Different Turnover of Fucose Residues in Plasma Membranes of Rat Liver and Morris Hepatoma 7777

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Half-lives and rate constants of degradation of protein-bound fucose have been determined in plasma membranes and total cell homogenates of rat liver and Morris hepatoma 7777. The existence of at least two dynamically different classes of fucose-containing glycoproteins could be demonstrated in both liver and hepatoma plasma membranes. The apparent half-lives were 8.4 and 24.5 h (host liver) and 11.5 and 33.9 h (Morris hepatoma). Since this biphasic loss of fucose residues was not observed for sialic acid [Harms & Reutter (1974) *Cancer Res.* **34**, 3165–3172], the differences are possibly related to specific functions of fucosylated glycoproteins of the plasma membrane.

Glycoproteins determine characteristic functions of plasma membranes, e.g. cell–cell recognition, growth control, information uptake mediated via hormones or toxins and the attachment of antibodies (for references, see Hughes, 1975). It is widely accepted that cell-surface carbohydrates are involved in oncogenicity (Warren *et al.*, 1978; Reutter & Bauer, 1978) and many changes of the cell surface in the course of malignant transformation have been reported. Such alterations may depend not only on changes in biosynthesis, but also on different rates of glycoprotein degradation. This second possibility has seldom been investigated (Harms & Reutter, 1974; Robbins *et al.*, 1974; Tauber & Reutter, 1978a; Leonard *et al.*, 1978). It has been demonstrated that the content of fucoproteins in the plasma membrane of Morris hepatoma 7777 is greatly increased (Vischer & Reutter, 1978). In the present paper we compare the degradation of glycoproteins in the whole plasma membrane of host liver and Morris hepatoma 7777 by using radioactively labelled L-fucose and report that they exhibit two different rates of turnover, emphasizing that there exist at least two dynamically different groups of fucose-labelled proteins in the plasma membranes of both tissues. Moreover, the fucose residues of the hepatoma show a prolonged half-life when compared with host liver.

Materials and Methods

Materials

Young adult male Buffalo rats (160–200 g) were

bred in our laboratory and fed with Altromin R (Altromin G.m.b.H., Lage/Lippe, Germany) and water was given *ad libitum*. Morris hepatoma 7777 (originally obtained from Dr. H. P. Morris, Howard University, Washington, DC, U.S.A.) were inoculated into both hind legs. The tumours were excised when 1–2 cm in diameter, and connective tissue and necrotic material were carefully removed. L-[5-³H]Fucose (sp. radioactivity 4.2 Ci/mol) and GDP-L-[U-¹⁴C]fucose (sp. radioactivity 140 Ci/mol) were from The Radiochemical Centre (Amersham, Bucks., U.K.). All other chemicals of analytical grade were purchased from E. Merck A.G. (Darmstadt, Germany).

Methods

Preparation of plasma membranes. The plasma membranes of liver were enriched by a modification of the procedure of Neville (1960), as described by Bachmann *et al.* (1977). The plasma membranes of hepatoma were prepared by an iso-osmotic/hypo-osmotic procedure as described previously Harms & Reutter, (1974).

Enzyme assays. The purity of the membrane fractions was checked by electron microscopy and by measurement of the following enzymes as summarized by Bachmann *et al.* (1977): 5'-nucleotidase (EC 3.1.3.5); glucose 6-phosphatase (EC 3.1.3.9); succinate 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyltetrazolium oxidoreductase (EC 1.3.99.1).

Chemical assays. Protein was determined by the method of Lowry *et al.* (1951), with bovine serum

albumin as a standard. For measurement of protein-bound L-fucose, the plasma membranes were hydrolysed for 1 h in 1M-HCl at 100°C and afterwards fucose was separated from other sugars by chromatography on Whatman no. 3 paper with butan-1-ol/pyridine/water (6:4:3, by vol.) as solvent. L-Fucose was determined by the cysteine/H₂SO₄ method described by Dische & Shettles (1948). GDP-L-fucose was measured as described previously (Vischer & Reutter, 1978).

Determination of radioactivity. The incorporation of labelled L-fucose into trichloroacetic acid-precipitable materials was measured by the method of Mans & Novelli (1961) as modified by Schreiber *et al.* (1971), but avoiding the heat step (Bauer *et al.*, 1976). Radioactive material in the lipid fraction was determined by extraction of the membrane fractions by the method of Folch *et al.* (1957). Radioactivity was measured in a model 3390 Tri-Carb scintillation spectrometer (Packard Instruments Inc., Oberlin, OH, U.S.A.) with Bray's (1960) scintillator for aqueous solutions and toluene scintillation mixture (Kallmann *et al.*, 1958) for radioactivity on paper.

