig fat-globule-membrane proteins by SDS/PAGE (Fig. 1, panel b, lane 1, asterisk; Johnson & Mather, 1985; Johnson *et al.*, 1985). In contrast, the glycoprotein does not stain with Coomassie Blue (Fig. 1, panel a, lane 1) and is relatively insensitive to the silver stain described by Merril *et al.* (1981).

PAS-I binds PNA after removal of sialic acid residues by treatment with neuraminidase (Fig. 1, panel c, lanes 1 and 2). Several other PNA-binding proteins were also detected, most notably a series of components with apparent M_r values from 65 000 to 80 000 (Fig. 1, panel c, square bracket).

The apparent M_r of PAS-I was estimated by comparing its electrophoretic mobility during SDS/PAGE with those of a set of protein standards. Values of 216 000, 211 000, 190 000 and 190 000 were obtained in 6, 8, 10 and 12% (w/v) polyacrylamide gels respectively. The asymptotic minimum M_r as defined by Segrest & Jackson (1972) was therefore 190 000. Neuraminidase treatment decreased the electrophoretic mobility of the protein (Fig. 1, panel b, lanes 1 and 2, asterisks), so that the apparent M_r rose to over 400 000. Given the large apparent size of PAS-I and its aberrant behaviour during electrophoresis, an accurate estimation of the protein's M_r is therefore not possible by these techniques.

PAS-I is markedly acidic. By the use of preparative electrofocusing over 90% of guinea-pig PAS-I focused within the pH range 2.1–3.6. Since this technique allows the formation of stable gradients down to approx. pH 2.0, a fraction of PAS-I may be even more acidic than is suggested by these estimates.

Purification of guinea-pig PAS-I

The acidic nature of PAS-I, compared with other components of guinea-pig fat-globule membrane, allowed the purification of this glycoprotein by a simple one-step procedure. Adsorption of detergent-solubilized membrane proteins at pH values between 3.5 and 4.5 on CM-cellulose resulted in binding of all the components of fat-globule membrane, except approx. 40% of PAS-I,

Fig. 1. Detection of PAS-I and PAS-I-like glycoproteins by SDS/PAGE and blotting techniques, in guinea-pig and human fat-globule membrane before and after treatment with neuraminidase

Guinea-pig and human fat-globule membrane (50 μ g of membrane protein/sample) were separated by SDS/PAGE in 6.0%-(w/v)-polyacrylamide gels, before and after treatment with neuraminidase. The separated proteins were either stained with (a, e) Coomassie Blue or (b, f) the PAS reagent, or transferred to nitrocellulose paper and incubated with either (c, g) peroxidase-conjugated PNA or (d, h) monoclonal antibody D-274. In Panels c and g, essentially all the stained proteins showed decreased binding or no binding to peroxidase-conjugated PNA in the presence of 50 mM-galactose, indicating specific binding to PNA. Guinea-pig and human proteins are shown to the left (a-d) and right (e-h) of the Figure respectively, and the junction between the stacking and separating gels is marked by a horizontal line spanning two lanes. Lanes 1 and 2, samples before and after neuraminidase treatments respectively. Symbols: *, PAS-I; ▲, xanthine oxidase, M_r 155000; ■, butyrophilin, M_r 63000; ▶, ▷, presumptive proteolytic fragments of PAS-I in samples before and after treatment with neuraminidase respectively. '[' is referred to in the text.

which was eluted in the void volume. Analysis by SDS/PAGE showed that these eluates contained a single major component that stained strongly with the PAS reagent, but not with Coomassie Blue, and had a similar electrophoretic mobility to that of PAS-I (Fig. 2). No Coomassie Blue-positive proteins were evident in the purified preparations.

Chemical characterization of PAS-I

Amino acid analysis of PAS-I (Table 1) showed that serine and threonine are the most abundant amino acids and comprise over 30 mol% of the total residues. PAS-I is composed of 30–50% carbohydrate by weight (Table 2). Major neutral sugars detected, in two separate preparations, were galactose, *N*-acetylgalactosamine, *N*-acetylglucosamine and mannose. Glucose was also present in both preparations. However, comparisons with a control sample showed that this was probably a contaminant introduced during isolation of the protein, e.g., during chromatography on CM-cellulose or during dialysis using cellulose tubing. Xylose was present at low levels in one sample. Since this sugar is not a common constituent of glycoproteins, the significance of this result is uncertain. PAS-I contains high levels of sialic acid (Table 2). On a molar basis, sialic acid accounted for 63.5% of the total sugar in sample 1 and 58.8% in sample 2.

