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INCORPORATION OF GALACTOSE FROM UDP-GALACTOSE INTO MICROSOMAL AND GOLGI MEMBRANES OF RAT LIVER

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Summary

Rough and smooth microsomes and Golgi membranes were incubated with UDP[^{14}C]galactose and the incorporation of radioactivity into the lipid extract and into endogenous protein acceptors were measured. Antagonistic pyrophosphatases were inhibited with ATP and interference from β-galactosidase activity was greatly decreased by carrying out the incubation at pH 7.8. After incubation the particles were centrifuged to remove free oligosaccharide residues. Radioactivity was found in the lipid extract from Golgi membranes but not from rough and smooth microsomes. This radioactivity, however, was not associated with dolichol or retinyl phosphates. The incorporation of radioactivity into proteins of the Golgi fraction was more than double than that of the microsomal fractions. In addition, the transferases in these two types of particles exhibited different properties. Trypsin treatment of intact rough microsomal vesicles, smooth vesicles and Golgi membranes removed about 5, 15 and 50%, respectively, of newly incorporated protein-bound galactose, indicating that the proportion of the newly galactosylated proteins, which are localized at the cytoplasmic surface of the membrane, is lowest in rough microsomes, intermediate in smooth, and highest in Golgi membranes.

Introduction

Most of the glycoproteins of the membranes of the endoplasmic reticulum and Golgi apparatus, as well as most of the secretory proteins synthesized on bound ribosomes and glycosylated during transport to the blood, contain galactose. During recent years a number of investigations have been performed on the nature and the site of galactose incorporation using UDP[^{14}C]galactose as substrate and microsomal and Golgi membranes with or without exogenous acceptors. The results obtained are contradictory, which is probably a result of the

different conditions used. Both the involvement and the non-involvement of lipid intermediates in galactosyl transfer reactions have been proposed [1—4] and the possibility that retinyl phosphate rather than dolichol phosphate is mediating this type of reaction in lung, intestinal, epidermal and mastocytoma cells has also been raised [5,6]. The transferase activity was found to be enriched in smooth microsomes contaminated by Golgi membranes [7,8], while other investigations localized this enzyme almost exclusively to the Golgi fraction [9—11]. On the other hand, recent studies have drawn attention to the fact that microsomes contain enzymes which hydrolyze UDPgalactose to galactose-1-phosphate and galactose [3,12].

The endoplasmic reticulum of the liver, together with the Golgi system, functions in the synthesis and transport of many blood proteins, most of which are glycoproteins. Independently of this synthetic process, the endoplasmic reticulum also synthesizes glycoproteins which are used in the construction of its own membrane [13]. It is possible that there are different mechanisms for the synthesis of these two types of proteins, since some of these membrane glycoproteins are on the cytoplasmic surface of microsomes. Chemical measurements and experiments with lectins established that about half of the protein-bound neutral sugars and sialic acid could be removed by enzymic hydrolysis of intact microsomal vesicles [14,15]. Also, the two microsomal cytochromes, *P*-450 and b_5, which are localized on the outer surface of the microsomes [16] have proven to be glycoproteins [17,18].

In order to obtain further information on the biosynthesis of membrane glycoproteins we have studied the glycosylation of endogenous acceptors in various subcellular fractions and we have also investigated the topology of newly galactosylated proteins in the transverse plane of the membrane.

Materials and Methods

Adult male albino rats weighing 180—200 g were used. All animals were starved for 20 h before being killed. Total microsomes and microsomal subfractions were prepared as described previously [19]. The total Golgi fraction was isolated according to Ehrenreich et al. [11] with the modification that the sucrose layers were 0.25 M, 1.10 M and 1.30 M sucrose, respectively. All fractions were washed by recentrifugation in 0.15 M Tris · HCl buffer, pH 8.0, to eliminate adsorbed proteins [19]. Trypsin treatment of microsomes was performed as described previously [16]. The permeability of microsomal vesicles to macromolecules was increased by the modified deoxycholate-salt procedure of Kreibich et al. [16,20].