Administration of radioactive isotopes. The radioactive precursor was diluted in 0.9% NaCl and injected intravenously via the tail vein. When studying the time-dependent incorporation of L-fucose into liver plasma membrane, serum proteins and total cell proteins, 2.5 mCi of L-[³H]fucose/kg body wt. was injected and the animal was killed at the intervals specified (15 min to 2 h). For measurement of the degradation rate of plasma-membrane glycoproteins 5 mCi of L-[³H]fucose/kg body wt. was given via the tail vein followed, 2 h later, by a chase with unlabelled fucose (20 mmol/kg body wt.). At 2–36 h after injection of the isotope, host liver and hepatoma were removed for plasma-membrane preparation.

Calculations of half-lives and rate constant of degradation. Rate constants of degradation (k_d) and half-lives ($t_{1/2}$) of total plasma-membrane glycoproteins were calculated from the decay of relative radioactivity, assuming a decay with first-order kinetics. Plotting the logarithms of the specific radioactivities against time, the slope of the straight line obtained is equivalent to the value of k_d . The half-life was calculated from the equation:

$$t_{1/2} = \frac{\log 2}{k_d}$$

as described previously (Tauber & Reutter, 1978b).

Results and Discussion

It has been shown in several different reports that L-fucose is primarily incorporated into glycopro-

teins without any appreciable conversion into other sugars (Coffey *et al.*, 1964; Bekesi & Winzler, 1967). The present results confirm these reports. [³H]Fucose-labelled plasma membranes of normal and tumour-bearing rats were hydrolysed and analysed as described in the Materials and Methods section. In both cases more than 90% of protein-bound radioactivity could be detected in L-fucose. In host liver and Morris hepatoma 7777 less than 7% of total membrane-bound radioactivity was soluble in chloroform/methanol, in agreement with previous reports describing only small amounts of labelled fucose in the lipid fraction (Atkinson, 1975; Snary *et al.*, 1976).

Fig. 1 shows the time-dependent incorporation of L-fucose into trichloroacetic acid-insoluble material of total host liver homogenate, plasma membrane and serum fraction. The relative radioactivity (d.p.m./mg of protein) of the trichloroacetic acid-soluble fraction of total homogenate is maximal 15 min after injection and decreases rapidly. Relative radioactivities of plasma membrane and serum fractions reach their maximum at 60 min and in the case of the serum remain at this value until 120 min, whereas in the membrane fraction during the same interval 30% of its radioactivity was lost. The

Fig. 1. Kinetics of L-[³H]fucose incorporation in liver plasma membranes, total homogenate and serum fraction

Normal rats were injected intravenously with L-[³H]fucose (2.5 mCi/kg body wt.) and at the times indicated plasma membranes (●), serum (■) and cell homogenate (▲) were prepared. The specific radioactivity was determined as described in the Materials and Methods section. A semi-logarithmic plot of specific radioactivity as a function of time is shown. Each point represents the mean $\pm$ S.D. for three animals.

decrease in relative radioactivity of total cell homogenate was accompanied by an increase in the relative radioactivity of plasma membrane and serum glycoproteins, supporting the findings by Franke *et al.* (1971) that glycoproteins synthesized in the liver cell are transported to the cell surface where they are incorporated into the membranes or released into the blood stream. A decrease in relative radioactivity in the membrane fraction in contrast with the constant value in the serum fraction indicates a high metabolic activity of fucose-labelled glycoproteins at the cell surface.

The rate of decay of fucose-labelled plasma-membrane glycoproteins was followed during a time interval of 2–36 h after the injection of labelled fucose. To minimize isotope reutilization, all animals were injected with unlabelled L-fucose. This chase caused a 30–40-fold increase in the GDP-fucose pool in host liver and hepatoma (Vischer & Reutter, 1978). The increase in the nucleotide sugar pool did not affect fucoprotein metabolism, as could be shown by omitting the chase with unlabelled fucose (P. Vischer & W. Reutter, unpublished work). In the case of Morris hepatoma 7777 there is a 2–3-fold higher incorporation of radioactivity compared with host liver. In both tissues a rapid loss of radioactivity in the total plasma-membrane fraction during the first 10 h is followed by a significantly slower rate during the following 26 h (Fig. 2). Half-lives calculated from two distinct regression lines of relative radioactivity yield times of 8.5 and 24.4 h for host liver and 11.5 and 33.9 h for Morris hepatoma 7777 plasma-membrane fractions (Table 1 and Fig. 2). Homogenate is also degraded in this biphasic manner, but with a slightly higher turnover in the first hours and a decreased rate in the later stage compared with the plasma-membrane fractions. A possible influence of the reutilization of labelled L-fucose was investigated by measuring the turnover of the intracellular pool of GDP-fucose in host liver

and Morris hepatoma 7777. Previously we showed that the pool remains unchanged after injection of [³H]fucose of high specific radioactivity (Vischer & Reutter, 1978). Comparing the loss of radioactivity in the GDP-fucose pool of hepatoma and host liver, there is no indication of differences in turnover between tissues (Fig. 3). However, it should be mentioned that tumour growth enhances the turnover of

