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A STUDY OF INTRACELLULAR TRANSPORT OF SECRETORY GLYCOPROTEINS IN RAT LIVER

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Summary

To study the transport of secretory glycoproteins in the endoplasmic reticulum of rat liver, the distribution of nascent glycoproteins in the membrane and luminal fraction of rough and smooth microsomes has been examined after short-time incorporation of radioactive glucosamine in vivo. 50—60% of the radioactivity was associated with the membranes of rough and smooth microsomes, whereas about 10% of the serum albumin was found in the same fractions. The relative amount of radioactivity in the membranes was the same whether the luminal content of the microsomal vesicles was released by sonication, French press, Triton X-100, Brij 35 or sodium deoxycholate. The distribution of labeled glycoproteins between the membrane and luminal fraction of rough and smooth microsomes did not change during the time interval of 15—120 min after administration of the isotope. The similarity of the labeling patterns obtained after sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis indicated that the same set of glycoproteins were located in the lumen and the membrane of rough and smooth microsomes. A specific precipitation of nascent glycoproteins from both the membrane and luminal fractions of rough and smooth microsomes was obtained with rabbit antiserum against rat serum. The nascent glycoproteins associated with the membranes were not released by high ionic strength or treatment with mercaptoethanol. A slow exchange between [^{14}C]glucosamine-labeled glycoproteins in the lumen and membrane fraction was, however, found.

Introduction

The main part of the plasma proteins is glycoproteins being synthesized in the endoplasmic reticulum of liver. The protein part is synthesized on membrane-bound ribosomes and glycosylated by a stepwise addition of carbohydrates as the nascent glycoprotein migrates through the rough and smooth endoplasmic reticulum and the Golgi apparatus before being secreted

[1,2]. The glycosyltransferases responsible for these reactions are located in the membranes of the endoplasmic reticulum [3]. Whereas it has been shown that serum albumin, a plasma protein containing no carbohydrates, is transported through the lumen of the endoplasmic reticulum [2,4], not much is known about the mode of movement of those proteins which are modified before being secreted. Redman and Cherian [4] suggested, from their in vitro experiments, that nascent glycoproteins remained associated with the membranes of rough microsomes and possibly also with the membranes of the smooth microsomes until galactose is added. Our studies of protein-bound carbohydrates in various submicrosomal fractions from rat liver revealed, however, substantial amounts of glycoproteins in both the luminal and membrane fraction [5]. In a more recent investigation by Kreibich et al. [6,7], nascent plasma glycoproteins were found in both lumen and membranes of rough and smooth microsomes from rat liver, after selective release of the lumen content by low concentrations of detergent.

The present work was carried out to examine the possibility for an involvement of the membranes in the transport of secretory glycoproteins through the endoplasmic reticulum. As adequate methods for (1) release of luminal content from vesicles and (2) the separation of luminal content and membranes were a prerequisite for this investigation, special attention was paid to this problem. The results obtained indicate that during the transport through the channels of the endoplasmic reticulum the nascent plasma glycoproteins interact with the membranes.

Materials and Methods

Animals. Male albino rats, 200 g, of the Wistar strain (Møllegaard, Havrup, Denmark) were used. The animals were fasted for 20 h before being killed by decapitation. The [^{14}C]glucosamine and [^{3}H]glucosamine were injected intraperitoneally except in the pulse-chase experiments where the isotope and the chase D-glucosamine were injected in the tail vein under pentobarbital (5-ethyl-5-(methylbutyl)barbituric acid)anesthesia.

Chemicals. Rabbit antiserum to rat serum albumin was obtained from Calbiochem., Calif., U.S.A., rabbit antiserum to rat serum was from Miles Lab., Inc., Ind., U.S.A. and rabbit antiserum to chicken serum was purchased from Behringswerke A.G., Germany. D-[1-^{14}C]Glucosamine · HCl (4 Ci/mol) and D-[1-^{3}H]glucosamine · HCl (3.2 Ci/mmol) were obtained from Radiochemical Centre, Amersham. Sodium deoxycholate, Triton X-100 and sodium dodecyl sulfate (SDS) were from Sigma Chemical Co., St. Louis, Mo., U.S.A. Brij 35 and rat serum albumin were from Koch-Light Lab., U.K.

Preparation of microsomal fractions. The preparation of rough and smooth microsomes was carried out as described by Helgeland et al. [5], using the method of Bergstrand and Dallner [8]. To prepare total microsomes the post-mitochondrial supernatant was centrifuged at 102 000 × *g* for 1 h in a 65 Spinco rotor. The microsomal fractions were washed once with 0.25 M sucrose and once with 50 mM Tris · HCl buffer (pH 7.8)/150 mM KCl/10 mM $MgCl_2$. An additional wash with Tris/salt medium did not remove any protein. After

centrifugation at 159 000 × *g* for 1 h, the pellets were stored at −20°C and used within 2 months.