For in vitro incorporation the incubation mixture contained (in a total volume of 400 μl): 30 mM Tris · HCl buffer, pH 7.8, 2.5 mM EDTA, 10 mM $MnCl_2$, 1.5 mM ATP (in the case of rough microsomes) or 2 mM ATP (in the case of smooth microsomes and Golgi membranes), 0.05 μCi of UDP[^{14}C]galactose (320 mCi/mol, Radiochemical Centre, Amersham) and 50 μl sample containing 1.5 mg (rough microsomes), 1.0 mg (smooth microsomes) or 0.6 mg (Golgi membranes) protein. The incubations were performed at 30°C for 30 min in thick-wall centrifuge tubes. After incubation the mixture was cooled and centrifuged at 105 000 × *g* for 60 min. This pellet was suspended in 0.5 ml water

and extracted with 3 ml chloroform/methanol (2 : 1, v/v) at 40°C for 20 min with occasional mixing. After centrifugation the upper water/methanol phase was removed and the surface rinsed with "upper phase" [21]. The chloroform phase was decanted, mixed with 1.5 ml upper phase, centrifuged and the surface was rinsed with upper phase. This washing procedure was repeated twice. The remaining protein was washed 3 times with 1.5 ml upper phase and extracted twice with 3 ml chloroform/methanol (2 : 1, v/v) at 40°C for 20 min. The three chloroform-methanol extracts were pooled (Lipid 1 fraction). The remaining fraction was extracted with 3×1 ml chloroform/methanol/H_2O (1 : 1 : 0.3, v/v) (Lipid 2 fraction). The protein pellet was washed with 1 ml H_2O and solubilized in 1 ml 2% sodium dodecyl sulphate. The two lipid extracts were evaporated and radioactivity was measured after addition of 10 ml Bray solution [22].

When total galactosyl transferase activity was measured the incubation medium was also supplemented with 4 nmol non-radioactive UDPgalactose, 40 mM mercaptoethanol and, as exogenous acceptor in some cases, 150 μg of desialidated mucin. At the end of the incubation the particles were precipitated with 7% trichloroacetic acid and dissolved in 2% sodium dodecyl sulphate for measurement of radioactivity. Bovin submaxillary gland mucin was desialidated by acid hydrolysis (0.05 N H_2SO_4 at 80°C for 1 h), neutralized and dialyzed against Tris · HCl, pH 7.5. After lyophilization the protein was dissolved in water (30 mg/ml).

Protein was estimated with the Biuret reaction [23]. The values in the tables and figures are the means of 5—8 experiments.

Results

A number of earlier investigations have demonstrated that the liver contains pyrophosphatase which hydrolyze nucleotide-bound sugars, e.g. UDP galactose [3,12]. One of the locations for these pyrophosphatases is the soluble cytoplasm and nonspecific adsorption to microsomes results in contamination of this fraction. Such contamination was reflected in our studies by the finding that reproducible results could only be obtained using microsomes washed with alkaline Tris-buffer. On the other hand, active pyrophosphatases, including enzymes splitting UDPgalactose, are present in many cytoplasmic and also in plasma membranes [24,25]. In the absence of ATP, most of the substrate in the incubation medium was hydrolyzed by rough and smooth microsomes and Golgi membranes, and the transferase activity measured as the appearance of radioactivity in the protein fraction was consequently low (Fig. 1). In the presence of ATP (1.5 mM in the case of rough microsomes and 2.0 mM in the case of smooth microsomes and Golgi membranes) the [^{14}C]galactose transfer increased 20—30 times in all three fractions. No lipid soluble counts were found after incubation with rough and smooth microsomes, while the chloroform-methanol extract after incubation with Golgi membranes in the presence of 2 mM ATP contained about 1000 cpm per mg protein. These results indicate that UDPgalactose transferase activity cannot be studied without inhibiting endogenous pyrophosphatase activity.

