BINDING OF GLYCOPROTEINS OF MICROSOMAL AND GOLGI MEMBRANES TO LECTINS

Lars Winqvist, Lennart Eriksson, Gustav Dallner and Bo Ersson

Department of Biochemistry, Arrhenius Laboratory, University of Stockholm, Department of Pathology at Sabbatsberg Hospital, Karolinska Institutet, Stockholm, and Department of Biochemistry, University of Uppsala, Uppsala, Sweden

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SUMMARY

Glycoproteins solubilized from microsomal and Golgi membranes with detergent bind to Concanavalin A and wheat germ agglutinin which have been coupled to Sepharose. The binding to wheat germ agglutinin is shown to depend on the presence of terminal sialic acid residues, the majority of which are localized at the cytoplasmic surface of rough endoplasmic reticulum and Golgi membranes and at the luminal surface of the smooth membranes. Not all the membraneous sialic acid residues are available for such binding.

The membranes of the endoplasmic reticulum (ER) and Golgi apparatus contain glycoproteins. The oligosaccharide chains of these proteins contain mannose, galactose, N-acetylglucosamine (GlcNAc) and (only in terminal position) N-acetyl neuraminic acid (NANA)(1).Completion of the oligosaccharide moiety of both secretory and membrane glycoproteins takes place during transport of the macromolecule from the rough ER via the smooth ER to the Golgi apparatus (2,3). Sialyl transferase is found only in the Golgi membranes; thus sialoproteins destined for incorporation into the ER must pass through the Golgi system during their synthesis. There are now a number of lectins available, such as Concanavalin A (Con A), which specifically binds oligosaccharides with a terminal α-mannose residue, wheat germ agglutinin (WGA), specific for GlcNAc, and Crotalaria lectin (Crot), specific for terminal galactose in β-linkage (4,5). A previous investigation revealed the presence of con A-binding proteins both in various microsomal fractions and in Golgi membranes (6). In the present study the binding of the three above mentioned lectins to the glycoproteins of these fractions and the localization of these glycoproteins on the membranes were studied.

MATERIALS AND METHODS

Adult, male Sprague-Dawley rats weighing 180-200 g and starved 20 hours before sacrifice were used. Glucosamine-^{3}H (Radiochemical Centre, Amersham, England, 10 Ci/mmol) was injected into the portal vein of rats under Nembutal anaesthesia (12 mg/rat), and the livers were removed 30 min. later. The total microsomal fraction, microsomal subfractions, and Golgi membranes were prepared as described earlier (7,8). In order to remove adsorbed and secretory proteins all fractions were subjected to the Tris-water-Tris washing procedure (7). The final pellet was suspended in 0.15 M Tris-HCl buffer, pH 8 (for the lectin binding experiments), in 0.15 M Tris buffer, pH 7 (for neuraminidase treatment) or in 0.25 M sucrose (for determination of NANA), to a protein concentration of 10 mg/ml. In the lectin binding experiments the incubation mixture consisted of 0.15 ml lectin-Sepharose; 0.65 ml of 0.15 M Tris-HCl, pH 8 or of 0.2 M inhibitor in 0.15 M Tris-HCl, pH 8; 0.1 ml 10 % sodium cholate and 0.1 ml microsomes (10 mg protein/ml). Incubation was performed for 30 min. at room temperature. The Sepharose beads were then sedimented by low-speed centrifugation, and the radioactivity in the pellet was determined in a Beckman liquid scintillation counter, using Bray's solution (9). The values in tables I-V are the means of 5-7 experiments.

Con A-Sepharose was purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Crot was prepared according to Ersson et al. (5). WGA was isolated from wheat germ lipase (Sigma Chemical Co, St. Louis) by adsorption to glucosamine coupled to CH-Sepharose (Pharmacia) followed by elution with 0.2 M GlcNAc. Crot and WGA were then bound to CNBr-activated Sepharose (Pharmacia). The inhibitors of lectin binding used were α-methyl mannoside (for Con A), GlcNAc and NANA (for WGA) and lactose (for Crot), all of which were purchased from Sigma.

