DEGRADATION OF FUCOPROTEINS AND SIALOPROTEINS IN THE PLASMA MEMBRANE OF NORMAL AND REGENERATING LIVER

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1. Introduction

Evidence is accumulating that alterations of free and protein-bound sugars are connected with changes in surface properties of solid tissue cells during different states of differentiation (reviewed [1]). L-Fucose and *N*-acetylneuraminic acid (NANA) are the terminal sugars of surface glycoconjugates of eukaryotic cells [2–4], exerting a key role in modifying intercellular connections in normal and transformed tissues [5–8]. Different turnover characteristics of L-fucose and NANA in the plasma membrane of rat liver and Morris hepatoma 7777 [9,10] suggest that the loss of these two sugars from the plasma membrane is regulated specifically. During the controlled rapid growth of regenerating liver after partial hepatectomy the degradation of proteins in the total cell homogenate [11] and in the plasma membrane [12] is reduced to one-half compared to normal liver. To contribute to the metabolic basis of a possible antagonism between L-fucose and NANA [13], the loss of protein-bound L-fucose and NANA from the plasma membrane of both normal and regenerating liver was compared.

2. Material and methods

Male Wistar rats (about 160–180 g each) were injected intraperitoneally with 300 μCi L-[6-^{3}H]fucose (25 Ci/mmol) or N-[^{3}H]acetyl-D-mannosamine (ManNAc) (Radiochemical Centre, Amersham) (500 mCi/mmol), 20 h before surgery. Partial hepatectomy was performed under slight ether anesthesia as in [14] removing 2/3 liver. When adrenalectomized rats were used, suprarenal glands were extirpated bilaterally 4 h before injection of label, i.e., 24 h before partial hepatectomy.

Plasma membranes and homogenates were prepared 1, 2, 4 and 6 days after injection of label as in [12]. Membrane purity was checked as outlined [12].

Protein-bound radioactivity was determined by a modified method of [15]. Filter disks were dried and counted in a Liquid-Scintillation Spectrometer (model 3390 of Packard Instruments, La Grange, IL) using a toluene/PPO/POPOP scintillator. Channel counting efficiency was determined by internal standard calibration. Protein content was determined as in [16] with bovine serum albumin (Behringwerke AG, Marburg) as a standard.

Rate constants of degradation k_d and half-lives $t_{½}$ were calculated from the decay of the specific radioactivity in normal liver and of the total radioactivity in regenerating liver as in [12], assuming a decay with first-order kinetics. k_d was calculated from the equation

$$C(t) = C_0 \cdot e^{-k_d \cdot t}$$

when C_0 is the radioactivity (dpm/mg protein) at time zero, $C(t)$ the radioactivity at times $t = t_1, t_2, \ldots t_x$ (days);

$$t_{½} = \ln 2/k_d.$$

Table 1

Rate constants of degradation and half-lives of protein-bound fucose and NANA in the plasma membrane and total cell homogenate of normal and regenerating liver

Radioactive precursor	Liver	Plasma membrane				Total cell protein			
		1–2 day after inj.		3–6 day after inj.		1–2 day after inj.		3–6 day after inj.	
		k_d	$t_{½}$ (h)	k_d	$t_{½}$ (h)	k_d	$t_{½}$ (h)	k_d	$t_{½}$ (h)
L-[6-^{3}H]Fucose	Normal	0.665 ± 0.06	23	0.356 ± 0.01	45	0.370 ± 0.03	45	0.400 ± 0.01	42
	Regenerating	0.371 ± 0.02	45	0.275 ± 0.03	60	0.174 ± 0.05	96	0.190 ± 0.01	86
		1–4 day after injection				1–4 day after injection			
		k_d	$t_{½}$ (h)			k_d	$t_{½}$ (h)		
N-[^{3}H]Acetyl-mannosamine	Normal	0.592 ± 0.02	28			0.433 ± 0.01	38		
	Regenerating	0.340 ± 0.03	49			0.203 ± 0.03	82		