Release of luminal content of microsomal fractions. Two media were used. Medium A: 50 mM Tris · HCl buffer (pH 7.8)/150 mM KCl/10 mM $MgCl_2$ and medium B: 50 mM Tris · HCl buffer (pH 7.8)/50 mM KCl/10 mM $MgCl_2$. Washed rough and smooth microsomes from 10 g of liver, and total microsomes from 2.5 g of liver, were used in each of the following treatments:

Sonication: Microsomes were suspended in 2.0 ml of medium A, sonicated as described previously [9], and the volume adjusted to 9.0 ml.

French press: Microsomes were suspended in 9.0 ml of medium A, and the suspension was run through a French press twice at a pressure of about 900 kg/cm^2 and a flow rate of 3 ml/min.

Detergents: Microsomes were suspended in 9.0 ml of medium B containing either 0.05% sodium deoxycholate, 0.035% Triton X-100 or 0.04% Brij 35. Suspensions were gently stirred for 30 min.

All steps were carried out at 2—4°C.

Separation of luminal content, membranes and ribosomes. After release of the luminal content as described above, the microsomal suspension was layered onto 2.0 ml of 0.25 M sucrose in the medium used in the release procedure. Centrifugation was carried out for 4 h at 149 000 × *g* in 65 Spinco rotor. The supernatant, including the sucrose layer, contained the luminal fraction, whereas the pellet consisted of the membranes and ribosomes. To solubilize the membranes prior to polyacrylamide gel electrophoresis, the pellets were suspended in 9.0 ml 2.5% Triton X-100 in medium A, and after gentle stirring for 30 min layered onto 2.0 ml medium A containing 0.25 M sucrose and 2.5% Triton X-100. Centrifugation was carried out for 90 min at 149 000 × *g* in a 65 Spinco rotor. The supernatant, including the sucrose layer, contained the solubilized membranes, and the pellet consisted of ribosomes. All steps were carried out at 2—4°C.

Radioactivity measurements. Proteins were precipitated by the addition of an equal volume of 12% trichloroacetic acid/1% phosphotungstic acid. After 30 min at 4°C the precipitates were filtered on 2.5-cm Whatman GF/C glass fibre filters and washed three times with 3.0 ml 6% trichloroacetic acid/0.5% phosphotungstic acid, three times with 3.0 ml chloroform/methanol (2 : 1, v/v), twice with 3.0 ml chloroform/methanol/water (10 : 10 : 1, v/v) and finally with 3.0 ml ether. The filters were dried in air, and the precipitates dissolved in 0.5 ml of Soluene 350 for 12 h at 50°C. 10 ml 0.5% PPO in toluene was added and the radioactivity counted in a Tri-Carb scintillation counter, Model 3385 Packard Instrument. Unstained gels from the SDS-polyacrylamide gel electrophoresis were cut in 2-mm slices and solubilized in 0.25 ml Soluene 350 for 15 h at 50°C. After the addition of 5 ml 0.5% PPO in toluene, the samples were counted as described above. Quench corrections were carried out by the channel ratio method.

SDS-polyacrylamide gel electrophoresis. Proteins were precipitated and washed as described above, and the precipitates dissolved in 0.01 M sodium phosphate buffer (pH 7.2)/2.0 M urea/1.0% SDS/0.01% EDTA/1% 2-mercaptoethanol by incubation for 15 min at 100°C. Electrophoresis was carried out according to Weber and Osborn [10]. 50 µl was layered on the gels (0.9 cm ×

0.5 cm) which contained 5% acrylamide and 0.133% methylenebisacrylamide. Electrophoresis was run for 4 h at 8 mA per tube with bromophenol blue as marker. The gels were washed with 12% trichloroacetic acid for 30 min, and 20% sulfosalicylic acid for 2 h, changing the solution 4—5 times. After washing with distilled water, the gels were stained with 0.25% Coomassie Brilliant Blue in 10% acetic acid/10% methanol for 2 h, and destained overnight in a BioRad diffusion destainer in 7.5% acetic acid/30% methanol.

Immunological methods. Rat serum albumin was estimated by radial immunodiffusion as described by Mancini et al. [11]. 1% agarose gels in 0.05 M veronal · HCl buffer (pH 8.6) containing 1% Triton X-100 with a final concentration of 10 μg/ml of antibody was used. After diffusion for 24 h at 37°C, the precipitates were stained with Amido black. Proteins of submicrosomal fractions labeled with [^{3}H]glucosamine were precipitated by adding rabbit antiserum against rat serum in excess and rat serum as carrier. Prior to the precipitation the samples were dialyzed against 0.05 M veronal · HCl buffer (pH 8.6), and treated with chicken serum and antiserum against chicken serum to remove unspecific precipitates. All precipitations were performed in the veronal buffer. After 1 h at 37°C and 20 h at 4°C, the precipitates were filtered on Whatman GF/C glass-fibre filters, washed and counted as described above.