Phospholipid (PL) was measured as reported earlier (10) and sialic acid was determined with the Warren procedure (11).

Neuraminidase (NANase) (Sigma, Type VI) treatment of membrane fractions was carried out by incubation of membrane protein (10 mg/ml) and neuraminidase (0.1 mg/ml) in 0.15 M Tris-HCl, pH 7 at 37°C for 30 min.

RESULTS AND DISCUSSION

The binding of solubilized membrane glycoproteins to lectin-Sepharose is demonstrated in Table I. To estimate the amount of non-specific binding, incubation was also carried out in the presence of inhibitor. As can be seen, binding of labelled glycoproteins to WGA-Sepharose is inhibited to the same extent by GlcNAc and NANA, indicating that WGA can bind both to GlcNAc and to NANA residues.

Glycoproteins from rough and smooth microsomes and from Golgi membranes interact with Con A and WGA but not with Crot (Table II). Thus, all three of these fractions contain oligosaccharides with terminal mannose residues and oligosaccharides with terminal GlcNAc or NANA residues or both. On the other hand, there is no galactose available for binding. After NANase treatment of

TABLE I

RADIOACTIVE GLYCOPROTEIN BOUND TO SEPHAROSE AND WGA-SEPHAROSE AFTER INCUBATION WITH LABELED LIVER MICROSOMES IN THE PRESENCE OF 1 % CHOLATE

	Cpm in pellet
Sepharose 4B	720
Sepharose 4B + GlcNAc	770
Sepharose 4B + NANA	650
WGA-Sepharose	3,471
WGA-Sepharose + GlcNAc	1,030
WGA-Sepharose + NANA	990

Liver microsomes (25,000 cpm total) from rats treated with glucosamine-^{3}H were incubated with Sepharose or lectin-Sepharose in the presence of 1 % cholate. The concentration of inhibitor (GlcNAc or NANA) was 0.13 M. Radioactivity bound to the sedimented Sepharose or lectin-Sepharose was measured.

TABLE II

BINDING OF GLYCOPROTEINS FROM MICROSOMES AND GOLGI MEMBRANES TO LECTINS

Fraction	Radioactivity bound to Con A	WGA	Crot
	% of total		
Non-treated			
Rough microsomes	35	11	1
Smooth microsomes	25	13	1
Golgi vesicles	17	16	1
NANase-treated			
Rough microsomes	32	1	5
Smooth microsomes	25	10	3
Golgi vesicles	19	4	8

Untreated or NANase-treated microsomes or Golgi membranes were incubated with lectin-Sepharose with and without inhibitor in 1 % cholate. Radioactivity bound to the sedimented lectin-Sepharose was measured; and the percentage of the total radioactivity specifically bound was calculated as the difference between the amounts of radioactivity bound in the non-inhibited and inhibited cases, divided by the total amount of radioactivity.

intact vesicles, no change in Con A-binding can be detected, while galactose residues become available for Crot binding in all three fractions. Thus, in these membrane glycoproteins, galactose sits next to the terminal NANA residue, as is known to be the case with secretory glycoproteins. WGA-binding decreases from 11 % to 1 % in rough microsomes and from 16 % to 4 % in Golgi membranes after NANase treatment of intact vesicles, indicating that WGA can bind to terminal NANA residues and that all of the NANA residues available to the lectin in rough microsomes are on the cytoplasmic side of the membrane. NANase treatment of smooth microsomes only decreases WGA-binding from 13 % to 10 %, and there is a possibility that the remaining WGA-binding represents binding to GlcNAc.

In order to also hydrolyze NANA on the inside surface of the membrane, microsomal vesicles were treated with NANase in the presence of 0.05 % DOC and 0.05 M KCl. Low DOC concentrations are known to make the vesicles permeable to macromolecules (12). Smooth microsomes treated in this manner lose all their WGA-binding sites (Table III). Thus, in the case of smooth membranes as well, WGA binds only to NANA. The majority of these binding sites in smooth vesicles are on the luminal side, which is different from the situation in rough and Golgi vesicles.