Male Wistar rats were injected intraperitoneally with 300 μCi L-[6-^{3}H]fucose or *N*-[^{3}H]acetyl-D-mannosamine/100 g body wt, 20 h prior to partial hepatectomy. Subcellular fractions were prepared as in section 2 20 h, 2 days, 4 days and as for fucose, additionally 6 days after the injection of label. The decay of specific radioactivity in normal liver and of total radioactivity in regenerating liver was followed. Rate constants of degradation k_d ± SEM were calculated mathematically as the slope of the straight lines obtained by linear regression, $t_{½} = \ln 2/k_d$. Groups of 6 animals were used for each date

3. Results and discussion

Protein-bound L-fucose and NANA were turning over more rapidly in the plasma membrane than in the total cell homogenate (table 1). In accordance with similar data of normal mouse liver [18] and Morris hepatomas [9] the present finding in regenerating liver confirms, that this is a characteristic feature of plasma membrane glycoproteins.

In normal liver the loss of L-fucose from the plasma membrane slowed down at about 44 h after injection of label, conversely to NANA which decayed at a constant rate. The biphasic decay may be caused by the existence of two classes of fucoproteins in the plasma membrane with different turnover characteristics. As fucose is not randomized into other sugars [19] a transition of the isotope into protein-bound sugars turning over more slowly cannot account for the second slope in normal liver. Reutilization of the isotope should also be taken into consideration. A biphasic decay of protein-bound fucose was also found in the plasma membrane of regenerating liver. On the first day after partial hepatectomy the half-life of fucose in the plasma membrane was nearly doubled compared to normal liver, but was rapidly readjusted to that of normal liver the following days. It may be assumed, that primarily the degradation of the short-lived class of fucoproteins is decreased in regenerating liver. In total cell homogenate, however, protein-bound fucose was lost at a uniform rate, that was reduced to one-half after partial hepatectomy.

When glycoproteins were labelled with [^{3}H] ManNAc as precursor of protein-bound NANA a linear decay of plasma membrane NANA was found in normal and regenerating liver. In this fast growing liver tissue half-lives of protein-bound NANA were doubled in the plasma membrane and in total cell homogenate, respectively. As NANA is taken up by the liver only to a negligible extent [9], reutilization of NANA from extrahepatic tissues is unlikely to account for this prolongation of half-life. ManNAc provides only CMP-NANA in the sugar nucleotide pools needed for glycoprotein synthesis [9], ensuring that only NANA is labelled after injection of ManNAc.

With respect to the possible role of corticosteroids in the regulation of protein metabolism [20], the half-lives of fucose were determined in normal and regenerating liver on the second day after adrenalectomy, i.e., in regenerating liver on the first day after partial hepatectomy. No marked alterations were found; especially in regenerating liver the reduced catabolism was not influenced (table 2).

In the context with the previous finding of a decreased protein degradation during liver regeneration [12], the present data point to a common feature in the regulation of the basic catabolic potential for proteins and glycoproteins. The different turnover characteristics of protein-bound fucose and NANA in the plasma membrane suggest that the turnover of the carbohydrate component of glycoproteins is specifically regulated, whether defined glycoproteins dissociate from the plasma membrane as a unit, or sugars are cleaved off separately by glycosidases independently regulated.

Table 2
Rate constants of degradation k_d and half-lives $t_{1/2}$ of protein-bound fucose in normal and regenerating liver in adrenalectomized rats

Liver	Plasma membrane		Total cell homogenate	
	k_d	$t_{1/2}$ (h)	k_d	$t_{1/2}$ (h)
Normal	0.544 ± 0.02	30	0.494 ± 0.05	34
Regenerating	0.386 ± 0.12	43	0.070 ± 0.11	10 days

Male Wistar rats were adrenalectomized 24 h prior to partial hepatectomy. L-[6-^{3}H]Fucose, 300 μCi, was injected intraperitoneally 4 h after adrenalectomy. Subcellular fractions were prepared 20 h and 44 h later. For calculation of k_d and $t_{1/2}$ see legend of table 1. Each k_d represents the mean ± SEM for 4 normal or 6 regenerating livers

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