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DISTRIBUTION OF PROTEIN-BOUND SUGAR RESIDUES IN MICROSOMAL SUBFRACTIONS AND GOLGI MEMBRANES

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SUMMARY

Liver microsomal subfractions and Golgi membranes free from adsorbed and secretory proteins have a characteristic sugar composition. The ratio of mannose to galactose is largest in rough microsomes, smaller in smooth I microsomes, still smaller in smooth II microsomes, and smallest in Golgi membranes. There is about twice as much glucosamine in Golgi membranes and 3 times as much in smooth II microsomes as in the other microsomal subfractions. Golgi membranes are rich in sialic acid in comparison to rough microsomes and it is present at even higher levels in the two smooth microsomal subfractions. Increasing concentrations of deoxycholate preferentially remove protein-bound mannose and glucosamine, while releasing significantly less galactose. About half of the microsomal mannose and galactose can be liberated from the surface of intact microsomal vesicles by treatment with trypsin. When trypsin is added to permeable vesicles where the inside surface can be also attacked, an additional 20% of the total mannose but no additional galactose is liberated.

INTRODUCTION

The microsomal fraction isolated from rat liver appears to contain glycoproteins tightly associated with the membrane structure [1, 2]. Rough and smooth microsomes contain both neutral and amino sugars in addition to sialic acid [3–5]. Golgi membranes have not yet been investigated in detail but evidently contain sialic acid [6]. The function of microsomal glycoproteins is not known, in contrast to the plasma membrane glycoproteins of erythrocytes [7]. The possibility has arisen that three minor microsomal enzyme proteins, nucleotide pyrophosphatase, adenosine monophosphatase (AMPase) and β-glucuronidase, may be glycoproteins [8–10]. This possibility is, however, far from sufficient to account quantitatively for the glycoproteins present in microsomes.

Studies on the glycoprotein composition of microsomal membranes involve two main problems. First, the microsomal fraction and subfractions have to be reasonably pure without sizeable contamination from other cytoplasmic membranes.

The second problem is that more than half of the protein in the microsomal fraction is nonmembranous but is adsorbed to the surface or is newly synthesized secretory protein entrapped in the vesicles [11]. The secretory proteins, which are to a large extent glycoproteins, are difficult to remove. In addition, released secretory proteins adsorb, at least in part, to the surface of microsomes during sedimentation.

An important question from the biological point of view is whether the transverse distribution of glycoproteins in the membranes of the endoplasmic reticulum is asymmetric. The localization of glycoproteins on the outside of the plasma membrane would require an accumulation of these glycoproteins inside the endoplasmic reticulum if membrane flow and fusion to the cell surface is occurring [12].

In this investigation the distribution of various protein-bound sugar residues in rough, smooth I and II microsomes, as well as in Golgi membranes were studied after application of a procedure which effectively removes cytosolic adsorbed proteins and secretory proteins free in the vesicle lumen. The distribution of microsomal glycoprotein in the transverse plane was analyzed using microsomal vesicles either intact (and thereby impermeable to macromolecules such as proteases) or in the presence of detergent (and thereby permeable to such macromolecules).

MATERIALS AND METHODS

Fractionation

Adult male albino rats weighing 180–200 g were used. The animals were starved for 20 h before sacrifice. Total microsomes and microsomal subfractions were prepared as described previously [13]. For isolation of Golgi membranes starved rats were given 1.2 g 50 % ethanol per 100 g body weight by stomach tube 90 min before decapitation [14]. The total Golgi fraction, floating on the top of the 1.15 M sucrose layer, was used. In some experiments smooth microsomes were flotated in a similar way in order to remove contaminating Golgi. Plasma membranes [15], mitochondria [16] and lysosomes [17] were isolated using procedures described earlier. For the preparation of lysosomes rats were injected intraperitoneally with 1 ml Triton WR-1339 (364 mg/ml) four days before sacrifice.

The various fractions were washed with alkaline Tris buffer in order to remove adsorbed proteins and then subjected to water thermal treatment, which releases the content of the vesicles [11]. A final washing of the subfractions with alkaline Tris buffer was also routinely performed.