It is possible that a lipid intermediate is saturated rapidly at the start of the

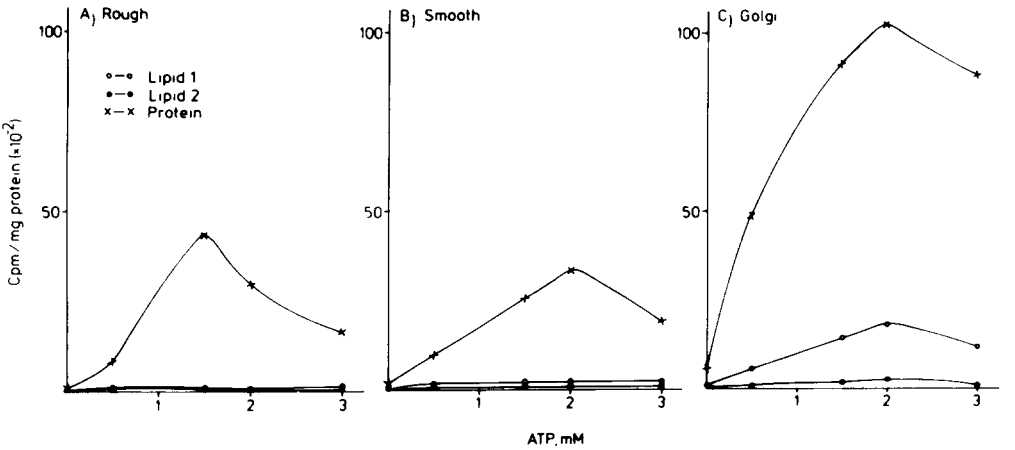

Fig. 1. Effect of ATP on the UDP[^{14}C]galactose transferase activity in rough (A), smooth (B) and Golgi membranes (C). After incubation the mixtures were centrifuged prior to extraction. Lipid 1 denotes the radioactivity soluble in chloroform/methanol (2 : 1, v/v) and Lipid 2 represents the radioactivity appearing in the chloroform/methanol/H_2O (1 : 1 : 0.3, v/v) extract.

incubation but no longer contains radioactivity after 30 min. For this reason we performed incubations for 1, 2 and 5 min (not shown in Table I). No glycosylation occurs in the absence or in the presence of dolichol phosphate with rough or smooth microsomes. The concentration of ATP present had no effect on the results.

Experiments with exogenous dolichol monophosphate demonstrated that this substance is not glycosylated by any of the three subfractions used (Table I). Furthermore, the presence of exogenous dolichol phosphate in the incubation medium did not stimulate sugar transfer to the protein acceptor.

In all our experiments, with the exception of the detergent studies, the particulate fraction was separated by ultracentrifugation at the end of the incubation, and the pellet was extracted with lipid solvents. The absolute necessity for this procedure is shown in Table II. The radioactivity in the protein fraction decreased after centrifugation to various extents; in the case of rough microsomes only one-third of the radioactivity remained. Some of the lipid extracts also contained significant radioactivity if no centrifugation was performed.

TABLE I

INCUBATION OF MICROSOMES AND GOLGI MEMBRANES WITH UDP[^{14}C]GALACTOSE IN THE PRESENCE OF ADDED DOLICHOL PHOSPHATE

The amount of dolichol phosphate added was 5 nmol. Radioactivity in Lipid 1, Lipid 2 and protein was determined as described in Materials and Methods. Results expressed in cpm/mg protein.

	— Dolichol phosphate			+ Dolichol phosphate		
	Lipid 1	Lipid 2	Protein	Lipid 1	Lipid 2	Protein
Rough microsomes	138	54	4 316	141	70	4 134
Smooth microsomes	197	54	5 844	125	64	5 778
Golgi membranes	1117	221	12 130	1003	189	11 292

TABLE II

EFFECT OF CENTRIFUGATION ON RADIOACTIVITY RECOVERED IN THE SUBFRACTIONS

After incubation the mixtures were extracted directly with chloroform/methanol (2 : 1, v/v) or centrifuged at 105 000 X *g* for 60 min prior to extraction. Results expressed in cpm/mg protein.

