Volume 222, number 2, 271–274 FEB 05164 October 1987

Metabolic stability of the fucose in rat transferrin

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Received 15 June 1987; revised version received 17 July 1987

The metabolic behaviour of the chitobiose core fucose that is a natural constituent of a large proportion of rat transferrin molecules was studied in rats comparatively to that of the polypeptide portion of the glycoprotein by using appropriate labels ([^{3}H]fucose and ^{125}I) and affinity chromatographic techniques (lentil-Sepharose). No evidence was obtained to suggest that this residue is cleaved from the glycan in significant amounts before removal of the entire glycoprotein for catabolism. Similarly, [^{14}C]fucose linked to GlcNAc residues in the antennae of human asialotransferrin was being eliminated in pigeons at the same rate as the polypeptide itself. It is concluded that in spite of transferrin's exposure to the cellular milieu, the fate of its fucose is distinctly different from that of the same in plasma membrane glycoproteins.

Fucose; Glycoprotein metabolism; Transferrin

1. INTRODUCTION

Precursor fucose, as shown by several investigations [1–4], is efficiently incorporated by the rat liver into newly synthesized membrane glycoproteins. An intriguing feature of fucose (and sialic acid) in hepatic membrane glycoproteins is its short half-life as compared with that of the peptide moiety. This observation was first made for dipeptidylpeptidase IV [5], and then extended over five additional individual glycoproteins [6] by Reutter and co-workers [7,8]. Hepatoma cell-membrane glycoproteins, whether in situ or transferred to fibroblasts, exhibit an analogous behaviour [9]. There is as yet no patent explanation for this phenomenon. Exposure of membrane glycoprotein glycans to subcellular hydrolases during endocytic passages, possibly in a prelysosomal compartment [8], seems a plausible hypothesis [6,9].

A plasma protein which is known to undergo repeated diacytic cycles is transferrin [10] and, by so doing, it might become exposed to a similar intracellular microenvironment as membrane glycoproteins do periodically. The question thus raised is: are the sugars that had been found to be short-lived in membrane glycoproteins, also prematurely removed from transferrin?

By employing lentil-lectin chromatography, which is specific for the presence of an α-linked fucose attached to the asparagine-linked *N*-acetylglucosamine of the chitobiose core in complex glycans [11], we found recently that approximately one rat transferrin molecule out of four is fucosylated [12]. This observation provided a means of investigating whether transferrin fucose and polypeptide are being eliminated simultaneously.

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2. MATERIALS AND METHODS

2.1. *Isolation and preparation of tracer proteins*

Rat transferrin (rTf) of the type that contains a standard biantennary glycan was isolated as in [13] from the plasmas of normal female Sprague-Dawley rats and from a rat which had received [^{3}H]fucose. The tracer (500 μCi, 55 Ci/mmol, ICN Radiochemicals, Irvine, CA) was injected i.p. and the recipient exsanguinated 6 h later. Fucose-

Published by Elsevier Science Publishers B.V. (Biomedical Division)
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containing and fucose-free forms of rTf were separated by affinity chromatography on a column (1 × 5 cm) of lentil-Sepharose (Sigma, St. Louis, MO) as before [13]. Samples (50–100 μg) of either form of rTf were labelled with ^{125}I (New England Nuclear, Boston, MA) by using tetrachloroglycoluril according to specifications given elsewhere [14]. Human asialotransferrin, the type possessing two biantennary glycans, was fucosylated in each of the four branch GlcNAc by incubation with GDP[^{14}C]fucose and α(1-3)fucosyltransferase from human milk under conditions given by Prieels et al. [15].

2.2. *Experimental procedures*

Adult rats (200–250 g) were used for studies with rTf and adult pigeons (390–480 g) for those with human asialotransferrin. Quantities of radioactivities injected were determined gravimetrically [13]. The protein doses per 100 g body wt were of the order of 10–20 μg for ^{125}I-, 170–190 μg for ^{3}H- and 90–100 μg for ^{14}C-labelled preparations. In experiments with ^{125}I, the animals' drinking water was supplemented with NaI (0.005%, w/v). Small (20–50 μl) venous plasma samples, anticoagulated with heparin, were obtained at intervals. They were brought to a standard volume and assayed in duplicate. ^{125}I was counted in a Packard model 5986 multichannel analyzer. Samples containing ^{3}H or ^{14}C were measured in a Beckman LS-230 counter before and after internal standardization. Total-body radiation was measured in an instrument described elsewhere [16].

3. RESULTS AND DISCUSSION

3.1. *General*

6 h after the i.p. administration of [^{3}H]fucose to a rat, 8% of the plasma radioactivity was still in a dialyzable form. Over 80% of the tracer that had been incorporated in plasma glycoproteins was bound by lentil-Sepharose, and a high proportion (70–80%) of the bound fraction represented fucosylated rTf.

3.2. *Observations with rTf*

Two rats received rTf biosynthetically labelled with [^{3}H]fucose and another two fucose-containing (i.e. lentil-binding) rTf labelled with ^{125}I. Data from one of these paired experiments are summarized in fig.1, and they show that the labels representing the polypeptide chain and the attached fucose were being eliminated, within experimental error, at identical rates. The finding was the same in the other pair of rats.

