BIOSYNTHESIS OF MEMBRANE GLYCOPROTEINS IN THE RAT SMALL INTESTINE

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1. Introduction

Considerable studies have been carried out recently to elucidate the intracellular sites and steps of biosynthesis of secretory glycoproteins in various tissues [1–3]. Intestine, liver, thyroid and other tissues have been shown to actively incorporate labelled sugar into their respective secretory glycoproteins [4–5]. These in vivo incorporation studies with radioactive sugars as well as studies of glycosyltransferases responsible for the addition of carbohydrates in the subcellular fractions of liver, thyroid glands and other tissues indicated that glycosylation of secreted glycoproteins may occur at both microsomes and Golgi membranes [6–9]. In contrast, there is a paucity of data concerning the biosynthesis of membrane glycoproteins. Prerequisite for this type of study on membrane glycoproteins is the preparation of submicrosomal membranes devoid of contamination with vesicular contents [10]. In the present study, using these membrane preparations the rate of incorporation of radioactive glucosamine into the submicrosomal and brush border membranes and the subcellular location of the glycosyltransferase enzymes were determined to elucidate the pathway for the biosynthesis of membrane glycoproteins.

2. Materials and methods

Rough and smooth membranes devoid of vesicular contents and brush border membranes were prepared from the mucosal scrapings of the small intestine of male Sprague–Dawley rats as described previously [10]. At various time intervals after the intraperitoneal injection of 25 μCi of [^{14}C]glucosamine, rats were killed and the membrane protein fractions prepared and the samples counted in a Packard Tri Carb liquid scintillation counter as described previously [3]. For the gel filtration study the labelled membrane proteins were dialyzed against the eluting buffer (0.05 M NH_4HCO_3, 1% SDS, pH 8.0) and chromatographed on a 1.2 X 80 cm column containing Sepharose 4B.

The incubation mixture for assay of the *N*-acetylglucosaminyltransferase contained 100 000 dpm of UDP-*N*-[^{14}C]acetylglucosamine (4 μCi/μmole), 1 mg α_1 acid glycoprotein acceptor, 1.6 μmoles of cacodylate–acetate buffer pH 6.9, 0.54 μmoles of ATP and 0.02–0.10 mg of enzyme protein in a final volume of 165 μl. The reaction was carried out at 37°C for 2 hr. The assay mixture for the galactosyltransferase contained 50 000 dpm of UDP-[^{14}C]galactose (6 μCi/μmole), 1 mg of the Fetuin II acceptor or ovine submaxillary mucin (OSM) acceptor, prepared as previously described [3], 3.3 μmoles of $MnCl_2$, 10 μl of 3.3% Triton X-100, 66 μmoles of cacodylate–acetate buffer, pH 6.5, and 0.2–0.10 mg of enzyme protein in a final volume of 330 μl. The conditions for assay of the fucosyltransferase were the same as those used previously [11] except that 0.5 mg of the Fetuin I acceptor desialyzed Fetuin was used and the incubation was for 2 hr at 25°C. The conditions for the assay of the sialyltransferase were as follows: 50 000 dpm CMP-*N*-[^{14}C]acetylneuraminic acid (20 μCi/μmole), 1 mg of the Fetuin I acceptor, 22 μmoles of cacodylate-acetate buffer pH 6.4, 10 μl of 1.1% Triton X-100 and 0.02–0.10 mg of enzyme protein in a final vol of 110 μl. A 2-hr incubation at 30°C was used.

3. Results

Fig. 1. shows that the smooth microsomal membrane is, initially, the most active site of incorporation. The rate of labelling of the rough membrane increased markedly 40 min after injection, its specific radioactivity reaching 80% of that of the smooth membrane at 80 min. Following a lag of 80 min, the labelling of the brush border membrane increased significantly, concomitant with a decrease in the specific radioactivity of the smooth and rough membranes. The homogenate was examined 20 min after injection for the presence of radioactive glucosamine and galactosamine. Over 90% of the label was associated with hexosamines, 60% with glucosamine and 30% with galactosamine, indicating the presence of an active epimerase in the intestinal mucosa. However, analysis of the radioactivity in all three membrane proteins after injection of labelled glucosamine showed that most of the radioactivity was associated with glucosamine (table 1).