Deoxycholate and trypsin treatment

Total microsomes were treated with increasing concentrations of deoxycholate as follows. The membranes were suspended (15 mg protein in 9 ml) in 0.15 M Tris · HCl buffer (pH 8.0) and 1 ml of a deoxycholate solution was added in order to obtain the final concentration of detergent desired. After mixing the suspension was centrifuged at 105 000 $\times g$ for 2 h and the pellet was analyzed. For trypsin treatment microsomes were suspended in 0.15 M Tris · HCl buffer (pH 7.5) mixed with trypsin (Boehringer, Mannheim; 50 μg/mg microsomal protein), and incubated at 30 °C for 15 min. After incubation the samples were cooled and centrifuged at 105 000 $\times g$ for 60 min. The pellets were washed with Tris buffer (0.15 M, pH 8.0). The permeability of vesicles to macromolecules was increased by adding microsomes (40 mg protein in

2 ml Tris buffer, 0.15 M, pH 7.5) with rapid mixing to 9 ml of a medium containing 50 mM KCl and 0.061 % sodium deoxycholate [18]. In appropriate cases trypsin was also added (50 μg/mg protein). After incubation at 30 °C for 15 min the microsomes were sedimented by centrifugation and the pellet was analyzed.

Quantitation of carbohydrate components

Neutral sugars were separated and estimated by gas-liquid chromatography of the alditol acetate derivatives. The delipidated protein (5–10 mg) from the different subcellular fractions was hydrolyzed in 2.5 ml 1 M HCl for 8 h at 100 °C in sealed tubes under a nitrogen atmosphere [2]. After hydrolysis the released neutral sugars were purified by ion-exchange chromatography on Dowex 50 (H^+) and Dowex 1 ($HCOO^-$) [19, 20]. The alditol acetate derivatives were prepared as described elsewhere [19, 21].

Amino sugars were released by hydrolysis of 10–15 mg protein in 2.5 ml 2 M HCl for 16 h at 100 °C under a nitrogen atmosphere [2]. The amino sugars were then purified on Dowex 50 (H^+) [22] and the alditol acetate derivatives prepared according to Niedermeier [23].

Neutral sugars were separated on a stainless steel column packed with Gas Chrom Q, 60–80 mesh, coated with 3 % ECNSS-M [19, 21]. The temperatures of the injection port, column oven and detector were 220, 172 and 220 °C, respectively. The carrier gas was argon and the flow rate was 30–40 ml/min.

Amino sugars were separated on a glass column packed with Gas Chrom Q, 100–120 mesh, coated with 3 % Poly A-103 [24]. The temperatures of the injection port, column oven and detector were 250, 230 and 250 °C, respectively. The flow rate of the carrier gas was 30–40 ml/min.

Chemical and enzymatic measurements

Protein was determined according to Lowry et al. [25] with bovine serum albumin as standard. Phospholipids were analyzed as described previously [26]. Determination of sialic acid was made by the Warren procedure after delipidation [27]. The various marker enzyme activities were measured according to previously described methods [28, 29]. For the measurement of cytidine monophosphate-*N*-acetyl neuraminic acid (CMP-AcNeu) transferase activity desialidated fetuin was used as acceptor [29].

RESULTS

Marker enzymes

The specific activities of various marker enzymes in the different subfractions are given in Table I and the results are in agreement with those published previously [15–17, 28]. The smooth microsomal subfractions are obviously contaminated by Golgi elements (for which CMP-AcNeu transferase activity is a marker), which may be removed to a large extent from total smooth microsomes by flotation. Flotation cannot be performed on isolated smooth I and II microsomes since separation of these two subfractions is based on selective aggregation by divalent cations [13]. The presence of AMPase activity in microsomal subfractions and in Golgi membranes is probably not the result of a sizeable plasma membrane contamination but may be

TABLE I

DISTRIBUTION OF MARKER ENZYMES IN LIVER SUBFRACTIONS

Smooth microsomes were flotated according to Ehrenreich et al. [14]. For the measurement of CMP-AcNeu transferase desialidated fetuin was used as acceptor [29]. The values are the means of 4 experiments. CMP-AcNeu = cytidine monophosphate-*N*-acetylneuraminic acid; AMP = adenosine monophosphate.

