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# STUDIES ON THE LATENCY OF UDP-GALACTOSE-ASIALO-MUCIN GALACTOSYLTRANSFERASE ACTIVITY IN MICROSOMAL AND GOLGI SUBFRACTIONS FROM RAT LIVER

GÖRAN N. ANDERSSON and LENNART C. ERIKSSON

*Department of Oral Pathology and Department of Pathology, Karolinska Institutet, Huddinge Hospital, S-141 86 Huddinge (Sweden)*

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## Summary

The activity of UDPgalactose-asialo-mucin galactosyltransferase (EC 2.4.1.74) in microsomal and Golgi subfractions was stimulated 2.4-fold after disruption of the membrane permeability barrier by hypotonic incubation. In the presence of Triton X-100, galactose transfer to asialo-mucin was increased 12-fold in rough microsomes and 5-fold in smooth microsomes both with and without hypotonic incubation; while in the Golgi subfractions no stimulation by detergent was observed. These experiments indicate differences in enzyme-lipid or enzyme-protein interactions in microsomes and Golgi membranes. Furthermore, these results strongly support the conclusion that the UDP-galactose-asialo-mucin galactosyltransferase activity in microsomal fractions is not due to contamination by Golgi vesicles but represents an enzyme activity endogenous to the endoplasmic reticulum.

Latency of membrane-associated enzyme activities is often due to the impermeability of the membrane bilayer to the substrate [1,2,8], and is used as evidence concerning the transverse orientation of membrane-bound enzymes. However, recent studies [3] have indicated the importance of clearly assessing whether stimulation of enzyme activities by detergents is caused by an altered substrate permeability, as is believed to be the case for mannose-6-phosphatase in microsomal membranes [2], or if other mechanisms of enzyme stimulation are involved. With purified microsomal galactosyltransferase [4] a rapid loss of activity on storage at −20°C was noticed if the sample was not supplemented with Triton X-100, suggesting a detergent effect not related to

membrane permeability properties. Mookerjea and Yung [5] and Bretz and Stäubli [1] found a stimulatory effect of detergents on galactosyltransferase activity in different subcellular fractions from rat liver. In the light of these considerations and of our findings on the inhibitory action of Triton X-100 on UDPgalactose-asialo-mucin galactosyltransferase (EC 2.4.1.74) activity in an isolated Golgi fraction [6], the present study was performed in order to evaluate the parameters responsible for the regulation of galactosyltransferase activity in microsomal and Golgi subfractions isolated from rat liver.

The total particulate fraction, rough and smooth microsomes, and Golgi I and II membranes were prepared from male Sprague-Dawley rats weighing 180—200 g starved for 20 h according to a sequential method developed recently in our laboratory [7]. Water-Tris treatment was performed by suspending the pelleted fractions in cold, distilled water to a final concentration of 1 mg protein per ml. The membrane suspension was then incubated at 37°C for 15 min and subsequently chilled on ice. After sedimentation at 105 000 × *g* for 90 min the fractions were resuspended in 0.15 M Tris-HCl buffer, pH 8.0, pelleted as described above and suspended in 0.25 M sucrose to a final concentration of 1 g liver tissue/ml. UDPgalactose-asialo-mucin galactosyl transferase activity in microsomal and Golgi subfractions was measured under the optimal conditions described for Golgi membranes, using desialylated mucin from bovine submaxillary gland as a galactose acceptor [6]. However, the reaction was terminated by adding 5% phosphotungstic acid in 2 M HCl instead of 8% trichloroacetic acid, a modification which resulted in complete precipitation of protein-bound radioactivity (Andersson, G.N. and Eriksson, L.C., unpublished data). β-Galactosidase and mannose-6-phosphatase activities were measured as described previously [6,8].

Two different approaches were used to reveal the latent portion of enzyme activities in microsomal and Golgi membranes. Hypotonic treatment has been used to remove intraluminal content and protein weakly bound (chiefly adsorbed) to the membrane [9]. In Table I is shown the effect of water-Tris treatment on the activity of mannose-6-phosphatase and β-galactosidase in microsomes and Golgi membranes, respectively. The difference in activity of mannose-6-phosphatase measured in the absence and presence of mild detergents such as sodium deoxycholate and Triton X-100 is widely used as a marker for microsomal membrane integrity [8]. Mannose-6-phosphatase activity is stimulated 12-fold in control rough and smooth microsomes in the presence of 0.75 mg Triton X-100 per mg protein. However, in water-Tris-treated microsomes the activity in the presence of detergent is inhibited to 25% of the value obtained in the absence of Triton X-100. The recovery of mannose-6-phosphatase activity in water-Tris-treated microsomes measured in the absence of detergent amounts to 85% of the activity in control microsomes incubated with detergent. Thus, if the latency of mannose-6-phosphatase is regarded as an indication of a membrane permeability barrier the water-Tris treatment can be used to remove the barrier to mannose-6-phosphate.