Other analytical methods. Protein was determined according to Lowry et al. [12] with crystalline bovine serum albumin as standard. RNA was measured by the orcinol method [13] with yeast RNA as standard. Lipids were extracted from 12% trichloroacetic acid/1% phosphotungstic acid precipitate (washed three times with 6% trichloroacetic acid/0.5% phosphotungstic acid) with chloroform/methanol (2 : 1, v/v). Phospholipid was measured by total phosphorus as described by Chen et al. [14]. The NADH-cytochrome *c* reductase activity was assayed as described by Ragnotti et al. [15] with a Spectronic 600 E recording spectrophotometer.

Results

Distribution of secretory glycoproteins in the luminal and membrane fractions of rough and smooth microsomes

To minize the degree of cross contamination between the luminal and membrane fraction of rough and smooth microsomes, centrifugation in a discontinuous sucrose gradient, as described in Materials and Methods, was used. The centrifugation time was determined experimentally as shown in Fig. 1. The amount of protein and phospholipid as well as the NADH-cytochrome *c* reductase activity in the supernatant (including the sucrose layer) decreased to a certain level after centrifugation for about 4 h. As judged from the activity of the membrane-bound enzyme NADH-cytochrome *c* reductase [16], negligible amounts of membrane fragments are present in the supernatant (luminal fraction) at this level. According to calculations no particles with a sedimentation coefficient below 5.5 S and only about 5% of particles with a sedimentation coefficient of 12 S will sediment under these conditions. This means that serum albumin which has a sedimentation coefficient of 4.5 S and molecular weight of about 70 000 will not sediment, and only a small part of globular proteins of a molecular weight of 200 000. As most plasma glycoproteins being

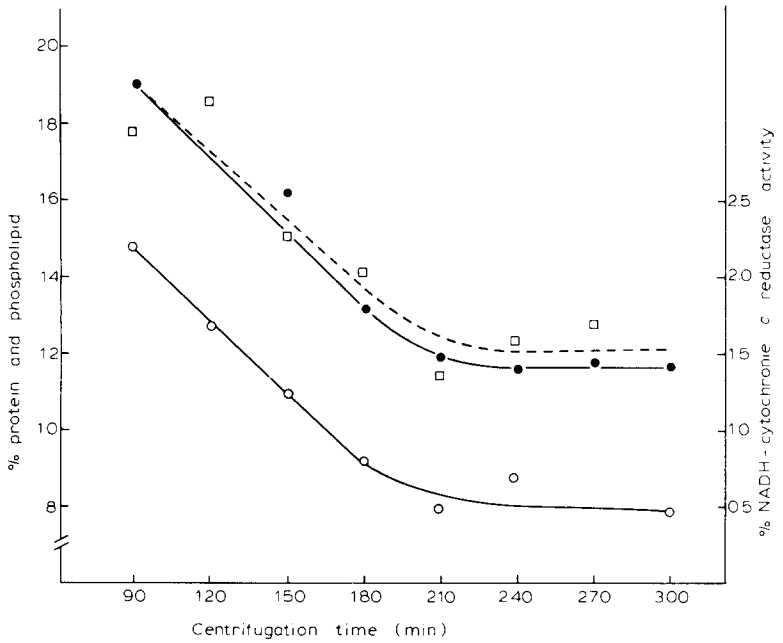

Fig. 1. Protein, phospholipid and NADH-cytochrome *c* reductase activity in the supernatant after varying periods of centrifugation of sonicated rat liver microsomes in a discontinuous sucrose gradient. Total microsomes from 5 g liver were sonicated, diluted to 9.0 ml and layered onto 2.0 ml 0.25 M sucrose in medium A (see Materials and Methods). After varying periods of centrifugation at 149 000 × *g* in 65 Spinco rotor, protein, phospholipid and NADH-cytochrome *c* reductase activity were determined in the supernatant and the pellet. The combined values obtained from supernatant and pellet were chosen as 100%. ●——●, protein; □- - - - -□, phospholipid; ○——○, NADH-cytochrome *c* reductase activity.

synthesized in liver have a molecular weight below 200 000 [17], the degree of separation obtained by this method seems acceptable for studies of plasma glycoproteins in liver microsomes.