In order to further analyze its distribution, NANA was measured chemically in rough and smooth microsomes (Table IV). NANase treatment of intact rough vesicles removes more than half of the NANA, but only a small amount can be removed in this manner from smooth membranes. When NANase was introduced into the vesicle, an additional 10-20 % of the original NANA could be released from both rough and smooth microsomes. It appears that only a limited portion of the NANA present is susceptible to digestion by NANase, particularly in smooth membranes. Pronase pretreatment of intact microsomes shows that, for smooth microsomes at least, a part of the protein-bound NANA on the outer surface is shielded by neighboring protein molecules from NANase action (Table V).

Even after NANase treatment of membranes dissolved in 0.6 % cholate some

30 % of the NANA still remained in protein-bound form. However, after pretreatment with phospholipase A, NANase removed all the NANA from the protein (not shown). Thus, only after extensive solubilization of the membrane is all of the NANA available for enzymatic release.

TABLE III

BINDING OF GLYCOPROTEINS FROM DOC-TREATED MICROSOMAL MEMBRANES TO LECTINS

	Radioactivity bound to		
	Con A	WGA	Crot
	% of total		
Microsomes pretreated with 0.05 % DOC			
Rough	25	8	1
Smooth	25	14	1
Microsomes pretreated with 0.05 % DOC + neuraminidase			
Rough	20	2	5
Smooth	25	1	6

Microsomes were made permeable to macromolecules with deoxycholate (12), treated with NANase (see Materials and Methods), and subsequently incubated with lectin-Sepharose as described in the legend to Table II.

TABLE IV

NANA CONTENT OF ROUGH AND SMOOTH MICROSOMES BEFORE AND AFTER NEURAMINIDASE TREATMENT

Treatment	NANA content in	
	Rough microsomes	Smooth microsomes
	μg/mg PL	
None	6.0	14.2
Neuraminidase	2.8	12.9
0.05 % DOC	5.8	13.5
0.05 % DOC + neuraminidase	2.1	10.1

NANase treatment (see Materials and Methods) was carried out in the presence or absence of 0.05 % DOC and 0.05 M KCl (12).

TABLE V

THE EFFECT OF COMBINED PRONASE AND NEURAMINIDASE TREATMENT OF MICROSOMES ON THEIR CONTENT OF NANA

Treatment	NANA content in Rough microsomes	NANA content in Smooth microsomes
	µg/mg PL	
None	5.9	13.9
Neuraminidase	3.0	12.7
Pronase	4.5	12.5
Pronase + neuraminidase	1.9	8.7

For pronase treatment microsomal vesicles were incubated with non-specific protease from Streptomyces griseus (Sigma) (20 µg/mg microsomal protein) at 37°C for 20 min.

Assuming that most of the membrane glycoproteins are labeled by our procedure, lectin binding gives a rough estimate of the relative frequency of different terminal sugars. Lectin-Sepharose was used in excess, but considerably less than 100 % of the radioactivity was bound. The explanation for this fact may be that some oligosaccharide chains are too short to reach the WGA molecule which is coupled directly to Sepharose.

These experiments clearly indicate that terminal NANA residues are responsible for the binding of microsomal and Golgi membrane glycoproteins to WGA. The molecular nature of this binding has not been further investigated and it is possible that electrostatic interactions are involved. The finding that NANA is localized on the cytoplasmic surface of the rough ER membrane was unexpected, since the biosynthesis of the oligosaccharide chain of glycoproteins is generally believed to be associated with the inner side of the membrane (3). Antibody precipitation experiments have shown that there are no NANA-containing serum glycoproteins in well-washed microsomes (13). It is thermodynamically improbable that a protein-bound oligosaccharide chain will pass freely through

the membrane, and it is possible that some glycoprotein synthesis on the rough ER is associated with the cytoplasmic side of the membrane.

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