ROUTES OF FUCOPROTEINS IN PLASMA MEMBRANE DOMAINS

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1. Introduction

The hepatocyte's surface membrane comprises three major subfractions which can be isolated according to their sedimentation properties and densities. Due to their different functions these three areas differ characteristically in morphology, composition, and enzyme- or receptor endowment [1–3]: The microvillus blood sinusoidal face (BS) represents approximately half of the cell surface membrane and is characterized by the presence of various hormone receptors. The bile canalicular site (BC) is a small microvillus area, whereas the contiguous face (CF) is a straight membrane directed to the adjacent cell which displays junctional complexes and desmosomes.

Glycoproteins are major constituents of all three plasma membrane domains. Their role includes enzyme activity, receptor function or cell-to-cell interaction. Terminal sugars of glycoconjugates as L-fucose or *N*-acetylneuraminic acid are related to these functions, although their role is not fully understood [4]. The content of plasma membrane fucoproteins, however, was found to be 4-fold increased in hepatoma tissue [5]. The common precursor pool of fucoprotein is the Golgi apparatus which contains the bulk of the fucosyltransferase activity [6]. Since L-fucose is incorporated within the Golgi complex into plasmalemma and secretory glycoproteins [7,8], this carbohydrate is an ideal precursor for investigating incorporation and degradation kinetics of glycoproteins.

2. Materials and methods

Male Wistar rats (150–180 g each) were injected intraperitoneally with L-[6-^{3}H]fucose (26 Ci/mol) or L-[4,5-^{3}H]leucine (40 Ci/mol) (The Radiochemical Centre, Amersham). Under light ether anesthesia the livers were removed at different times after the pulse. Plasma membranes of bile canalicular and lateral origin were prepared from a hypo-osmotic low-speed pellet by zonal centrifugation according to Pfleger et al. [9] using the Kontron rotor TZT 32 (Kontron Analytic, Zürich). The method of Touster et al. [10] was used to enrich the blood sinusoidal plasma membranes. The final sucrose gradient of this procedure was modified to obtain the sinusoidal and the Golgi membrane-enriched fractions, collected between 34 and 29% (w/w) and 29 and 27% (w/w) interfaces, respectively. The purity of the fractions was checked by use of marker enzymes (table 1) and electron microscopy. Protein-bound radioactivity was determined by a modified method of Mans and Novelli [11,12]. Electrophoresis of solubilized membranes was performed essentially as described previously [13]. For radioautography the gels were processed according to Laskey and Mills [14].

3. Results

3.1. *Incorporation and degradation kinetics of L-[^{3}H]fucose*

Pulse chase experiments were performed as indicated in the legend of fig.1. At 15 min after the injection of the precursor the maximum of the protein-bound radioactivity was found associated with the Golgi and the BS membranes and followed by a rapid decay until 4 h after the pulse. In the BC and CF fractions, however, the highest specific radioactivity was reached at 30 min and a minor loss of radioactivity was observed thereafter. At 24 h after the injection a remarkable loss of radioactivity could be detected in all fractions followed by an increase within the next 12 h.

Table 1
Characterization of the membrane fractions by marker enzymes

	Golgi-enriched Fraction	BS	BC	CF	Homogenate $\mu mol \times h^{-1} \times mg^{-1}$ protein
	Relative specific activity				
Galactosyltransferase	80.3	27.9	n.m.	0.7	5.3×10^{-3}
5'-Nucleotidase	7.5	11.4	31.5	18.7	3.4
INT-Oxidoreductase	0.03	0.05	0.05	0.3	1.6
Glucose-6-phosphatase	1.0	1.2	0.5	0.5	5.4

Enzyme activities were determined as described previously [11,13]. Relative specific activities were calculated as the ratio of the specific activity of the respective membrane fraction to that of the homogenate. The data are average values of 3–5 membrane preparations (n.m., not measurable).

3.2. *Polyacrylamide gel electrophoresis and radioautography*

When comparing the polypeptide patterns (figs.2a and 3a) a common basic structure becomes evident. In addition, there are characteristic bands only appearing in one domain: e.g. the only occurrence of polypeptide bands in CF of 30 000 and in the range below 20 000 apparent molecular weight (MW). Injecting L-[^{3}H]fucose as early as 8 min later some polypeptides from the Golgi as well as from the BS membranes are labelled (fig.2b). However, 8 min after the injection with L-[^{3}H]leucine (fig.3), all fractions show a rather identical labelling pattern. The rapid disappearance of heavily labelled bands also present in the Golgi fraction, is most pronounced in BS within the first hour. During the next 5 h changes of the labelling pattern are rather identical in all 4 membrane fractions, e.g. the disappearance of a band of 75 000 apparent MW. Furthermore, the existence of a long-lived fucose-containing polypeptide (MW about 60 000) should be pointed out.