For Golgi membranes it is more difficult to find an appropriate biochemical indicator of membrane permeability properties. Farquhar et al. [10] demonstrated, using cytochemical techniques, that products resulting from

TABLE I

EFFECT OF HYPOTONIC INCUBATION AND ALKALINE BUFFER WASHING ON THE ACTIVITY OF MANNOSE-6-PHOSPHATASE IN ROUGH AND SMOOTH MICROSOMES (a) AND OF β-GALACTOSIDASE IN GOLGI I AND II MEMBRANES (b)

The results are the mean of three experiments. Results for mannose-6-phosphatase activity are expressed as μmol inorganic phosphorus released/5 min per g liver. Incubations with detergent were performed in the presence of 0.75 mg Triton X-100 per mg protein. Results for β-galactosidase activity are expressed as nmol *p*-nitrophenylate formed per 30 min per g liver. The supernatant fraction was prepared by pooling the supernatants after hypotonic incubation and Tris-wash performed as described in the text.

| (a) | Mannose-6-phosphatase | |
|---|---|---|
| | Control | Water-Tris |
| Rough microsomes | 0.41 | 4.1 |
| Rough microsomes + detergent | 4.8 | 1.05 |
| Smooth microsomes | 0.15 | 1.50 |
| Smooth microsomes + detergent | 1.85 | 0.42 |

| (b) | β-Galactosidase | | |
|---|---|---|---|
| | Control | Water-Tris | |
| | | Pellet | Supernatant |
| Microsomes | 0.970 | 0.140 | 0.810 |
| Golgi I | 0.015 | 0.002 | 0.013 |
| Golgi II | 0.047 | 0.003 | 0.041 |

the activity of lysosomal hydrolases were located within the lumen of isolated Golgi elements. In Table Ib is shown the loss of β-galactosidase activity after water-Tris treatment, compared to control Tris-washed membranes, which illustrates the altered permeability properties of the Golgi membrane. Only 15, 12 and 7% of the total activity finally remains associated with microsomes, Golgi I and Golgi II membranes, respectively, after this treatment. The remaining fraction of the enzyme activity can be recovered in the supernatants. Furthermore, morphological analyses of the isolated microsomal and Golgi subfractions after dilution, hypotonic incubation and sedimentation show disrupted membrane profiles and complete loss of lipoprotein particles from the lumen of Golgi elements (Andersson, G.N. and Eriksson, L.C., unpublished data).

In previous studies [1,11] a 2- to 3-fold stimulation of galactosyltransferase activities in Golgi-enriched fractions in the presence of non-ionic detergents like Triton X-100 was observed. However, on measuring the galactosyltransferase activity with asialo-mucin as exogenous acceptor protein no latent enzyme activity could be detected in our Golgi fractions [6]. The enzyme was assayed 6 h after killing of the animals so that the absence of latent enzyme activity can hardly be attributed to ageing phenomena.