	No centrifugation			Centrifugation		
	Lipid 1	Lipid 2	Protein	Lipid 1	Lipid 2	Protein
Rough microsomes	491	190	5 759	149	81	3 903
Smooth microsomes	374	42	6 743	214	56	3 237
Golgi membranes	986	471	18 023	420	314	11 069

Apparently, the situation is the same as with microsomes incubated with GDP-mannose [26] and UDP-*N*-acetylglucosamine [27], that is, liberated oligosaccharides [27] contaminate the protein and lipid fractions.

Under our conditions radioactivity was present in the chloroform-methanol extract only from Golgi membranes and the possibility naturally arose that a

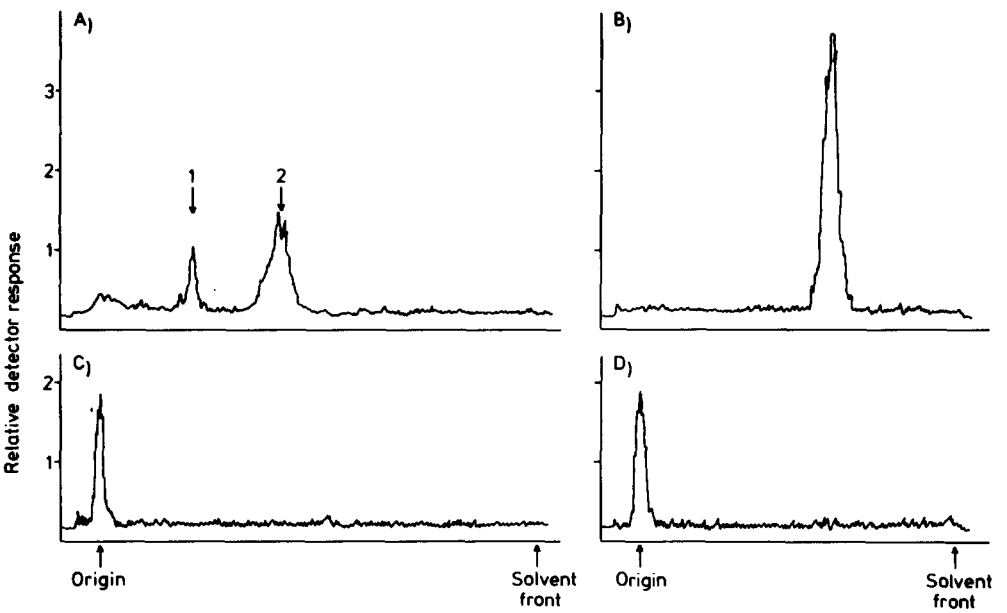

Fig. 2. Thin layer chromatography of lipid soluble radioactivity from Golgi membranes incubated with UDP[^{14}C]galactose. A: The chloroform/methanol (2 : 1, v/v) extract of rough microsomes after incubation with GDP[^{14}C]mannose and retinyl phosphate was purified by chromatography on DEAE-cellulose [28]. The fraction containing retinyl phosphate-mannose was mixed with the chloroform/methanol (2 : 1, v/v) extract of rough microsomes incubated with GDP[^{14}C]mannose and dolichol phosphate. Thin layer chromatography was performed on Silica gel 60 plates (Merck, Darmstadt) developed with chloroform/methanol/H_2O (60 : 25 : 4, v/v). The radioactivity was detected using a thin layer scanner. Peak 1 represents retinyl phosphate-mannose and peak 2 dolichol phosphate-mannose. B: The lipid extract of rough microsomes after incubation with GDP[^{14}C]mannose and dolichol phosphate was chromatographed on silica gel. The solvent system was *n*-propanol/H_2O (8 : 2, v/v). C: Golgi membranes were incubated with UDP[^{14}C]galactose and the chloroform/methanol extract was subjected to chromatography as in A. D: The chloroform/methanol extract of Golgi membranes after incubation with UDP[^{14}C]galactose was chromatographed as in B.