Fig. 2. Loss of fucose label from host-liver and Morris-hepatoma plasma-membrane fractions
Tumour bearing rats labelled with [³H]fucose as described in Table 1 were killed at the times indicated and plasma membranes were prepared. The incorporation of radioactivity into trichloroacetic acid-precipitable material measured and expressed as d.p.m./mg of plasma-membrane protein. The logarithms of the relative radioactivities (ordinate) are plotted against the time (abscissa). Regression lines were calculated using the least-squares method. Symbols: ●, host liver; ▲, Morris hepatoma 7777.

Table 1. Rate constant of degradation (*k_d*) and half-lives (*t_{1/2}*) of protein-bound fucose in host liver and Morris hepatoma 7777

Tumour-bearing rats were injected intravenously with 500 μCi of L-[³H]fucose/100 g body wt., followed 2 h later by injection of excess unlabelled fucose. At six time points during the first 10 h after pulse labelling and at 12, 24 and 35 h after administration of label animals were killed and plasma membranes were prepared. The rate constant of degradation (*k_d*) of trichloroacetic acid-precipitable radioactivity and the resulting half-lives were calculated as described in the Materials and Methods section. Each *k_d* value represents the mean ± s.d. for six to twelve animals.

	Plasma membrane		Total cell homogenate	
	<i>k_d</i>	<i>t_{1/2}</i> (h)	<i>k_d</i>	<i>t_{1/2}</i> (h)
Liver	0.0346 ± 0.0070	8.7	0.0222 ± 0.0110	13.5
	0.0123 ± 0.0003	24.5	0.0110 ± 0.0050	27.5
Hepatoma	0.0264 ± 0.0070	11.5	0.0453 ± 0.0060	7.0
	0.0088 ± 0.0006	33.9	0.0082 ± 0.0003	37.5

Fig. 3. Time dependent decrease of GDP-[^3H]fucose in host liver and Morris hepatoma 7777

Tumour-bearing rats were injected with 3 mCi of L-[^3H]fucose/kg body wt. and at the times indicated the specific radioactivity (d.p.m./nmol) of GDP-fucose was determined. Each point represents the mean $\pm$ S.E.M. for three animals. Symbols: ●, host liver; ▲, Morris hepatoma 7777.

GDP-fucose in both tissues (results not shown), supporting previous findings that fucose metabolism of host liver is influenced by hepatoma (Bauer *et al.*, 1976). From the data reported in the present paper it appears that host liver and Morris hepatoma possess two distinct groups of fucose residues with different half-lives and that both pools have significantly lower rates of degradation in hepatoma cells. This biphasic turnover of overall membrane fractions is obviously reflecting the heterogeneity of the turnover of distinct membrane glycoproteins, because preliminary studies with polyacrylamide-gel electrophoresis of isolated plasma-membrane fractions labelled with fucose show heterogeneous rates of glycoprotein metabolism (P. Vischer, R. Tauber & W. Reutter, unpublished work).

More recently a similar heterogeneity of fucose residues could be demonstrated in neuroblastoma cells (Hudson & Johnson, 1977) and hepatoma cells in culture (Doyle *et al.*, 1978).

This biphasic loss of radioactive label seems to be a characteristic feature of fucose residues in plasma membrane of liver and hepatoma, because the degradation rate of *N*-acetylneuraminic acid in the overall plasma-membrane fraction appears to exhibit uniform degradation kinetics (Harms & Reutter, 1974; Grünholz *et al.*, 1977). The physiological significance of these findings may be related (a) to specific functions of fucoglycoproteins on the cell surface, e.g. as receptor or structural membrane

proteins, which may require different rates of turnover and (b) to the position of fucose in the oligosaccharide chain. Fucose can occur in at least three different positions in asparagine-linked sugar chains (Paulson *et al.*, 1978; Schachter, 1979). Removal or attachment of fucose in specific linkages may possibly not only alter the function of fucosylated glycoproteins, but may also have a dramatic influence on glycoprotein degradation (Priels *et al.*, 1978) and perhaps cause prolongation of the normal degradation time as shown in the present paper and previously (Vischer & Reutter, 1978) for Morris hepatoma 7777. Further studies on purified plasma-membrane glycoproteins should allow us to elucidate the influence of fucose on the degradation of cell-surface glycoproteins. In the meantime, it has been shown in this laboratory that in a 110000-mol.wt. glycoprotein of liver plasma membrane the half-life of protein-bound L-fucose is 12.5 h, whereas that of protein-bound *N*-acetylneuraminic acid is 33 h (Kreisel *et al.*, 1980).