Fraction	Glucose-6-phosphatase*	CMP-AcNeu** transferase	AMPase*	Cytochrome*** *c* oxidase	Acid phosphatase*
Rough microsomes	2.2	4.2	0.03	0.05	0.09
Smooth I microsomes	2.3	41.8	0.06	0.04	0.17
Smooth II microsomes	0.8	20.5	0.08	0.02	0.19
Total smooth microsomes (flotated)	2.5	12.8	0.08	0.04	0.15
Golgi fraction	0.2	851	0.10	0.02	0.20
Mitochondria	0.5	—	—	5.01	—
Lysosomes	—	—	—	—	1.12
Plasma membranes	—	—	0.73	—	—

* μmol P_i/min per mg protein
** pmol AcNeu transferred/10 min per mg protein
*** μmol O_2/min per mg protein

explained by the multimodal distribution of this enzyme, i.e. AMPase may also be localized on the endoplasmic reticulum and Golgi system [30, 31]. The levels of mitochondrial and lysosomal marker enzyme activities in the various microsomal subfractions and Golgi membranes are low. The latter fraction also exhibits 10 times lower glucose-6-phosphatase activity on a protein basis than the microsomal fractions.

Effect of washing

The presence of adsorbed and secretory proteins in the isolated microsomal

TABLE II

EFFECT OF WASHING ON THE NEUTRAL SUGAR CONTENT OF TOTAL MICROSOMES

Microsomes sedimented in 0.25 M sucrose (105 000 × *g*, 60 min) were suspended in water for analysis on non-treated microsomes. For Tris wash the microsomal pellet was suspended in 0.15 M Tris · HCl (pH 8.0) to give a concentration of 40 mg protein/10 ml and sedimented by centrifugation. For the water-thermal treatment the microsomal pellet was suspended in cold distilled water to give a concentration of 1 mg protein/ml, incubated at 30 °C for 15 min, cooled rapidly in an ice-water bath and sedimented by centrifugation. The different treatments were done consecutively as indicated in the table and after each treatment an aliquot was withdrawn for analysis. The values represent the mean of 3 experiments.

Procedure	Protein		Mannose		Galactose	
	mg/g liver	% of total	μg/g liver	% of total	μg/g liver	% of total
None	22.3	100	96.5	100	28.7	100
First Tris wash	15.7	70.2	86.1	89.2	24.9	86.8
Water-thermal treatment	11.3	50.6	67.0	69.4	23.1	80.4
Second Tris wash	10.0	44.8	60.2	62.4	22.2	77.4

fractions is obviously highly undesirable if one is measuring chemical components. Adsorbed protein, about 30 % of the total, can be removed effectively by alkaline washing (Table II). Luminal secretory proteins, which make up about 25 % of the total microsomal protein, can be released and removed by water thermal treatment followed by washing with Tris buffer. An accurate determination of the sugar composition of microsomes is dependent on the use of an effective washing procedure. The presence of nonmembranous glycoproteins is indicated by a decrease in the content of protein-bound sugar during washing; about 40 % of the total mannose and 20 % of the galactose are removed by the washing procedures employed here.

A number of experiments were performed to study the effect of the Tris-water-thermal treatment on microsomes (Table III). Labeled serum proteins were mixed with the homogenate and part of these serum proteins proved to be associated with the microsomal fraction prepared from the homogenate (Table III, experiment 1). The washing procedure employed removed 90 % of this adsorbed protein. The

TABLE III

EVALUATION OF THE TRIS-WATER-THERMAL WASHING PROCEDURE

In experiment 1 labeled total serum proteins were prepared by intraportal injection of 0.2 mCi [^{14}C] leucine (Radiochemical Centre, Amersham, U.K.) followed by decapitation 60 min later. The serum was passed through a Sephadex G-25 column (Pharmacia Fine Chemicals, Uppsala, Sweden) and an aliquot of the protein peak was mixed with a liver homogenate and after preparation of microsomes Tris-water-thermal washing was performed. In experiment 2 phospholipid and enzyme activities and amounts were measured before and after Tris-water-thermal treatment of microsomes. In experiment 3 microsomes were prepared after the injection of 0.2 mCi [^{3}H]glucosamine (Radiochemical Centre) into the portal vein of rats 60 min before decapitation. The washed microsomes were treated with rabbit antiserum to rat serum protein by a two-step antibody precipitation procedure as described previously [33]. Total radioactivity and radioactivity in isolated *N*-acetylneuraminic acid (AcNeu) in the total microsomes and in the precipitate was determined. The results given are the means of 3–5 experiments.