lipid carrier could be involved in the transfer of galactose to protein. Thin layer chromatography with chloroform/methanol/H_2O as the developing system (Fig. 2A) demonstrated that retinyl phosphate-mannose and dolichol phosphate-mannose synthesized enzymatically with rough microsomes have R_F values of about 0.2 and 0.4 respectively. In *n*-propanol/H_2O/dolichol phosphate-mannose has an R_F value of about 0.6 (Fig. 2B). Chromatography of the chloroform/methanol extract from Golgi membranes after incubation with UDPgalactose showed that the radioactive peak at the origin did not move upon chromatography in these two systems (Fig. 2C and D). Thus, it is unlikely that lipid intermediates are involved in the galactosyl transferase reaction under our conditions.

Another question which requires consideration is the presence of water soluble glycolipids which could remain in the final protein residue after chloroform/methanol (2 : 1, v/v) and chloroform/methanol/water (1 : 1 : 0.3, v/v) extraction. Hydrophilic glycolipids have been characterized in erythrocytes [29,30] and it is possible that such lipids are also present in intracellular membranes. In our experiments the protein residue was washed with water which in fact removes some radioactive compounds. These are apparently, as described in connection with Table II, liberated oligosaccharides. Consequently, in agreement with previous findings [31], it is improbable that under our in vitro conditions, glycolipids are present in the protein residue. In order to further investigate this problem we performed extensive labeling experiments using sugar, lipid and protein precursors followed by the extraction of the final protein residue according to the procedure of Kościelak et al. [29,30]. In the phosphate buffer phase, in which the hydrophilic glycolipids of erythrocytes appear, only a small portion, around 10% of the total galactose radioactivity was present. The nature of this minor water soluble radioactivity was investigated using a number of chromatographic procedures. The majority of the galactose incorporated under in vitro conditions in the phosphate buffer was found to be associated with small peptides or was present in free oligosaccharide chains. Therefore, we conclude that the extraction procedure used in these experiments removes all lipids, and no water soluble glycolipids, like those described in erythrocytes [29,30], are present in the final protein residue.

The effect of Triton X-100 on the transfer of galactose to protein is shown in Fig. 3. The transfer by rough and smooth microsomes was influenced only to a small extent; but the transferase activity in Golgi membranes was inactivated by increasing concentrations of the detergent.

A serious problem in studies of glycosyl transferase is the presence of various glycosidases which attack mainly the protein-bound product. In the case of α-mannosidase it is known that this type of enzyme is present not only in lysosomes but also in Golgi membranes and in the supernatant [32]. When the β-galactosidase activity in the membrane fractions used was measured, all three displayed high hydrolytic activity at pH 4 (Fig. 4). At pH 6 these activities were greatly decreased and at pH 7.8 they were low and probably no longer significant. For this reason all our investigations were carried out at pH 7.8.

In most glycoproteins galactose is situated at the terminal part of the oligosaccharide chain, which should greatly limit the number of acceptor