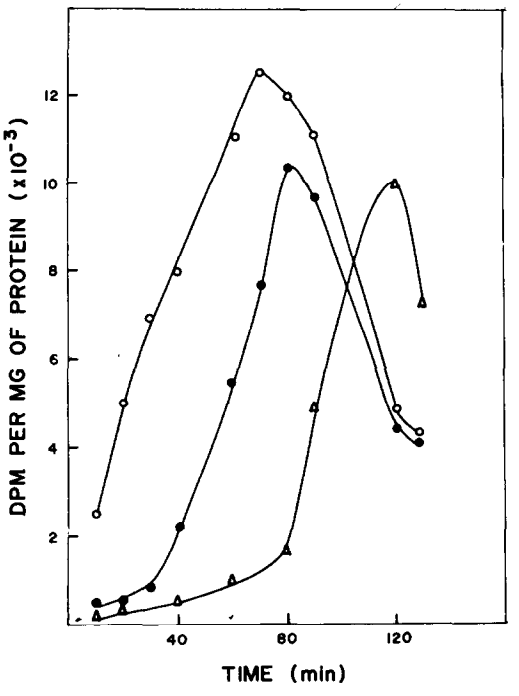

Fig. 1. Time course of incorporation of radioactivity into membrane proteins after injection with [^{14}C]glucosamine. Rats were injected intraperitoneally with 25 μCi of [^{14}C]-glucosamine, sacrificed at different times and membrane proteins isolated. The profiles are: smooth membranes (○———○), rough membranes (●———●) and brush border membranes (△———△). Results are means of 5 experiments.

Table 1
Percent distribution of ^{14}C in hexosamines of rough, smooth and brush border membrane proteins

Membrane	Hexosamine	% Distribution		
		20 Min	70 Min	120 Min
Rough	GlcN[a]	82.9	82.2	–[c]
	GalN[b]	17.1	17.8	–
Smooth	GlcN	80.7	68.1	–
	GalN	19.3	31.9	–
Brush border	GlcN	81.6	80.1	86.1
	GalN	18.4	19.9	13.9

[a]Glucosamine.
[b]Galactosamine.
[c]Not determined.
Rough, smooth and brush border membrane proteins were isolated 20, 70, and 120 min after injection of 25 μCi of [^{14}C]glucosamine and were hydrolyzed in 4 N HCl at 100°C under vacuum and glucosamine and galactosamine separated by an amino acid analyzer. The column effluent was collected and counted in a water miscible scintillator. Counts corresponding to the elution time of each hexosamine were integrated and the % calculated from those values.

Fig. 2 shows that the majority of the radioactivity was present in the two peaks both in the rough and the smooth membrane. Most of the label was associated with the larger molecular weight species (M.W. 93 000) in the rough membrane while in the smooth fraction, the radioactivity was distributed almost equally between the two peaks. The brush border membrane had a single broad radioactive peak that eluted in the range of the lower molecular weight peak (M.W. 20 000).

Table 2 shows that the specific activities of the glycosyltransferases examined were up to 93 times higher in the smooth membranes than in the other membrane fractions. The specific activities of all the glycosyltransferases studied were lowest in the brush border membranes.

4. Discussion

Carbohydrates may be incorporated into the glycoproteins or glycolipids of membranes. Examina-