In another set of studies, fucose-free and fucose-containing rTf preparations were labelled with ^{125}I for separate injections in two rats each. 1 h before administering the dose as well as 24 h later, the animals were given 0.75 mg Fe/100 g body wt as the nitrilotriacetate complex. (This treatment saturates circulating rTf with Fe for hours and thereby promotes transcellular movement of the protein [17].) Plasma samples (0.1 ml) obtained 24 and 48 h after injecting the labelled proteins were chromatographed on lentil-Sepharose in order to determine whether there were any time-dependent changes in the affinity behaviour of rTf still in the circulation. No portion of the fucose-free rTf acquired any affinity for the lectin during the observation period. However, the proportion of molecules lacking affinity for lentil in the fucose-containing preparation increased from 0.86% in the dose solution to 1.55% after 24 h and to 2.51% after 48 h.

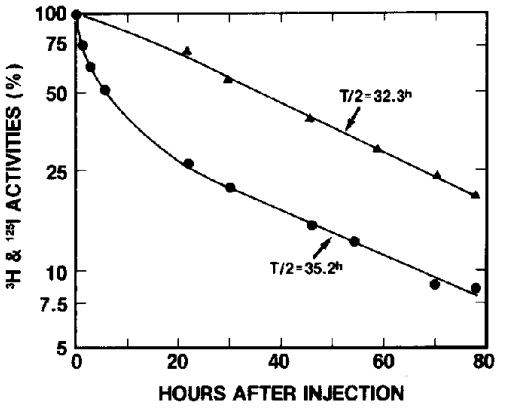

Fig.1. Comparison of the catabolic rates of the fucose and polypeptide moieties of rTf in two rats. One rat, denoted by circles, received at 0 h biosynthetically labelled [^{3}H]fucose-rTf (4.4×10^{5} dpm), and the other, denoted by triangles, lentil-positive rTf labelled with ^{125}I (approx. 60 μCi). The upper curve represents ^{125}I total-body radiation whereas the lower one concentrations of ^{3}H activity per ml of plasma. Half-lives were calculated by semilogarithmic regression from the single-exponential sections of the curves.

These findings suggest that the fucose in rTf, unlike in hepatic plasma membrane glycoproteins, is basically a steady constituent: The fucose-free form of the glycan does not acquire any fucose post-secretionally, and only a minor portion of rTf that possesses fucose initially loses it subsequently. A loss of $<2\%$ in 48 h seems biologically insignificant when the metabolic half-life of the whole glycoprotein molecule is taken into account. The intermediate position of rTf with respect to fucose content between human (not fucosylated [18]) and porcine (fully fucosylated [18]) transferrins is therefore biosynthetic in its origin.

Interpretation of the data in fig.1 would suffer if [^{3}H]fucose hydrolyzed from the injected rTf were reutilized to a large extent during de novo synthesis of rTf. However, in the light of cellular fucose metabolism [19], this seems unlikely. An alternative source of error, namely denaturation of rTf on iodination [14], would have been detectable from the shape of total-body radiation curves [20].

3.3. *Observations with fucosylated human asialotransferrin*

One conceivable explanation of the unequal stability of fucose in rTf and plasmalemma glycoproteins could be a difference in attachment sites, considering that fucose in the chitobiose core, as in rTf, is much less accessible to fucosidase than that in the antennae [21]. To explore this, the elimination of [^{14}C]fucose linked α(1-3) to GlcNAc residues of the antennae of human asialotransferrin was investigated in three pairs of pigeons comparatively to the degradation of the peptide moiety. (Avian species lack a hepatic recognition system for asialoglycoproteins [22].) No significant difference was found between the disappearance rates of the sugar and protein labels (fig.2).

The present studies show that the unique behaviour of fucose in hepatic membrane glycoproteins, outlined in section 1 cannot be simulated by rTf. Co-ordinate turnover of the carbohydrate and peptidyl portions was also reported for β-glucuronidase and β-galactosidase in mouse peritoneal macrophages [23]. It is unclear at present whether the stable character of rTf fucose is due to a difference in the subcellular routing of rTf or to the number of transcellular cycles rTf accomplishes by comparison to the structures having short-lived terminal sugars. Finding the answer may cast new light on intricate details of the intracellular handling of glycoproteins in general.

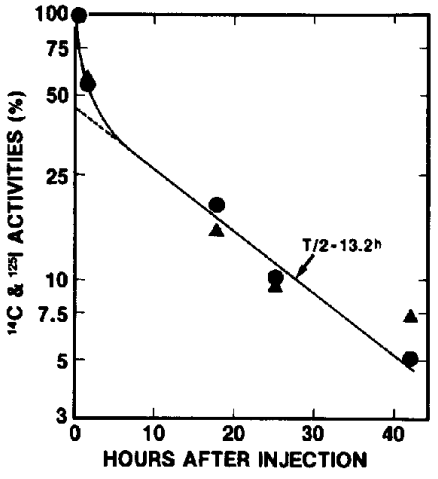

Fig.2. Comparison of the catabolic rates of the fucose and polypeptide moieties of human asialotransferrin in two pigeons. One pigeon, denoted by circles, received at 0 h ^{125}I-labelled fucosylated human asialotransferrin (16 μCi). The other pigeon, denoted by triangles, was given the same protein, not iodinated but traced by its [^{14}C]fucose content (1.56×10^5 dpm). Points represent concentrations of ^{14}C and protein-bound ^{125}I per ml of plasma, expressed as percentages of the first samples obtained 5 min after injection. The initially multiexponential appearance of the curve reflects equilibration of the dose with the extravascular space [20]. The slope and half-life refer to the experiment with ^{125}I.

ACKNOWLEDGEMENT

This work was supported by the Medical Research Council of Canada.

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