Binding of monoclonal antibodies to PAS-I

Five monoclonal antibodies (D-16, -40, -256, -274 and -345; Johnson & Mather, 1985) were used to determine

the presence of PAS-I in the mammary gland and in other organs and tissues. The specificity of these antibodies was established by immunoblotting procedures, in which bound antibody was detected with either peroxidase-conjugated (Fig. 1, panel d) or 125 I-labelled second antibodies (Johnson & Mather, 1985). Each antibody bound to PAS-I, both before, and to a lesser extent after, treatment with neuraminidase (see, for example, Fig. 1, panel d, for D-274). Binding of antibody to minor components of lower apparent M_r than PAS-I was also detected (Fig. 1, panel d, closed and open arrow-heads). These components are most probably proteolytic degradation products of PAS-I produced either *in vivo* or during isolation of fat-globule membrane (Greenwalt *et al.*, 1985).

A major immunoreactive protein with the same apparent M_r as PAS-I was also detected in crude microsomal membranes from lactating mammary tissue with all of the antibodies (see, for example, Fig. 3, lanes 1 and 2, for D-274). This tissue form of PAS-I was demonstrated to bind PNA after treatment with neuraminidase (results not shown).

Distribution of PAS-I epitopes in guinea-pig mammary gland and other tissues

To determine if PAS-I is present at all stages of mammary development, guinea-pig mammary tissue was obtained from mature virgin guinea pigs as well as from pregnant and lactating animals. Antibody-binding sites increased as the gland developed and reached a maximum during lactation (Fig. 4). PAS-I appeared to be absent, or

present at extremely low levels, in the mammary gland of virgin guinea pigs (Fig. 4, panels a and b). Tissue from mid-pregnant animals showed a 'patchy' apical fluorescence, and secretory material in developed alveoli was positively stained (Fig. 4, panels c and d). Lactating

Fig. 2. Analysis of protein fractions, before and after purification of PAS-I, by SDS/PAGE

Samples of guinea-pig fat-globule membrane (lanes 1), the Triton X-100-solubilized extract (lanes 2) and the purified fraction after CM-cellulose chromatography (lanes 3) were separated by SDS/PAGE on a 6%-(w/v)-polyacrylamide gel, stained with the PAS reagent (panel b) and then counterstained with Coomassie Blue (panel a). Note the absence of Coomassie Blue-positive proteins in the purified fraction (panel a, lane 3). Symbols: ▶, PAS-I; ▲, xanthine oxidase, M_r 155 000; ■, butyrophilin, M_r 63 000.

tissue exhibited very bright staining along the entire apical surface of the secretory epithelial cells (Fig. 4, panels e and f).

PAS-I or PAS-I-like proteins were not detected either by immunofluorescence microscopy (results not shown) or immunoblotting techniques in liver, lung, pancreas, heart, intestine, uterus, salivary gland (Fig. 3, lanes 3–5 and 7–10), ovary and spleen (results not shown). In immunoblots there was no apparent reaction between kidney microsomal proteins and any of the antibodies (Fig. 3, lane 6). However, in tissue sections the glomerulus and distal convoluted tubules were brightly stained (results not shown). This inconsistency may be because the antibody-defined epitopes in kidney are irreversibly denatured by SDS/PAGE before transfer of the proteins to nitrocellulose for immunoblotting.

Distribution of PAS-I epitopes in human fat-globule membrane

Large glycoproteins have been identified in the fat-globule membranes of several species including rat, cow

Table 1. Amino acid composition of guinea-pig PAS-I

Values for serine and threonine assume 10% destruction of these residues during the 72 h hydrolysis. Abbreviation: n.d., not determined.

Amino acid	Composition (mol/100 mol)	Amino acid	Composition (mol/100 mol)
Ala	7.2	Lys	5.3
Arg	3.8	Met	1.8
Asx	5.2	Phe	2.9
Cys	2.1	Pro	4.2
Glx	2.7	Ser	15.2
Gly	6.2	Thr	15.1
His	8.0	Trp	n.d.
Ile	3.8	Tyr	2.7
Leu	5.6	Val	7.9

Table 2. Sugar composition of guinea-pig PAS-I

Sugar	Control‡	Amount of sugar (µg/mg of protein*)		Molar ratio† relative to N-acetylglucosamine	
		Sample 1 §	Sample 2	Sample 1	Sample 2
Galactose	0.30	156.3	76.6	5.2	3.1
N-Acetylgalactosamine	0	29.9	32.0	0.8	1.1
Glucose	4.29	–11.1	25.8	–	–
N-Acetylglucosamine	0	37.2	30.0	1.0	1.0
Mannose	0.63	34.3	11.0	1.1	0.4
Sialic acid	0	734.0	337.0	14.1	8.0
Xylose	0.39	0	8.4	–	–

* Portions (50 µg) of PAS-I protein were analysed; protein was determined with the bicinchoninic acid reagent; values have been corrected for sugars detected in the control sample.