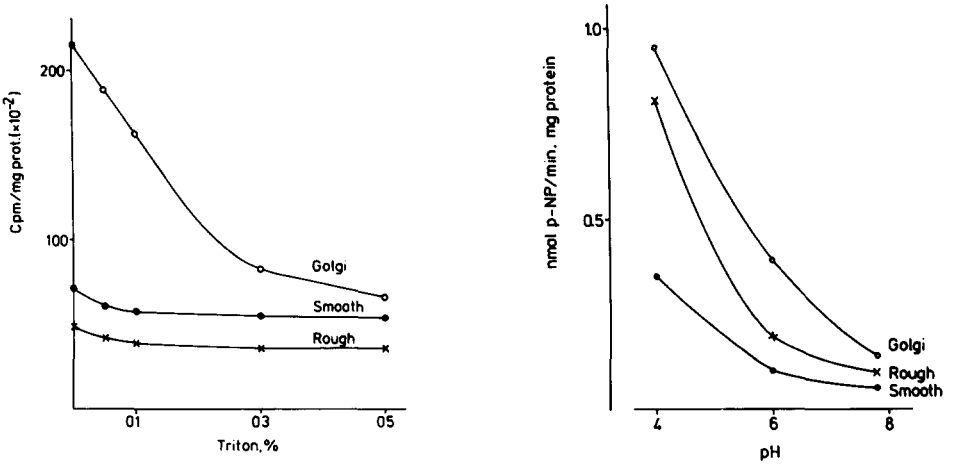

Fig. 3. Effect of Triton X-100 on the incorporation of galactose from UDP[^{14}C]galactose. Triton was added to the incubation medium to give the indicated final concentrations and after incubation the mixture was extracted directly with chloroform/methanol and chloroform/methanol/H_2O. The figure gives only the data for the protein fraction.

Fig. 4. β-Galactosidase activity of various fractions at different pH. The incubation medium contained 0.2 ml citrate/phosphate buffer, 0.05 M, pH 4.0 or 6.0, or Tris-buffer, 0.08 M, pH 7.8; 20 μl Triton X-100, 1%; 100 μl *p*-nitrophenylgalactose pyranoside, 10 mM, and sample in a final volume of 0.5 ml. After incubation at 37°C for 30 min the reaction was stopped with 0.5 ml 12% trichloroacetic acid, centrifuged and 0.5 ml 2 M Trizma base was added to the supernatant. Absorption was measured at 400 nm.

molecules. In order to produce more acceptor molecules for galactose, preincubation of rough microsomes and Golgi membranes with UDP-*N*-acetylglucosamine and GDPmannose (Table III) was performed. With both these fractions only GDPmannose was able to increase the incorporation of galactose into protein. Thus, it seems probable that the sugar moiety preceding galactose

TABLE III

THE EFFECT OF PREINCUBATING ROUGH MICROSOMES AND GOLGI MEMBRANES WITH UDP-*N*-ACETYL-GLUCOSAMINE AND GDP-MANNOSE

Rough microsomes and Golgi membranes were incubated with 10 nmol of non-labeled UDP-*N*-acetylglucosamine (GlcNAc) or GDPmannose using the incubation medium described in Materials and Methods without UDP[^{14}C]galactose. After incubation at 30°C for 10 min the particles were isolated by ultracentrifugation and suspended in an incubation medium containing UDP[^{14}C]galactose as substrate. After incubation the mixtures were centrifuged prior to extraction. Radioactivity in the protein fraction is shown in the table.

Preincubation	Incubation with UDP[^{14}C]galactose	
	cpm/mg protein	%
Rough microsomes		
None	3 824	100
UDPGlcNAc	3 900	102
GDPmannose	5 621	147
Golgi membranes		
None	11 264	100
UDPGlcNAc	10 986	98
GDPmannose	16 989	151

TABLE IV

REMOVAL OF THE SECRETORY PROTEINS FROM TOTAL MICROSOMES AFTER INCUBATION WITH UDP[^{14}]GALACTOSE

Microsomes were incubated as described in Materials and Methods, recentrifuged, washed with alkaline Tris-buffer and incubated at 0 or 30°C with 0.05% deoxycholate/50 mM KCl for 10 min. After ultracentrifugation the pellets were extracted and radioactivity in the protein fractions were determined.

Experiment	Treatment	Radioactivity in protein	
		cpm/g liver	%
1	None	33 310	100.0
	Deoxycholate, 0°C	26 715	80.2
2	None	36 373	100.0
	Deoxycholate, 30°C	29 208	80.3

on the oligosaccharide chain is, at least in part, mannose.