Experiment	Homogenate	Microsomes	
		Non-washed	Tris-water-thermal treated
1 Radioactivity from added labeled serum proteins[a]	948 000	26 700	2900
2 Phospholipid[b]		5.4	5.2
NADPH-cytochrome *c* reductase[c]		0.11	0.10
Cytochrome b_5[d]		1.82	1.87
Nucleoside diphosphatase[e]		1.60	1.52
3 Radioactivity precipitable with antiserum[f]			18
Radioactivity in AcNeu precipitable with antiserum[g]			0

[a] total cpm;
[b] mg/g liver;
[c] μmol NADPH oxidized/min per mg phospholipid;
[d] nmol/mg phospholipid;
[e] μmol P_i/min per mg phospholipid;
[f] % of total microsomal radioactivity;
[g] % of total radioactivity in AcNeu.

amount of phospholipid and the amount or activity of some microsomal enzymes were determined in order to study the possibility that Tris-water-thermal treatment may extract some membrane components (Table III, experiment 2). The washing procedure did not remove any phospholipid; and three microsomal enzymes which are known to be loosely bound to the membrane structure [32], NADPH-cytochrome *c* reductase, cytochrome b_5 and nucleoside diphosphatase displayed unchanged activity or amount. The removal of the serum protein from the luminal compartment was controlled by subjecting the washed microsomes to a two-step antibody precipitation procedure. This was achieved using rabbit antiserum against rat serum protein and, for the removal of all antigen-antibody complexes, purified sheep IgG against rabbit IgG [33]. Some labeled glycoproteins are precipitated with the antibody but none of the glycoproteins containing AcNeu (Table III, experiment 3) indicating thereby the presence in the washed microsomes of only a limited amount of serum proteins which are in the process of biosynthesis and which are known to occur in membrane-bound form during core completion [34]. The above experiments show that the washing procedure used is effective in removing adsorbed and luminal secretory proteins. The fact that loosely bound microsomal enzymes are not removed by the washing procedure is no direct evidence against the possible removal of some membranous glycoproteins but such removal seems to be improbable.

Sugar composition

Washed microsomal membranes contain the neutral sugars mannose and galactose (Table IV). Highly variable amounts of glucose were also detected on the gas chromatograms; this glucose probably originates from remaining sucrose or glycogen. Rough and smooth I microsomal fractions are relatively rich in protein-bound mannose. Smooth II microsomes contain even more protein-bound mannose, while Golgi membranes contain less, on a phospholipid basis.

On the other hand, the protein-bound galactose varies to a greater extent between the subfractions being least in rough, higher in smooth I, and highest in smooth II microsomes. Golgi membranes are also relatively rich in protein-bound galactose. Consequently, the ratio of protein-bound mannose to galactose is highest in rough, lower in smooth I, still lower in smooth II microsomes, and lowest in Golgi membranes.

Relatively large amounts of glucosamine are present in all the subfractions examined and the distribution pattern is similar to that found for galactose (Table V). The amount on a phospholipid basis is lowest in rough, higher in smooth I, and still higher in Golgi membranes; and again, smooth II microsomes contain 3 times as much glucosamine as the other microsomal fractions. Small amounts of galactosamine were also identified on the gas chromatograms, but the significance of this finding is questionable. The low amounts of this sugar present in all fractions made a realistic statistical evaluation impossible.

Protein-bound AcNeu varies greatly between the subfractions. About 5 μg AcNeu/mg phospholipid were found in washed rough microsomes; 1.7 times this amount was recovered in Golgi membranes; while the amount in smooth I and II microsomes is about 2 times greater than that in rough microsomes.

The distribution of marker enzymes shown in Table I demonstrates that the only significant contaminant of smooth microsomes is Golgi membranes. It is improb-

TABLE IV

NEUTRAL SUGAR COMPOSITION OF LIVER MICROSOMAL MEMBRANES

All subfractions were subjected to the Tris-water-thermal washing procedure as described in Table II. Determination of the neutral sugars is described in Materials and Methods. The values are the means ± S.E.M. ($n = 7$). PL = phospholipid.