† For the purposes of calculating molar ratios, values for glucose and xylose were omitted (see the text).

‡ Values represent µg of sugar detected in a 'blank' sample fractionated by ion-exchange chromatography, concentrated and analysed by g.l.c.-m.s. (see the text).

§ Samples 1 and 2 were prepared from different batches of guinea-pig fat-globule membrane

|| This negative value reflects larger amounts of glucose in the control sample than in sample 1.

Fig. 3. Distribution of PAS-I in guinea-pig tissues determined by SDS/PAGE and immunoblotting techniques

Samples of guinea-pig fat-globule membrane (50 μ g of protein) and microsomal-membrane fractions (approx. 100 μ g of protein/sample) from guinea-pig tissues were separated by SDS/PAGE in 8%-(w/v)-polyacrylamide gels, and the separated proteins were electrophoretically transferred to nitrocellulose paper. PAS-I was localized by using monoclonal antibody D-274 and peroxidase-conjugated goat anti-(mouse IgG) antibody. (a) Coomassie Blue-stained proteins; (b) corresponding immunoblot. Guinea-pig fat-globule membrane in lane 1 was compared with microsomal membrane fractions in lanes 2–9 from the following tissues: 2, lactating mammary gland; 3, liver; 4, lung; 5, pancreas; 6, kidney; 7, heart; 8, intestine; 9, uterus; and 10, salivary gland. A major immunoreactive protein in lactating mammary gland is indicated by an arrowhead ($\blacktriangledown$).

and man (Kobylka & Carraway, 1972; Snow *et al.*, 1977; Huggins *et al.*, 1980; Shimizu & Yamauchi, 1982; Shimizu *et al.*, 1986). The human glycoproteins are only partially resolved by SDS/PAGE and remain in 4% (w/v) stacking gels as a series of Coomassie Blue-negative, PAS-positive, bands (Fig. 1, panels e and f). As found with guinea-pig PAS-I, neuraminidase treatment decreased the electrophoretic mobilities of a fraction of these proteins (Fig. 1, panel f). Similarly, the human glycoproteins were positive for the T antigen, although unlike guinea-pig PAS-I, PNA bound to the human proteins both *before* and after treatment with neuraminidase (Fig. 1, panel g). All five monoclonal antibodies to guinea-pig PAS-I cross-reacted with the human glycoproteins and a presumptive degradation product (closed and open arrowheads) both before and after removal of sialic acid residues (Fig. 1, panel h).

DISCUSSION

Heavily glycosylated proteins of high M_r have been isolated from human fat-globule membrane (Shimizu & Yamauchi, 1982; Fischer *et al.*, 1984; Sekine *et al.*, 1985; Shimizu *et al.*, 1986), malignant breast carcinoma cells (Sekine *et al.*, 1985) and various rodent mammary-cell lines, including adenocarcinomas of the mouse (Cordington *et al.*, 1972; Van den Eijnden *et al.*, 1979) and rat (Sherblom *et al.*, 1980; Steck *et al.*, 1987). These glycoproteins share several properties with guinea-pig PAS-I, including high levels of serine and threonine, the presence of sugars common to *O*-glycosidic bonds and PNA-binding sites. Additionally, epitopes of guinea-pig PAS-I, defined by five monoclonal antibodies, are associated with the human fat-globule mucins.

Despite this, there are also significant species differences, even when glycoproteins from the same source (e.g., fat-globule membrane) are compared. Thus PAS-I and the human fat-globule mucins differ markedly in size and in the molar amounts of certain sugars and amino

acids [compare the present study with those of Shimizu & Yamauchi (1982) and Shimizu *et al.* (1986)]. Most notably, guinea-pig PAS-I contains mannose, indicating the presence of *N*-glycosidic bonds, and much higher amounts of sialic acid than the human mucins. PAS-I also contains approximately one-third the amount of proline. This amino acid is commonly abundant, together with serine and threonine, in human mucin-like glycoproteins (see, e.g., Donald, 1973; Creeth *et al.*, 1977). However, it should be noted that comparisons of amino acid composition are complicated by the polymorphic nature of the protein core of the human fat-globule mucins (Swallow *et al.*, 1987).