The distribution of labeled glycoproteins between the membrane and luminal fractions of rough and smooth microsomes was determined after short-term (45 min) labeling of the rats with [^{14}C]glucosamine. The luminal content of rough and smooth microsomes was released by sonication or French press, or by treatment with the non-ionic detergents Triton X-100 and Brij 35 and the anionic detergent sodium deoxycholate. The concentration of detergents necessary for a selective release of the luminal content without disassembling the membranes depends on the concentration of microsomes and the ionic strength of the medium [6,7], and had therefore to be determined. The amounts of serum albumin recovered in the luminal fractions were taken as a measure of the release of the vesicle content [2,4]. Any effect of the detergents on the membranes was examined by comparing the SDS-acrylamide gel electrophoretic patterns of the fractions and by measurement of solubilized phospholipids. At the concentration of microsomes and ionic strength used (see Materials and Methods), 0.05% (1.3 mM) sodium deoxycholate, 0.035% (0.57 mM) Triton X-100 and 0.04% (0.35 mM) Brij 35 were found to release the vesicle content with undetectable effect on the membranes. The SDS electrophoretic patterns of the luminal content fraction obtained with the

TABLE I

RELEASE OF PROTEIN, [^{14}C]GLUCOSAMINE-LABELED PROTEIN, RNA AND SERUM ALBUMIN FROM ROUGH AND SMOOTH MICROSOMES BY MECHANICAL OR DETERGENT TREATMENT

Rats were injected with [^{14}C]glucosamine (5 μCi per rat) and killed after 45 min. Luminal and membrane fraction (from 10 g liver) were prepared as described in Materials and Methods. The values given are expressed as released material in percent of the total amounts in smooth and rough microsomes.

Substance tested	Rough microsomes					Smooth microsomes				
	Mechanical treatment		Detergent treatment			Mechanical treatment		Detergent treatment		
	Sonication	French press	0.050% deoxycholate	0.035% Triton X-100	0.040% Brij 35	Sonication	French press	0.050% deoxycholate	0.035% Triton X-100	0.040% Brij 35
Protein	15.9	16.4	13.7	12.2	11.2	17.0	20.3	15.6	17.3	17.2
^{14}C-labeled protein	45.6	50.6	53.0	35.4	49.9	45.9	45.5	41.9	38.6	35.9
RNA	—	1.3	1.1	1.3	1.8	—	—	—	—	—
Serum albumin	84.2	86.2	92.1	87.8	77.6	86.4	82.4	90.8	88.8	89.1

—, not detectable.

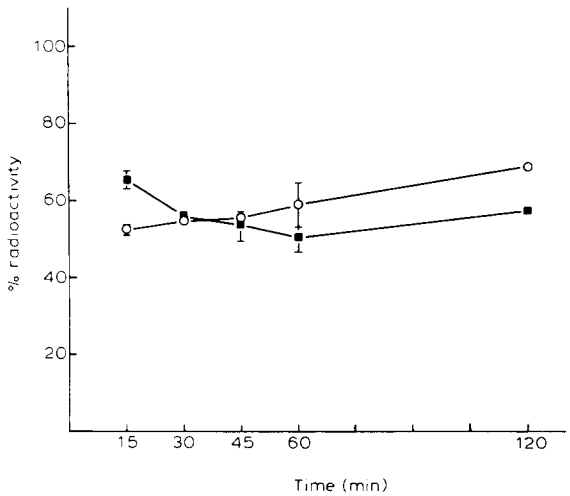

Fig. 2. Time course for the relative amount of ^{14}C-labeled protein in the membrane fraction from rough and smooth microsomes of rat liver after incorporation of [^{14}C]glucosamine in vivo. Rats were injected with [^{14}C]glucosamine (5 μCi per rat). Rough and smooth microsomes from 10 g liver were sonicated, luminal and membrane fraction prepared, and protein-bound radioactivity determined as described in Materials and Methods. The values for 15, 45 and 60 min after injection are based on 2—4 experiments, the vertical bars giving the range, whereas the other time points represent single experiments. The amount of protein-bound radioactivity in the luminal and membrane fraction was chosen as 100%. ■———■, rough membrane fraction; ○———○, smooth membrane fraction.

different methods were similar.

To solubilize the membranes of rough and smooth microsomes, Triton X-100 was chosen as the non-ionic detergents were found to be more efficient than sodium deoxycholate. Moreover, Triton X-100 is preferable since it was found not to interfer in the immunological techniques employed, which is in agreement with earlier observations [18]. After treatment with detergent, the ribosomes were removed by centrifugation, and as a measure of the efficiency of membrane solubilization, the RNA/protein ratio in the pellet was determined. For purified ribosomes from eukaryotes this is reported to be 1.1 [19].