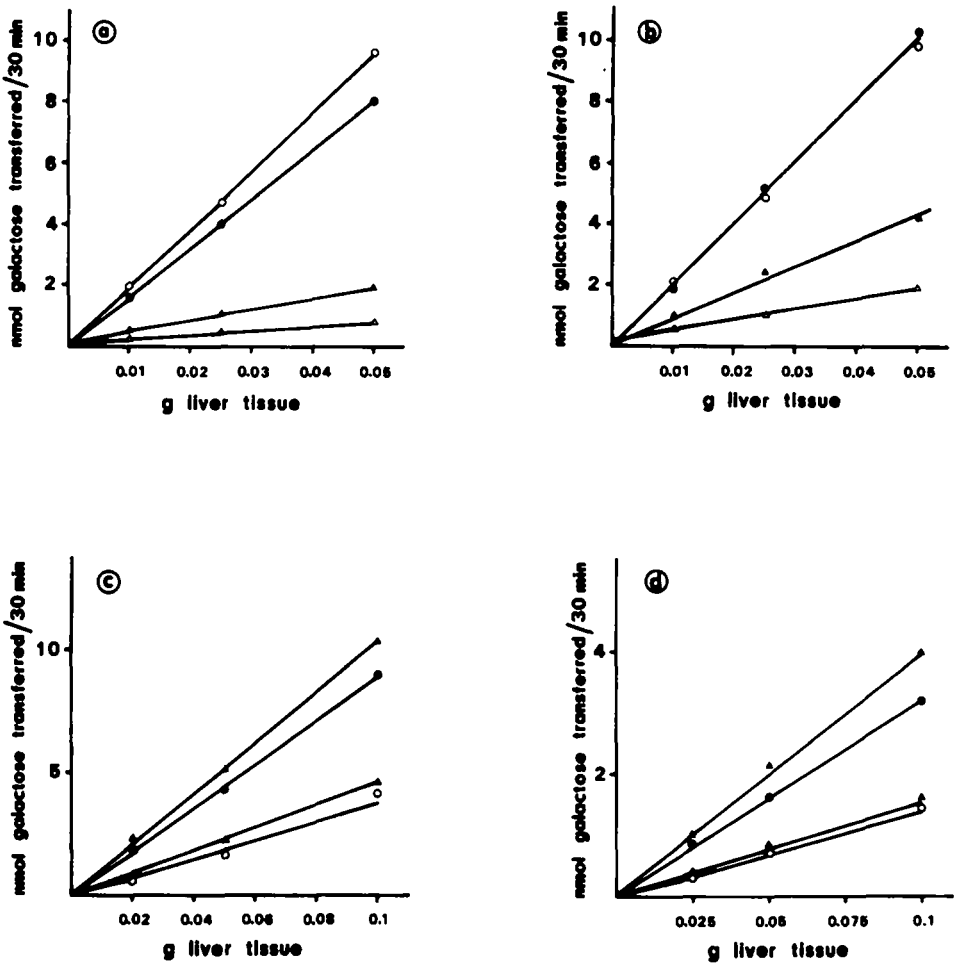


Fig. 1. Galactose transfer to exogenous desialylated mucin in control (open symbols) and water-Tris treated (filled symbols) subcellular fractions in the absence (△—△, ▲—▲) and in the presence (○—○, ●—●) of 1 mg Triton X-100 per mg protein. Aliquots of the fractions were incubated in a medium containing 30 mM Tris-HCl, pH 7.5, 5 mM $MnCl_2$, 30 mM β-mercaptoethanol, 7.5 mg desialylated mucin and 0.92 mM UDPgalactose at a specific radioactivity of 0.75 mCi/mmol, at 37° C for 30 min. The results are the mean of four experiments. a, rough microsomes; b, smooth microsomes; c, Golgi I; d, Golgi II.

Upon assaying UDPgalactose-asialo-mucin galactosyltransferase activity in control and water-Tris-treated membranes (Fig. 1a—d), we found interesting differences between rough and smooth microsomes and between Golgi I and Golgi II membranes. With both control and water-Tris-treated rough and smooth microsomes, maximal activity was obtained after incubation with Triton X-100 (1 mg/mg protein) at 37° C for 30 min. Disruption of the microsomes with water-Tris treatment alone increased galactosyltransferase activity 2.4- and 2.2-fold, respectively. In the presence of detergent, a 12-fold stimulation was seen with rough microsomes, while with smooth microsomes a 5-fold stimulation was obtained. In Golgi fractions (Fig. 1c, d) water-Tris treatment alone results in a 2.5- and 2.3-fold stimulation without any further stimulation after addition of detergent.

We interpret the results of the experiment shown in Table I and Fig. 1 as indicating that incubation in a hypotonic medium followed by one wash in a slightly alkaline buffer disrupts the permeability barrier of rough and smooth microsomes and Golgi membranes. For the microsomal membrane permeability changes are only of minor importance in the activation of the galactosyltransferase, while the effect of the detergent on protein-lipid or protein-protein interactions is more important. In Golgi membranes, however, the permeability of the membrane is a key factor in determining the rate of galactose transfer. These results suggest fundamental differences in the organization of the membrane components and in the relation of the galactosyltransferase to other membrane constituents in endoplasmic reticulum and Golgi. The possibility of two different enzymes catalyzing galactose transfer in microsomes and Golgi membranes cannot at present be excluded.

The optimal absolute and specific activities of UDPgalactose-asialomucin galactosyltransferase in the various fractions are shown in Table II. 37% of the activity measured in the total particulate fraction can be recovered in the unfractionated microsomal fraction. There is an equal distribution in the activity per g liver between rough and smooth microsomes. Also, by comparing the patterns seen in Fig. 1a and b, there seems to be a very close relationship between rough and smooth microsomes with respect to stimulation of galactosyltransferase activity. This enzyme activity is widely used as a biochemical marker for the Golgi apparatus and the Golgi fractions are enriched about 16-fold in galactosyltransferase activity per mg protein compared to the rough microsomal fraction. A substantial portion of the total activity can be recovered in the endoplasmic reticulum and since the properties of the enzyme activity in this organelle differ from those of Golgi membranes with respect to detergent stimulation, we conclude that the microsomal enzyme is present on fragments of the endoplasmic reticulum and is not due to contamination by Golgi vesicles.

TABLE II

DISTRIBUTION OF UDP-GALACTOSE-ASIALO-MUCIN GALACTOSYLTRANSFERASE ACTIVITY IN ISOLATED SUBFRACTIONS OF RAT LIVER

Fractions were isolated as described previously [7]. The assay for the galactosyltransferase activity was performed as described in Fig. 1. All fractions except Golgi I and Golgi II were incubated with 1 mg Triton X-100 per mg protein. The results are the mean of 10 experiments. Values of absolute activity expressed as nmol galactose transferred per 30 min per g liver; specific activity as nmol galactose transferred per 30 min per mg protein.

| | UDPgalactose-asialo-mucin galactosyltransferase | |
|---|---|---|
| | Absolute activity | Specific activity |
| Total particulate fraction | 2282 ± 138.9 | 14.5 ± 0.95 |
| Microsomes | 825 ± 33.5 | 35.9 ± 1.45 |
| Rough microsomes | 185 ± 11.7 | 18.5 ± 1.10 |
| Smooth microsomes | 200 ± 12.4 | 40.0 ± 2.40 |
| Golgi I | 101.4 ± 15.2 | 337.0 ± 50.5 |
| Golgi II | 39.9 ± 6.0 | 306.9 ± 46.0 |

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