Under in vivo conditions both secretory and membrane glycoproteins are glycosylated in the membranes of the endoplasmic reticulum. Therefore, incorporation of galactose into these kinds of proteins under in vitro conditions was tested (Table IV). After incubation of total microsomes with UDP[^{14}C]galactose the membranes were treated with deoxycholate at a concentration which is known to increase the permeability of the vesicles to macromolecules without solubilization of the membrane itself [20]. When the microsomes were treated either at 0 or 30°C, about 20% of the protein-bound label was removed,

TABLE V

EFFECT OF TRYPSIN TREATMENT OF THE VARIOUS FRACTIONS

The fractions were incubated with trypsin (50 μg/mg protein) at 30°C for 15 min, centrifuged and suspended for incubation with UDP[^{14}C]galactose. After incubation the mixtures were centrifuged prior to extraction. When trypsin treatment was performed after incubation the incubated fractions were pelleted, washed with alkaline Tris-buffer and trypsin-treated as above. After the proteolytic treatment, the recentrifuged pellets were extracted and the radioactivity in the protein fractions is shown in the table.

Treatment	Incorporation cpm/g liver	
	Protein	%
Rough microsomes		
None	35 696	100.0
Trypsin before incubation	34 197	95.8
None	31 383	100.0
Trypsin after incubation	29 343	93.5
Smooth microsomes		
None	10 486	100.0
Trypsin before incubation	11 768	112.5
None	8 535	100.0
Trypsin after incubation	7 143	83.7
Golgi membranes		
None	3 696	100.0
Trypsin before incubation	702	19.0
None	3 502	100.0
Trypsin after incubation	1 881	53.7

TABLE VI

GALACTOSYL TRANSFERASE ACTIVITY OF ISOLATED CYTOPLASMIC MEMBRANES

Galactosyl transferase activity in the absence or presence of desialidated bovine submaxillary gland mucin was measured as described in Materials and Methods.

Subfractions	pmol galactose transferred/mg protein	
	Endogenous acceptor	Exogenous acceptor
Rough microsomes	2.6	7.4
Smooth microsomes	20.4	55.2
Golgi membranes	131.6	1673.9

indicating that some secretory glycoproteins were also glycosylated under our conditions.

The topology of the newly synthesized membrane glycoproteins was studied using proteolytic treatment (Table V). In the case of rough microsomes only about 5% of the radioactivity in the protein fraction could be removed by treatment with trypsin before or after incubation. The results with smooth microsomes were not affected by proteolytic treatment before incubation but, in comparison with the rough subfraction, a larger amount (15%) of the labeled protein could be removed after incubation. In the Golgi membranes the glycoprotein acceptors and the transferase system are associated with the outer surface to a large extent. Proteolysis before incubation abolished the sugar transfer almost completely and trypsin treatment after incubation liberated about half of the newly glycosylated acceptors. Therefore, it appears that a small amount of newly galactosylated glycoproteins in smooth microsomes and a larger amount in Golgi vesicles are located on the cytoplasmic side of these organelles.

In all of the above experiments galactosylation of endogenous protein acceptors was studied without taking into consideration the actual level of galactosyl transferase activity in the fractions. As expected, the glycosylation is low when using only endogenous acceptors (Table VI). Clearly, the amount of acceptors present is very limiting and this amount is lowest in rough microsomes, intermediate in smooth, and highest in Golgi membranes. When galactosylation of desialidated mucin is measured in the presence of excess substrate and acceptor, the capacity for glycosylation was 10-fold greater in smooth than in rough microsomes and 30-fold greater in Golgi membranes than in smooth microsomes. Clearly, Golgi membranes have by far the highest capacity for galactosylating the acceptor used in this case.

Discussion

In this paper the incorporation of galactose from UDP[^{14}C]galactose was investigated in order to determine the extent of glycosylation and the localization of endogenous acceptor proteins. Under the conditions used in this study transferase activity and acceptor proteins were found in rough and smooth microsomes and in Golgi membranes, but the glycosylation exhibited different properties in the microsomes and in the Golgi fraction.