Fraction	Protein (mg/g liver)	PL (mg/g liver)	Mannose		Galactose		Mannose/ Galactose
			μg/g liver	μg/mg PL	μg/mg liver	μg/mg PL	
Total microsomes	11.0 ± 1.8	5.28 ± 0.37	60.2 ± 3.2	11.4	22.2 ± 1.8	4.20	2.71
Rough microsomes	5.85 ± 0.70	2.65 ± 0.32	27.3 ± 3.3	10.3	8.69 ± 1.5	3.28	3.14
Smooth I microsomes	3.92 ± 0.67	1.40 ± 0.04	15.7 ± 1.1	11.2	8.33 ± 0.58	5.95	1.88
Smooth II microsomes	0.37 ± 0.06	0.43 ± 0.05	7.05 ± 0.85	16.4	5.93 ± 0.42	13.8	1.19
Golgi membranes	0.30 ± 0.04	0.28 ± 0.02	1.88 ± 0.13	6.71	2.83 ± 0.34	10.1	0.66

TABLE V

DISTRIBUTION OF HEXOSAMINES AND *N*-ACETYLNEURAMINIC ACID IN LIVER MICROSOMAL MEMBRANES

Measurement of amino sugars and AcNeu was performed as described in Materials and Methods. The values are the means ±S.E.M. ($n = 6$). In the case of galactosamine the values obtained were low and difficult to quantitate on the chromatogram. For this reason only approximative values are given in the Table. AcNeu = *N*-acetylneuraminic acid; PL = phospholipid

	Glucosamine		Galactosamine (μg/mg PL)	AcNeu	
	μg/g liver	μg/mg PL		μg/g liver	μg/mg PL
Total microsomes	61.5 ±3.7	11.6	0.5–1	38.1 ±1.62	7.22
Rough microsomes	23.7 ±1.5	8.94	~0.5	13.0 ±0.92	4.92
Smooth I microsomes	16.9 ±1.9	12.1	0.5–1	15.8 ±1.0	11.3
Smooth II microsomes	12.9 ±1.9	30.1	1–2	4.29 ±0.14	9.97
Golgi membranes	6.52 ±0.22	23.3	1–2	2.31 ±0.25	8.26

TABLE VI

EFFECT OF FLOTATION ON SMOOTH MICROSOMES

Sucrose was added to total smooth microsomes to give a final concentration of 1.17 M. This suspension (30 mg protein/10 ml) was placed in a tube of the SW27 rotor and overlayered with 7 ml 1.10 M sucrose followed by 20 ml 0.25 M sucrose. After centrifugation at 74 000 × *g* for 3 h the fraction remaining in the 1.17 M sucrose was diluted, recentrifuged and the ensuing pellet was washed as in previous experiments. The values are the mean of 3 experiments. PL = phospholipid.

	PL (mg/g liver)	Mannose		Galactose	
		μg/g liver	μg/mg PL	μg/g liver	μg/mg PL
Total smooth microsomes	1.80	18.9	10.5	10.2	5.67
Total smooth microsomes (flotated)	1.72	18.3	10.6	9.62	5.59

able that the neutral sugar content of microsomal fractions is significantly influenced by Golgi contamination, but this possibility was tested directly as follows. Smooth microsomes were flotated by the procedure of Ehrenreich et al. [14] in order to remove Golgi contaminants (Table VI). The remaining purified microsomal fraction had protein-bound mannose and galactose contents very similar to those of the non-flotated smooth microsomes, indicating that the small amount of Golgi membranes present in isolated smooth microsomal subfractions does not seriously interfere with determination of the neutral sugar composition.