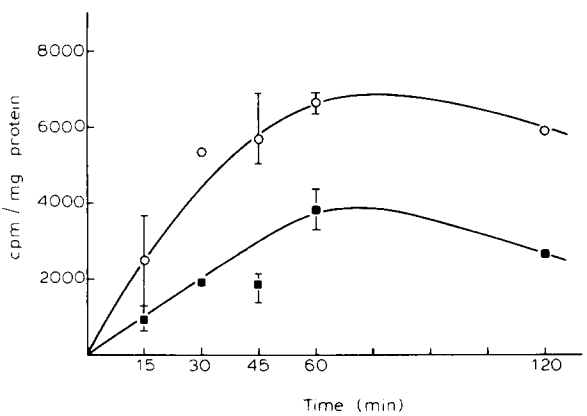

Fig. 3. Time course of protein-bound radioactivity in rough and smooth microsomes of rat liver after incorporation of [^{14}C]glucosamine in vivo. Experimental details as under Fig. 2.

After one extraction of the microsomes with 2.5% Triton X-100, a RNA/protein ratio of 1.04 was obtained.

Table I shows the release of labeled glycoproteins obtained with the various treatments of microsomes. As seen, 40—50% of the protein-bound radioactivity was released from both rough and smooth microsomes, and about 16% of the total protein. Concomitantly, about 90% of the serum albumin was recovered in the luminal fraction which indicates a nearly complete release of the luminal content. The different methods gave similar results. A time course study of the distribution of labeled glycoproteins (Fig. 2) between the membrane and luminal fraction of rough and smooth microsomes revealed no major changes during 15—120 min after the administration of the isotope. Fig. 3 shows that the time course pattern of protein-bound radioactivity after intraperitoneal injection of [^{14}C]glucosamine is similar in rough and smooth microsomes, although with a higher specific radioactivity in smooth microsomes. In pulse-chase experiments, carried out with 10-min pulses, the same distribution of protein-bound radioactivity as shown in Table I was found after 10 and 45 min (experiments not shown). In these experiments the vesicle content was released by sonication. Taken together, the results indicate that 50—60% of the nascent glycoproteins are associated with the membranes of both rough and smooth microsomes at any stage of synthesis and transport which has been investigated.

By repeated extractions of rough and smooth microsomes with 0.05% sodium deoxycholate for 30 min (Table II), negligible amounts of protein-bound radioactivity was released by the second and third treatment. About 13—16% was released, however, if the second treatment with deoxycholate lasted for 16 h. This indicates that the labeled glycoproteins are confined to two compartments, one which is located in the lumen and one which is associated with the membranes and only slowly released upon treatment with 0.05% sodium deoxycholate.

Treatment of rough and smooth microsomes with ultrasonics in the presence of 2% mercaptoethanol had no effect on the distribution of labeled glycoproteins, excluding binding by disulfide bridges to proteins integrated in the

TABLE II

RELEASE OF PROTEIN AND PROTEIN-BOUND RADIOACTIVITY FROM ROUGH AND SMOOTH MICROSOMES BY REPEATED TREATMENTS WITH 0.05% SODIUM DEOXYCHOLATE

Rats were injected with [^{14}C]glucosamine (5 μCi per rat) and killed after 45 min. Rough and smooth microsomes from 10 g liver were treated with 0.05% sodium deoxycholate for 30 min, and the luminal content separated from the membrane fraction as described in Materials and Methods. The membrane fraction was resuspended in 0.05% sodium deoxycholate and stirred for 30 min or 16 h at 4°C. The treatment was repeated. The values are expressed as released material in percent of total amounts in rough and smooth microsomes. —, not detectable.

Treatment No.	Treated for	Protein		^{14}C-labeled protein	
		Rough	Smooth	Rough	Smooth
1	30 min	16.9	20.2	54.3	44.5
2	30 min	—	—	3.1	4.2
	16 h	5.2	6.8	13.1	15.7
3	30 min	—	—	2.5	3.8
	16 h	—	1.6	2.1	7.5

membranes. Upon sonication of rough and smooth microsomes at high ionic strength (1.5 M KCl), increased amounts of protein and phospholipid were found in the luminal fraction, whereas the distribution of labeled glycoproteins was not affected (experiments not shown). Microsomal membranes are permeable for charged substances of molecular weight up to 90 [20]. This indicates that electrostatic interactions with membrane proteins or hydrophilic components of membrane lipids not are involved in the binding of nascent glycoproteins to the membranes.