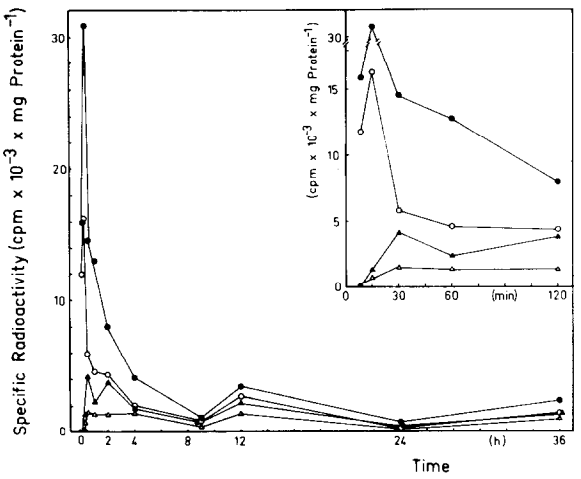

Fig.1. Kinetics of L-[^{3}H]fucose incorporation into the Golgi fraction and plasma membrane domains. Three rats were injected i.p. with 500 μCi L-[^{3}H]fucose per kg of body wt. each. The pulse was followed by a chase with 100 mg of unlabelled L-fucose per animal 20 min, 3 h and every 12 h later. The animals were killed at different times after the pulse and the membrane fractions were prepared from the pooled livers as described in section 2. Specific radioactivity was determined in a Golgi (•), BS (○), BC (▲) and CF (△) membrane enriched fraction. Each point represents the mean value from two sets of experiments with a difference in the range below 15%. The inserted figure redisplays the values obtained at times up to 2 h by use of a wider time-scale.

4. Discussion

The rapid labelling, and the strikingly similar labelling pattern of the fucose-containing polypeptides is a characteristic feature of both the Golgi as well as the BS membranes (figs.1 and 2). This demonstrates their secretory function [15] being further supported by the appearance of labelled fucoproteins in the serum at the same time [16,17]. In the BC and CF fraction, however, no radioactivity can be detected 8 min after the injection of L-[^{3}H]fucose. When this process is finished by the chase 30 min after the pulse further radioactive bands appear which represent the basic glycopolypeptide structure of the domains. In contrast, the protein components of this structure can already be visualized 8 min after a pulse with