Deoxycholate treatment

Deoxycholate solubilizes microsomal components in a non-random fashion since at relatively low concentrations some of the microsomal electron transport enzymes are liberated while others remain in a tightly bound form [35]. In order to study the manner in which microsomal glycoproteins are associated with the vesicles, total microsomes free from adsorbed and secretory proteins were treated with increas-

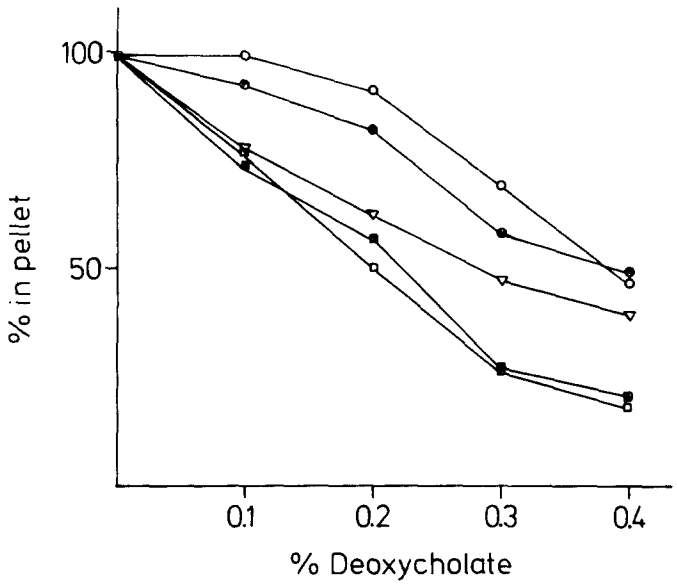

Fig. 1. Effect of deoxycholate on microsomal protein-bound sugars. Washed total microsomes (1.5 mg protein/ml) were subjected to appropriate concentrations of deoxycholate as described in Materials and Methods and the various chemical components remaining in the pellet after centrifugation were determined. Each value represents the mean of 5–7 experiments. (○) galactose; (■) glucosamine; (□) mannose; (●) phospholipid; (▽) protein.

ing concentrations of deoxycholate (Fig. 1). With a protein concentration of 1.5 mg/ml and a deoxycholate concentration up to 0.2 % only a small amount of phospholipid is solubilized and practically no galactose-containing glycoproteins are released. In contrast, at this deoxycholate concentration about 50 % of both protein-bound mannose and glucosamine are liberated. At a deoxycholate concentration of 0.4 %, where half of the phospholipid is solubilized, half of the protein-bound galactose but only 20 % of the microsomal protein-bound mannose and glucosamine can be recovered in the pellet. Similar results were obtained when rough and smooth microsomal subfractions instead of total microsomes were treated with increasing concentrations of deoxycholate (not shown). It appears that galactose-rich microsomal glycoproteins are more tightly bound and less easy solubilized than those enriched in mannose and glucosamine.

Effect of treatment with trypsin

Trypsin treatment of intact microsomes removes about 40 % of the microsomal protein, but only enzymes and other proteins localized on the cytoplasmic surface are liberated [36]. Table VII demonstrates that about half of the protein-bound mannose and galactose are solubilized by such treatment. Repeated incubations with trypsin do not remove more of these sugars. Incubation of 4 mg microsomal protein/ml in the presence of 0.05 % deoxycholate and 0.5 M KCl does not solubilize any microsomal protein or phospholipid but makes the microsomal vesicles permeable to macromolecules like trypsin [18]. When trypsin is introduced into the intramicrosomal space by this procedure, about 10 % of the protein of the inner surface is solubilized together

TABLE VII

INTRAMEMBRANOUS LOCALIZATION OF NEUTRAL SUGARS IN MICROSOMAL MEMBRANES

Protein, phospholipid, mannose and galactose were determined in control total microsomes and in microsomes after various treatments described in detail in Materials and Methods. The control microsomes as well as those used for further treatments were washed with the Tris-water-thermal procedure. All treatments were performed on trypsin-treated microsomes, also washed with Tris · HCl 0.15 M, pH 8.0 ("Trypsin microsomes"). Each value represents the mean of 6 experiments.

Fraction	Treatment	Protein		Phospholipid		Mannose		Galactose	
		mg/g liver	% of control	mg/g liver	% of control	μg/g liver	% of control	μg/g liver	% of control
Total microsomes (control)	None	10.2	100	5.35	100	61.5	100	21.3	100
Trypsin microsomes	None	5.63	55	4.81	90	32.5	53	13.3	63
	Trypsin	5.15	50	4.51	84	28.9	47	12.4	58
	50 mM KCl; 0.05 % deoxycholate	4.85	48	4.65	87	28.5	46	11.9	56
	Trypsin; 50 mM KCl; 0.05 % deoxycholate	3.66	36	4.52	84	16.6	27	12.3	58

with an additional 20 % of protein-bound mannose. In contrast, no galactose is released by proteolytic treatment of the inner surface.