The most striking feature of the composition of guinea-pig PAS-I is the surprisingly high level of sialic acid. Sialic acid is most commonly found as single terminal residues on protein-associated oligosaccharide chains. However, on a molar basis, sialic acid accounted for approx. 60% of the total sugar analysed. This is clearly more neuraminic acid than can be accounted for as single terminal residues. There are at least two possible explanations for these data. PAS-I may contain an unusual sialic acid derivative or other component that reacts anomalously with the thiobarbituric acid reagent, resulting in an erroneously high estimate for 'sialic acid' content. Alternatively PAS-I may contain poly(sialic acid) residues, possibly of the kind described by Hoffman *et al.* (1982) and Finne *et al.* (1983) in the neural-cell adhesion molecule, 'N-CAM'. In agreement with this latter possibility, highly acidic oligosaccharides can be released from PAS-I by alkaline borohydride treatment that separate on DEAE-cellulose in a manner similar to, but not identical with, partially hydrolysed colominic acid [poly(sialic acid)] standards (P. J. Madara, D. A. Zopf and I. H. Mather, unpublished work).

As a possible reflection of the large amount of sialic acid in PAS-I, neuraminidase treatment caused a pronounced change in the electrophoretic mobility in SDS/polyacrylamide gels. This paradoxical observation, in

Fig. 4. Detection of PAS-I in guinea-pig mammary tissue at various stages of development using immunofluorescence techniques and monoclonal antibody D-274

Mammary tissue was fixed in 'methacorn' (Warburton *et al.*, 1982) and embedded in paraffin. Sections were treated sequentially with monoclonal antibody D-274, horse anti-(mouse IgG) antibody conjugated with biotin and fluorescein-avidin. Mammary tissue was from panels a and b, an 8-week-old virgin; panels c and d, the fourth week of pregnancy; panels e and f, the seventh day of lactation. Panels a, c and e, immunofluorescence micrographs; panels b, d and f, corresponding phase-contrast micrographs. The bar represents 50 μ m.

which removal of material leads to an *increase* in apparent size, has been observed following similar treatment of other glycoproteins, such as glycophorin (Gahmberg & Andersson, 1982). The native charge on most glycoproteins is effectively masked after binding of large quantities of negatively charged SDS. In glyco-

phorin and PAS-I, however, the high levels of sialic acid apparently contribute a significant additional negative charge to the SDS-protein complexes. As a consequence, electrophoretic mobilities during SDS/PAGE are higher than would be expected from the actual size of the protein (Gahmberg & Andersson, 1982). The mobility of

desialylated proteins, therefore, more closely parallels the expected behaviour of such proteins during electrophoresis. Bearing this in mind, the estimated M_r of the native form of PAS-I is likely to be in excess of 400 000.

It should be noted that the PAS-I analysed in the present study is only a fraction of the glycoprotein identified by SDS/PAGE. It remains to be determined whether the samples of PAS-I analysed are representative of the total membrane-bound form(s) of this glycoprotein. However, both purified PAS-I and 'total' PAS-I share many of the same properties, including cross-reactivity with monoclonal antibodies, the presence of PNA-binding sites and an identical change in electrophoretic mobility after neuraminidase treatment.

Guinea-pig PAS-I is one of several glycoproteins of fat-globule membrane that appear to be maximally expressed in lactating mammary tissue. Other examples include the high- M_r human mucins (Arklie *et al.*, 1981), guinea-pig and bovine butyrophilin (Franke *et al.*, 1981; Johnson & Mather, 1985) and guinea-pig GP-55 (Johnson & Mather, 1985). The PAS-I specific antibodies discussed here should be useful as markers of terminally differentiated epithelial cells and the apical surfaces of these cells during lactation. The restricted distribution of PAS-I epitopes in guinea-pig tissues also suggests that these antibodies may be useful as specific 'mammary-cell markers'. Antibody D-274 has been used to identify mammary cells in a variety of human pathological conditions, including benign fibrocystic disease and infiltrating and non-infiltrating duct carcinoma (Greenwalt *et al.*, 1985).

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