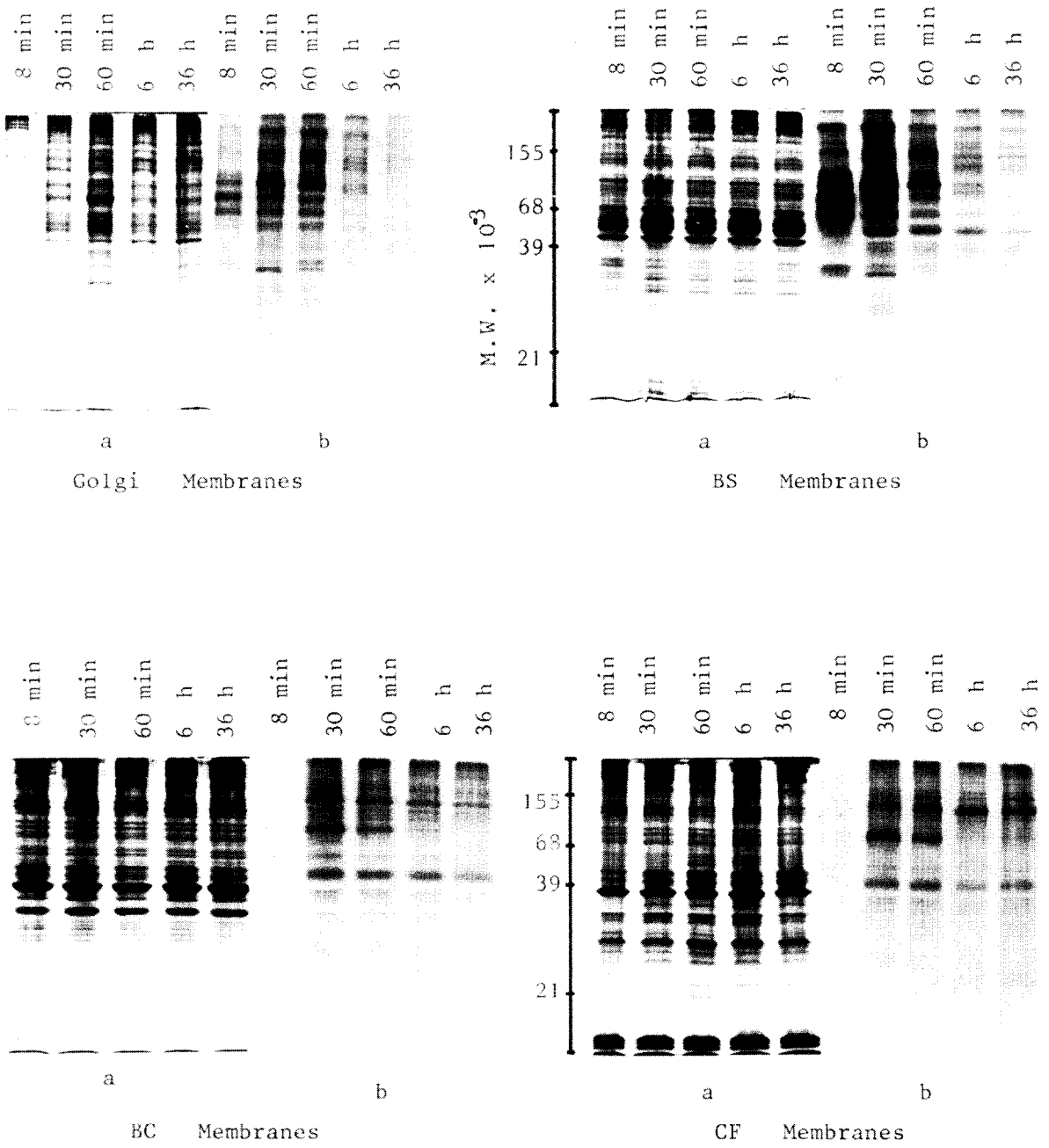

Fig.2. Distribution of L-[^{3}H]fucose among different glycopolypeptides in the Golgi fraction and plasma membrane domains. Three rats were injected intraperitoneally with L-[^{3}H]fucose (20 mCi/kg of body wt.) followed by injections of unlabelled L-fucose as indicated in the legend of fig.1. At different times after the pulse the membrane fractions were prepared from the pooled livers and submitted to electrophoresis on 10% polyacrylamide slab gels containing SDS. 60 μg of protein were applied to each lane. The gels were stained for protein with Coomassie brillant blue (a) and exposed for radioautography (b).

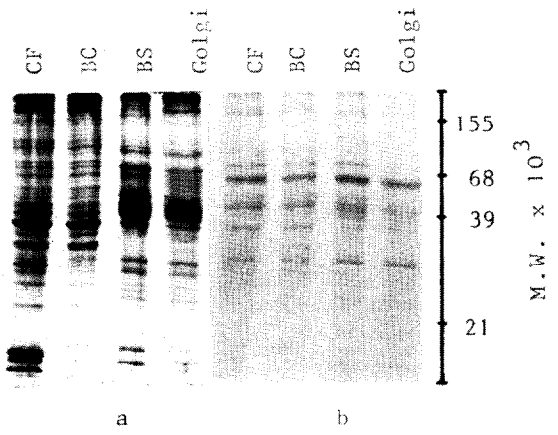

Fig.3. Distribution of L-[^{3}H]leucine among different polypeptides in the Golgi fraction and plasma membrane domains. Three rats were injected intraperitoneally with 20 mCi L-[^{3}H]leucine per kg of body wt each. 8 min later the rats were killed and the membrane fractions were prepared from the pooled livers. Electrophoresis was performed on 10% polyacrylamide slab gels in the presence of SDS. 60 μg of protein were applied to each lane. The gels were stained for protein (a) and exposed for radioautography (b).

L-[^{3}H]leucine. This difference can be explained by different synthesis and degradation mechanisms and kinetics of the carbohydrate and protein moiety [18] which include the existence of plasma membrane bound glycosidases [19] and glycosyltransferases [20]. In addition, one subpopulation of the Golgi complex may synthesize glycoprotein components of one cell surface domain only. Furthermore, from our results the possibility arises, whether some proteins can even be inserted into the membrane bypassing the Golgi system. The regulation of the biosynthetic routes [21] is even more complex considering different degrees of glycosylation of proteins [18,22] as well as the possible action of signal peptidases [23]. At later times redistribution processes take part in the modification of the membrane components. These mechanisms (fig.2) consist in both the lateral diffusion as well as the internalization followed by the re-insertion of membrane constituents as supported by findings in intestinal cells [24,25]. The exact routes and mechanisms of these redistribution processes must be detailed by further experiments firstly using drugs interfering with the action of microtubules and microfilaments and secondly studying the kinetics of an isolated plasma membrane glycoprotein.

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