DISCUSSION

The sugar composition of glycoproteins in various cytoplasmic membranes, as well as the manner in which these glycoproteins are associated with the membranes, were investigated. It is concluded that the glycoproteins of different membranes display specific sugar compositions and that some of these glycoproteins are tightly bound membrane components.

Several investigations performed to date have led to the conclusion that microsomal fractions contain glycoproteins [1–6]. The presence of contaminating particles, in our case mainly Golgi membranes, is insufficient to influence significantly the distribution pattern of various sugars. What is more important is the removal of adsorbed and secretory proteins, since many of these proteins are glycoproteins. Microsomal and Golgi membranes washed with alkaline buffer and subjected to the water thermal procedure do not differ qualitatively in carbohydrate composition. All of these fractions contain the neutral sugars mannose and galactose, glucosamine as their main amino sugar, and sialic acid. The almost total absence of galactosamine indicates that the only type of binding between protein and the oligosaccharide chains in the glycoproteins present is *N*-glycosidic linkage between asparagine and *N*-acetyl glucosamine [37].

With respect to quantitative sugar composition these four types of membranes differ significantly from each other and display characteristic patterns. The highest carbohydrate content is found in smooth II microsomes. The nature and origin of this subfraction is not yet established but recent subfractionation and enzymatic analyses indicate that these membranes are part of the endoplasmic reticulum and are specialized for specific functions [38]. The high mannose to galactose ratio in rough microsomes distinguish this fraction from smooth I microsomes; the corresponding ratio in the Golgi fraction is particularly low. Glucosamine content is lowest in rough microsomes, higher in smooth I microsomes, and still higher in Golgi membranes. The sialic acid content displays a still different pattern, being present in the Golgi fraction and smooth I vesicles in amounts that are 1.7 and 2 times, respectively, the amount found in rough microsomes.

The different glycoproteins within one subfraction are associated with the membrane in different ways. When membranes are solubilized with detergents, proteins rich in mannose and glucosamine are easily solubilized, thus exhibiting a loose binding similar to that of NADPH-cytochrome *c* reductase and cytochrome b_5, for example [35]. On the other hand, proteins rich in galactose are tightly bound and not liberated to any great extent by detergent, this behavior is similar to that of phospholipids, ATPase and glucose-6-phosphatase [35]. Previous investigations revealed similar behavior for microsomal protein-bound sialic acid [4].

The carbohydrate portions of the plasma membrane glycoproteins of erythrocytes and other cell types are known to have an asymmetric transverse distribution; all of the sugar residues (identified as antigens, receptor sites, etc.) are found on the outer surface [7, 39]. If microsomal and Golgi vesicles move to the plasma membrane and fusion occurs involving an inversion of the inner surface of these vesicles to

the outer surface of the plasma membrane, one would expect that most of the glycoproteins of microsomes would be found at the inner surface of the vesicle [12]. This is clearly not the case. A sizeable part but not all of the neutral sugar residues may be removed by proteolytic digestion of the cytoplasmic surface. When trypsin is introduced into the vesicle lumen some mannose but practically no galactose is liberated. In earlier studies neuraminidase treatment of the inner microsomal surface did not remove protein-bound sialic acid [4]. The experimental data strongly suggest that the majority of microsomal membrane glycoproteins are not located at the inner surface but display a more or less symmetrical distribution.

No explanation can be offered at present as to why the carbohydrate composition of various subfractions is different and why some glycoproteins are tightly bound to membranes while others are loosely bound. One possibility is that a number of glycoproteins are enzymes participating in different functions. Several microsomal proteins and lipids are known to be distributed asymmetrically in the transverse plane of the membrane [40]. It is also possible that some of the membrane glycoproteins change location during their synthesis, moving to new sites where completion of the oligosaccharide chain may take place [33]. Such movement is established for some secretory proteins which, during core completion, are in a membrane-bound form [34].

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