Comparison of secretory glycoproteins in the luminal and membrane fraction of rough and smooth microsomes from rat liver

When labeled glycoproteins from the luminal and membrane fraction of rough and smooth microsomes were compared by SDS-polyacrylamide gel electrophoresis, the patterns appeared to be very similar (Fig. 4). [^{3}H]Glucosamine was used to obtain a high specific radioactivity. The similarity makes it

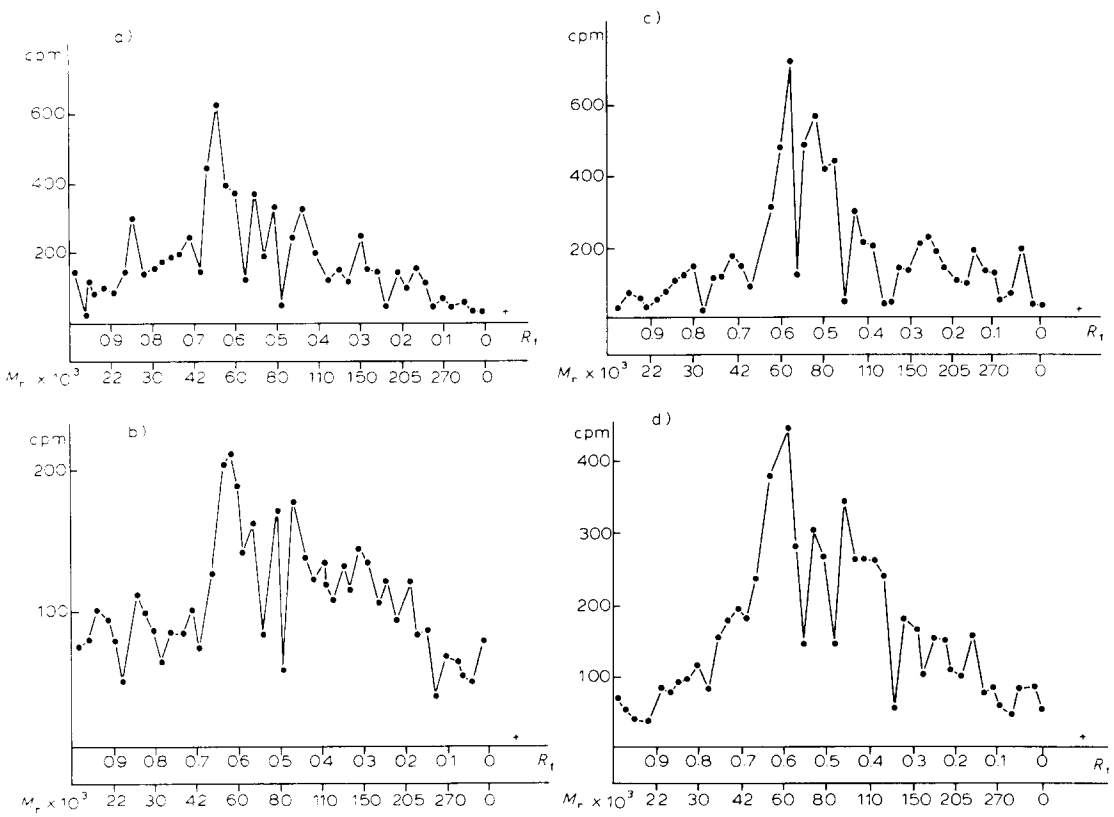

Fig. 4. SDS-polyacrylamide gel electrophoretic patterns of protein-bound radioactivity from labeled subfractions of rough and smooth microsomes. Rats were injected with [^{3}H]glucosamine (100 μCi per rat) and killed after 45 min. The luminal fraction was prepared by sonication, and membranes were separated from ribosomes as described in Materials and Methods. a, luminal fraction of rough microsomes; b, membrane fraction of rough microsomes; c, luminal fraction of smooth microsomes; d, membrane fraction of smooth microsomes.

TABLE III

IMMUNOPRECIPITATION OF PROTEIN-BOUND RADIOACTIVITY IN LUMINAL AND MEMBRANE FRACTION OF ROUGH AND SMOOTH MICROSOMES FROM RAT LIVER WITH RABBIT ANTISERUM AGAINST RAT SERUM

Rats were injected with [^{3}H]glucosamine (5 μCi per rat) and killed after 45 min. 15 μl chicken serum and and excess of antiserum against chicken serum were added to 0.5 ml of the luminal (obtained by sonication) or membrane fraction (see Materials and Methods). After removal of the precipitate, 10 μl rat serum and an excess of antiserum against rat serum were added. Incubation and determination of radioactivity are described in Materials and Methods. The amount of ^{14}C-labeled protein in the sample prior to precipitation with antisera was chosen as 100%.