During this investigation an attempt was made to inhibit enzymes present in the liver and capable of hydrolyzing the substrate or the product. In agreement with a previous investigation [3] ATP (at about 2 mM concentration under our conditions) is capable of inhibiting pyrophosphatase activity to a large extent; and the β-galactosidase activity of the various fractions is greatly inhibited by the alkaline pH used for incubation. However, complete inhibition of these antagonistic enzymes is not possible. Furthermore, the presence of free oligosaccharide residues in the lipid extracts and in the protein fraction [26,33] show that considerable hydrolysis of the sugar moiety occurred during the incubation. Obviously, direct analysis of the lipid extracts and of protein precipitates without the prior centrifugation step gives false results and centrifugation or some other procedure for removing radioactivity not bound to lipid or protein is absolutely necessary. We could not find evidence in our experiments that some type of lipid intermediate is involved in the transfer of galactose from UDPgalactose to protein. There was either no radioactivity in the lipid extract (microsomes) or the radioactivity was not associated with lipids (Golgi membranes).

The amount of endogenous acceptor is very limited and consequently the extent of glycosylation has no quantitative relation to the transferase activity. For this reason the total galactosyl transferase activity was also determined using an excess of substrate and exogenous acceptor. It was found that galactosylation of desialidated mucin takes place mainly in Golgi membranes and occurs only to a small extent in rough and smooth microsomes. A complete analysis of the subcellular localization of galactose transferase activities, however, would require the use of different types of acceptors both with simple and complex saccharide chains. It is possible that galactosylation of acceptors with other types of chains shows a pattern different from that described in Table VI, e.g., high activity in microsomes and lower activity in the Golgi fraction.

The galactosylation of rough and smooth microsomes differs significantly from that of the Golgi membranes. Triton X-100 does not influence the incorporation into microsomal proteins but greatly decreases the radioactivity appearing in the Golgi fraction. Trypsin treatment removes only small amounts of labeled proteins from the surface of the microsomes while the amount liberated from the Golgi fraction is about half of the total. Obviously, the acceptor proteins and probably also the transferase itself are distributed differently in the transverse planes of these different membranes.

An explanation for these differences is likely to be found in the type of acceptor glycosylated. It is reasonable to suppose that galactosylation in the microsomes involves proteins having oligosaccharide chains consisting of only a few sugars. On the other hand, galactosylation at the Golgi level involves the terminal part of the oligosaccharide chain in which the core is completed in the rough and smooth endoplasmic reticulum. In this case galactosylation in microsomes and in Golgi membranes is mediated by different enzymes with different acceptor specificities.

The trypsin sensitive part of intact microsomal vesicles contains only a small part (5—15%) of the newly galactosylated protein acceptors. In contrast, the outer surface of both rough and smooth microsomes has about 50% of the total

membrane protein-bound galactose [14]. There are several possible explanations for these findings. If there are two different enzyme systems operating in the membrane two different types of glycoproteins may be synthesized. Alternatively, the same galactosyl transferase may glycosylate two different types of proteins at the outer and the inner surfaces of the membrane. The theoretical possibility that all glycoproteins are galactosylated at the inner surface and appear at the cytoplasmic surface by a "flip-flop" mechanism is improbable [34]. No experiments to date have demonstrated such a movement. The lack of protein acceptors at the cytoplasmic surface of the endoplasmic reticulum is, furthermore, in good agreement with recent findings concerning the biosynthesis and transport of microsomal glycoproteins [35,36]. According to this concept some of these proteins, after synthesis on bound ribosomes, move in the lateral plane of the membranes, at the outer surface, from rough to smooth endoplasmic reticulum and then to Golgi membranes. During this process the proteins are glycosylated and, after completion in the Golgi apparatus released to the cytoplasm as a lipoglycoprotein complex and later incorporated into membranes of the endoplasmic reticulum as integral membrane components. In such a process completed, galactosylated glycoproteins are transferred from the cytoplasm to the outer surface of the membranes, which may explain the presence of the large amount of protein-bound galactose and the low amount of galactose acceptors at this surface.

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