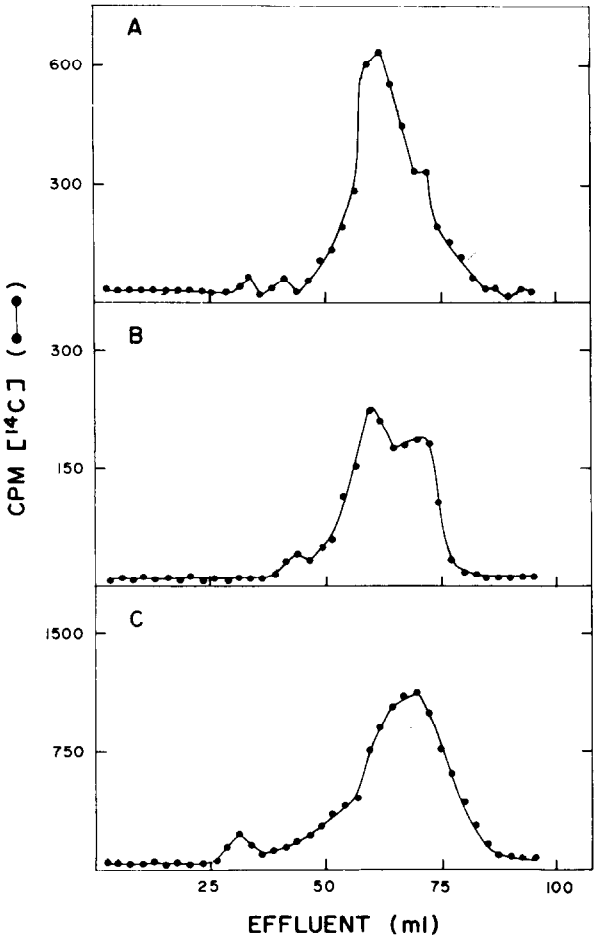

Fig. 2. Sepharose 4B gel filtration of labelled membrane proteins. Membrane proteins were labelled in vivo by injection of [^{14}C]glucosamine (●———●) 70 min before sacrifice for the smooth and rough membranes and 120 min for brush border membranes.

tion of [^{14}C]glucosamine into membrane glycoproteins shows a very rapid uptake by the smooth membranes shortly after injection. The incorporation into the rough membranes soon increases after the initial lag and approaches that of the smooth membranes. The lag in the labelling of the rough membrane is difficult to explain, but a similar phenomenon has also been reported by Hallinan et al. [12] in rat liver and by Cook et al. [13] in Ehrlich ascites carcinoma cells. The time course of incorporation of labelled sugar into brush border membranes showed an even longer lag period as was observed previously by Forstner [14] The lag in labelling of the rough and brush border membranes is probably not due to the time required for the conversion of glucosamine to other substances which are subsequently incorporated into these membranes, since the distribution of radioactivity into glucosamine and galactosamine following injection of labelled glucosamine is similar in the three membrane fractions.

Table 2
Distribution of glycosyltransferases in smooth, rough membranes and brush border membranes

Glycosyltransferases	Acceptor	Enzyme activity[a] (nmoles/mg protein)		
		Rough	Smooth	Brush border
Galactosyltransferase	OSM[b]	0.15 ± 0.03	3.62 ± 1.14	0.12 ± 0.02
Sialytransferase	Fetuin I[c]	0.22 ± 0.07	2.87 ± 0.54	0.12 ± 0.03
Fucosyltransferase	Fetuin I	0.05 ± 0.01	0.93 ± 0.36	0.01 ± 0.00
N-Acetylglucosaminyl-transferase	α_1 glycoprotein[d]	0.61 ± 0.20	8.80 ± 2.10	0.80 ± 0.22
Galactosyltransferase	Fetuin II[e]	0.24 ± 0.06	4.28 ± 1.28	0.08 ± 0.02

Rough, smooth and brush border membranes were dialyzed overnight against water and assayed for glycosyltransferase by the methods described in the text.
[a]Results are means ± S.D. of 5 experiments of 3 rats each.
[b]Ovine submaxillary mucin with terminal *N*-acetylgalactosamine.
[c]Fetuin with terminal galactose.
[d]α_1 acid glycoprotein with terminal mannose.
[e]Fetuin with terminal *N*-acetylglucosamine.

The activities of all five glycosyltransferases examined were localized predominantly in the smooth microsomal membranes and the minimal enzyme activities observed in the rough and brush border membrane fractions could well be accounted for by the inevitable trace contamination with the smooth membranes. This difference in the localization of glycosyltransferases is apparently not due to the presence of an activator or the absence of an inhibitor in the membrane preparations since mixtures of the membrane fractions in different proportions yielded the expected additive enzyme activity. One must be cautious in drawing a conclusion from the labelling pattern alone. However the high degree of hexosamine labelling in the rough and brush border membranes coupled with the data showing negligible glycosyltransferase activity in these fractions indicates that the glycoproteins present in these membranes are probably synthesized elsewhere. The substantial labelling at early time periods of the smooth membranes and the predominant localization within these membranes of enzymes required for glycosylation strongly suggest that the smooth membrane is the main site of glycosylation of glycoproteins destined to be a part of the rough and brush border membranes. The lag in the incorporation of glucosamine into rough microsomal and brush border membranes observed in the present study is probably due to the time required for the synthesis of membrane glycoproteins at the smooth microsomal membranes and their transport and eventual uptake by the rough microsomal and brush border membranes.

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