Fraction	Unspecific precipitation (%)	Specific precipitation (%)
Rough luminal fraction	9.9	35.2
Smooth luminal fraction	9.3	35.4
Rough membrane fraction	10.5	18.2
Smooth membrane fraction	6.3	18.6

unlikely that the protein-bound radioactivity associated with the membranes represents a certain fraction of secretory glycoproteins.

The labeled glycoproteins of the luminal and membrane fractions of rough and smooth microsomes were characterized by immunological precipitation with rabbit antiserum against rat serum. Table III shows that 35% of the protein-bound radioactivity in the luminal fractions and 18% in the membrane fractions were precipitated with rabbit antiserum against rat serum, which establish the presence of nascent serum glycoproteins in both the lumen and the membranes. The results obtained with rough and smooth microsomes were similar.

As shown in Table IV, an exchange of labeled glycoproteins between the luminal and the membrane fraction could be demonstrated. To make the microsomal vesicles permeable for proteins, the medium contained 0.05% sodium deoxycholate [6]. The transfer of labeled glycoproteins was about the same with labeled luminal fraction and unlabeled membranes or vice versa. The distribution of protein between the luminal and membrane fraction was similar in the different samples, excluding a net transfer of protein from one fraction to another. The exchange was time dependent, and more pronounced in smooth than in rough microsomes.

Discussion

The large recovery of serum albumin (90%) in the luminal fraction isolated by various methods revealed an efficient release of the luminal content of the microsomal vesicles. About 16% of the total protein of microsomes was released which is in agreement with earlier observations [6,21]. Based on NADH-cytochrome *c* reductase activity and SDS gel electrophoretic pattern of the different fractions, the treatment of microsomes with detergents, ultrasonic or French press apparently did not produce any disassembly of the membranes. Furthermore, with the method used to separate the luminal and

TABLE IV

EXCHANGE OF PROTEIN-BOUND RADIOACTIVITY BETWEEN LUMINAL CONTENT AND MEMBRANES OF ROUGH AND SMOOTH MICROSOMES FROM RAT LIVER BY MIXING LABELED AND UNLABELED FRACTIONS

Rats were injected with [^{14}C]glucosamine (5 μCi per rat) and killed after 45 min. Rough or smooth microsomes (10 g liver) from labeled and unlabeled rats were treated by sonication followed by a separation of the luminal and membrane fraction as described in Materials and Methods. Luminal fractions were adjusted to 0.05% with respect to deoxycholate. Expt. A: Labeled membranes were suspended in 9.0 ml of unlabeled luminal fraction. Expt. B: Unlabeled membranes were suspended in 9.0 ml of labeled luminal fraction. After incubation for 30 min or 16 h at 4°C with stirring, luminal and membrane fraction were separated, and protein and radioactivity determined as described in Materials and Methods. The amount of protein or radioactivity in the luminal and the membrane fraction was chosen as 100%.

		Time of exchange	Protein in supernatant (%)	Radioactivity in supernatant (%)	Radioactivity in pellet (%)
Expt. A	^{14}C-labeled membranes + unlabeled luminal fraction				
	Rough microsomes	30 min	15.1	8.3	
		15 h	17.3	14.1	
	Smooth microsomes	30 min	14.2	18.2	
		15 h	16.8	25.6	
Expt. B	^{14}C-labeled luminal fraction + unlabeled membranes				
	Rough microsomes	30 min	19.5		7.4
		15 h	15.2		13.3
	Smooth microsomes	30 min	17.1		15.6
		15 h	23.0		22.6

membrane fractions, cross contamination was minimized. It is therefore assumed that the methods used to prepare the luminal and membrane fraction of rough and smooth microsomes are adequate.

The finding that only a part of the protein-bound radioactivity was released from the microsomal vesicles by various treatments is in agreement with the results of Kreibich and Sabatini [7] who showed that newly synthesized glycoproteins were found in both the luminal and membrane fraction after selective release of the luminal content by low concentration of sodium deoxycholate. The fact that we found about 50% of the protein-bound radioactivity in the luminal fraction, independent of the method used for release, whereas the recovery of serum albumin was about 90%, suggests that the distribution of glycoproteins between the luminal and membrane fraction is different from that of non-glycosylated proteins such as serum albumin. Any contamination of the membrane fraction by aggregates of luminal protein sedimenting under the centrifugation conditions used is unlikely as the relative amount of protein-bound radioactivity in the membrane fraction was the same whether the centrifugation was carried out for 90 or 240 min. Furthermore, an adsorption of luminal glycoproteins to the microsomal membrane is not probable since sonication in a medium of high ionic strength and repeated short time treatment with 0.05% deoxycholate did not alter the distribution of protein-bound radioactivity. The problem of vesicle membranes being contaminated by protein from the vesicle content has recently been emphasized by Castle et al. [22]. Taken together, our observations indicate that a large part of the nascent

glycoproteins are associated with the membranes in vivo. By prolonged treatments with 0.05% deoxycholate, however, the release of ^{14}C-labeled protein from rough and smooth microsomes amounted to about 70% of the total content. It thus seems probable that the glycoproteins in the microsomes are found in the two fractions, one which is easily extracted from the microsomes with 0.05% deoxycholate and represents the luminal content, and one which is released slowly to the water-soluble phase, and is associated with the membranes in vivo.