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References

- Atkinson, P. H. (1975) *J. Biol. Chem.* **250**, 2123–2134
- Bachmann, W., Harms, E., Hassels, B., Henninger, H. & Reutter, W. (1977) *Biochem. J.* **166**, 455–462
- Bauer, C., Hassels, B. & Reutter, W. (1976) *Biochem. J.* **154**, 141–147
- Bekesi, J. G. & Winzler, R. J. (1967) *J. Biol. Chem.* **242**, 3873–3879
- Bray, G. A. (1960) *Anal. Biochem.* **1**, 279–285
- Coffey, J. W., Miller, O. N. & Sellinger, O. Z. (1964) *J. Biol. Chem.* **239**, 4011–4017
- Dische, R. & Shettles, L. B. (1948) *J. Biol. Chem.* **175**, 595–603
- Doyle, D., Baumann, H., England, B., Friedman, E., Hou, E. & Tweto, J. (1978) *J. Biol. Chem.* **253**, 965–973
- Folch, J., Lees, M. & Sloane-Stanley, S. (1957) *J. Biol. Chem.* **226**, 497–509
- Franke, W. W., Morré, J. D., Deumling, B., Cheetham, R. D., Jarasch, E.-D. & Zentgraf, H.-W. (1971) *Z. Naturforsch.* **26b**, 1031–1039
- Grünholz, H.-J., Vischer, P. & Reutter, W. (1977) *Hoppe-Seyler's Z. Physiol. Chem.* **358**, 1209
- Harms, E. & Reutter, W. (1974) *Cancer Res.* **34**, 3165–3172
- Hudson, E. J. & Johnson, T. C. (1977) *Biochem. J.* **166**, 217–223
- Hughes, R. C. (1975) *Essays Biochem.* **11**, 1–36
- Kallmann, H., Hayes, N. E. & Furst, M. (1958) *Liquid Scintillation Counting*, p. 56, Pergamon Press, New York

- Kreisel, W., Volk, B., Büchsel, R. & Reutter, W. (1980) *Proc. Natl. Acad. Sci. U.S.A.* in the press
- Leonard, J. G., Hale, A. H., Roll, D. E., Conrad, H. E. & Weber, M. J. (1978) *Cancer Res.* **38**, 185–188
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mans, R. J. & Novelli, G. D. (1961) *Arch. Biochem. Biophys.* **94**, 48–53
- Neville, D. M. (1960) *J. Biophys. Biochem. Cytol.* **8**, 415–422
- Paulson, J. C., Prieels, J.-P., Glasgow, L. R. & Hill, R. L. (1978) *J. Biol. Chem.* **253**, 5617–5624
- Prieels, J.-P., Pizzo, S. V., Glasgow, L. R., Paulson, J. C. & Hill, R. L. (1978) *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2215–2219
- Reutter, W. & Bauer, Ch. (1978) in *Morris Hepatomas, Mechanisms of Regulation* (Morris, H. P. & Criss, W. E., eds.), pp. 405–437, Plenum Press, New York
- Robbins, P. W., Wickus, G. G., Branton, P. E., Gaffney, B. J., Hischberg, C. B., Fuchs, P. & Blumberg, P. M. (1974) *Cold Spring Harbor Symp. Quant. Biol.* **39**, 1173–1180
- Schachter, H. (1979) in *The Glycoconjugates* (Horowitz, M. J. & Pigman, W., eds.), vol. 2, pp. 88–181, Academic Press, New York
- Schreiber, G., Urban, J., Zähringer, J., Reutter, W. & Frosch, U. (1971) *J. Biol. Chem.* **246**, 4531–4538
- Snary, D., Allen, A. K., Faulkes, R. A. & Crumpton, M. J. (1976) *Biochem. J.* **153**, 75–78
- Tauber, R. & Reutter, W. (1978a) *FEBS Lett.* **87**, 135–138
- Tauber, R. & Reutter, W. (1978b) *Eur. J. Biochem.* **83**, 37–45
- Vischer, P. & Reutter, W. (1978) *Eur. J. Biochem.* **84**, 363–368
- Warren, L., Buck, C. A. & Tuszynski, G. P. (1978) *Biochim. Biophys. Acta* **516**, 97–127