The distribution of ^{14}C-labeled protein between the luminal and membrane fraction did not change essentially during a time interval of 15—120 min after administration of the isotope. As the same results were obtained with rough and smooth microsomes this shows that the nascent glycoproteins have affinity for the membranes in both types of the endoplasmic reticulum. This is not in agreement with the suggestion that the transport of secretory glycoproteins might occur in the membranes of the rough and in the lumen of the smooth endoplasmic reticulum [4,2], based on the in vitro experiments of Redman and Cherian [4] who recovered 90% of the mannose-labeled protein from the membrane fraction of rough microsomes, whereas 89% of the galactose-labeled proteins were found in the luminal fraction of smooth microsomes. Difference in experimental conditions might explain this discrepancy. In their experiments, the luminal fraction was prepared by treating microsomes with a high concentration of deoxycholate (0.25%), and the method used for the separation of the luminal and membrane fractions was not adequate. These methods have also been criticized by Sauer and Burrow [23].

By precipitation with antiserum against rat serum, part of the ^{14}C-labeled glycoproteins in the luminal and membrane fraction could be identified as serum glycoproteins. The uncomplete precipitation of ^{14}C-labeled glycoproteins could to some extent be due to the fact that antiserum against serum proteins was used whereas microsomes contain the whole range of plasma proteins except the immunoglobulins. The probability also exists that all antigen determinants are not formed at this stage and/or that antigen determinants are masked. The latter could particularly apply to the membrane-associated ^{14}C-labeled protein [4]. Furthermore, the presence of intracellular glycoproteins with high turnover cannot be excluded. An incomplete immunoprecipitation, being specially pronounced with the membrane fraction, has been reported previously [4,7].

The similarity of the SDS-polyacrylamide gel electrophoretic pattern of the luminal and membrane fraction from rough and smooth microsomes indicates that the same set of secretory glycoproteins are found in the two fractions. Similar observations have recently been reported by Kreibich and Sabatini [7]. The possibility of two separate transport mechanisms for different types of secretory glycoproteins can therefore be excluded. It appears from the electrophoretic patterns that a large part of the ^{14}C-labeled protein associated with membranes represents secretory glycoproteins. To what extent structural glycoproteins of the microsomal membranes are labeled during short time of incorporation is unknown. Although it has been suggested that the turnover of structural membrane glycoproteins is lower than that of secretory glycoproteins, present knowledge is meager [24—27]. The finding, however, that

there was no major change in the relative amount of ^{14}C-labeled protein in the membrane fraction during 15—120 min, support the assumption that the ^{14}C-labeled proteins associated with the membranes do not represent structural glycoproteins to any large extent.

The slow exchange of nascent glycoproteins between the lumen and membrane in the presence of 0.05% deoxycholate at 4°C, without any net transfer of protein from one fraction to another, might indicate that nascent glycoproteins in smooth and rough microsomes equilibrate between a water-soluble and a membrane-bound form. It is possible that the exchange would have been more rapid if physiological conditions, i.e. temperature and any cofactors involved in the binding, had been fulfilled. Jamieson [28] has suggested that specific receptor sites on the luminal side of the reticulum membrane of pancreatic exocrine cells might be involved in the transport of exportable proteins. Possible candidates could be membrane-bound glycosyltransferases [28]. In the present work, however, it was found that binding of nascent glycoproteins to the membranes by disulfide bridges or electrostatic interactions is not very likely. Hydrophobic bonding is a possibility, but so far we have not been able to provide any evidence for this.

It is well established that serum albumin is transported through the channels of the endoplasmic reticulum [2]. For glycoproteins, however, which are modified by successive attachments of carbohydrates to the protein as this migrates through the rough and smooth endoplasmic reticulum, a frequent contact between the membrane-bound glycosyltransferases and the substrate is expected.If there are specific receptor sites for the nascent secretory glycoproteins at the luminal side of the reticulum membrane, the glycoproteins might equilibrate between the membrane and the water phase in the lumen, and thus the channels of the endoplasmic reticulum might also serve as the pathway for the transport of glycoproteins. The results obtained in the present investigation, however, does not exclude the possibility of transport in both the lumen and membranes of rough and smooth endoplasmic reticulum.

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