Heterogeneous turnover of terminal and core sugars within the carbohydrate chain of dipeptidylaminopeptidase IV isolated from rat liver plasma membrane

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Dipeptidylaminopeptidase IV, a plasma membrane-bound glycoprotein, is characterized by an intramolecular heterogeneous turnover of the protein backbone and carbohydrate chain. The faster turnover of the latter is restricted only to the outer sugars. The inner core sugars D-mannose and *N*-acetyl-D-glucosamine turn over at the same rate as the protein backbone.

Plasma membrane *Glycoprotein* *Dipeptidylaminopeptidase IV*
Intramolecular heterogeneous turnover *D-Mannose* *D-Glucosamine*

1. INTRODUCTION

In a previous publication on the turnover of a glycoprotein, it was shown that the half-lives of L-fucose, D-galactose and *N*-acetylneuraminic acid (12.5, 20 and 33 h, respectively) are much shorter than the half-life of the protein backbone (70–78 h); thus, the terminal carbohydrates turn over several times during the lifespan of the glycoprotein [1]. The glycoprotein isolated from rat liver plasma membrane was identified as the monomer of dipeptidylaminopeptidase IV (DPP IV, EC 3.4.14.5) [2]. The question arose as to whether the core sugars D-mannose and *N*-acetyl-D-glucosamine, in *N*-glycosidically-linked carbohydrate chains [3], turn over at the same rate as the peripheral sugars or as the protein. Our results show that the half-lives of D-mannose and *N*-acetyl-D-glucosamine are in the same range as that of the polypeptide backbone. It is concluded that the rapid turnover within this membrane glycoprotein is restricted to the peripheral carbohydrate residues.

2. MATERIALS AND METHODS

All chemicals were of analytical grade and were purchased from Roth (Karlsruhe) and Merck (Darmstadt). Isotopes were obtained from the Radiochemical Centre (Amersham). Male Wistar rats, 180–200 g, had free access to water and commercial diet (Altromin R, Lage-Lippe) containing 19% protein. Plasma membranes were isolated as in [4] with some modifications [5] and checked for purity as in [6]. Isolation of DPP IV and determination of the respective half-lives were performed as described [1]. After hydrolysis of the glycoprotein in 1 M HCl for 1 h at 100°C, followed by removal of the HCl, neutral sugars were separated on Whatman paper no.3 by descending chromatography. Radioactivity on paper strips was measured in a liquid scintillation spectrometer using a toluene scintillator.

3. RESULTS AND DISCUSSION

After labelling with [^{35}S]methionine and

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[^{14}C]guanidinoarginine the apparent half-life of the protein moiety of DPP IV was 70–78 h [1]. Since in such experiments [8] a reutilization of the label cannot be completely excluded, the true half-life of the protein part might be shorter. Using [^{14}C]carbonate as the label for DPP IV, a half-life of 40 h was measured (fig.1). Since about 15% of radioactivity is incorporated into carbohydrates (e.g., D-galactose) which have half-lives shorter than 40 h [1], the real half-life of the polypeptide must be longer than 40 h and possibly shorter than 70 h. After labelling with [^{14}C]-D-mannose, about 90% of the protein-bound radioactivity was identified as D-mannose and about 10% as D-galactose. In these experiments the half-life of the label was 58 h, being in the same range as the half-life of the protein part. After injection of [^{3}H]-D-glucosamine, radioactivity is incorporated into protein-bound *N*-acetyl-D-glucosamine and *N*-acetylneuraminic acid and to a lesser extent into *N*-acetyl-D-galactosamine [9]. In the experiments of DPP IV performed to measure the half-life of *N*-acetyl-D-glucosamine, the pool of CMP-*N*-acetylneuraminic acid has been increased by i.p. injection of 50 mg *N*-acetyl-D-mannosamine/100 g body wt 2 h before administration of [^{14}C]-D-glucosamine, thus diluting the pool of labelled CMP-*N*-acetylneuraminic acid [10]. A chase of 50 mg D-glucosamine/100 g body wt was given after 2 additional hours. At 12 h and at each subsequent 12-h interval, a further dose of D-glucosamine and *N*-acetyl-D-mannosamine was injected. In these experiments we obtained a mean half-life of 43 h (fig.1), lying between the half-lives of D-mannose (58 h) and the outer core sugars L-fucose (12 h), D-galactose (20 h) and *N*-acetyl-

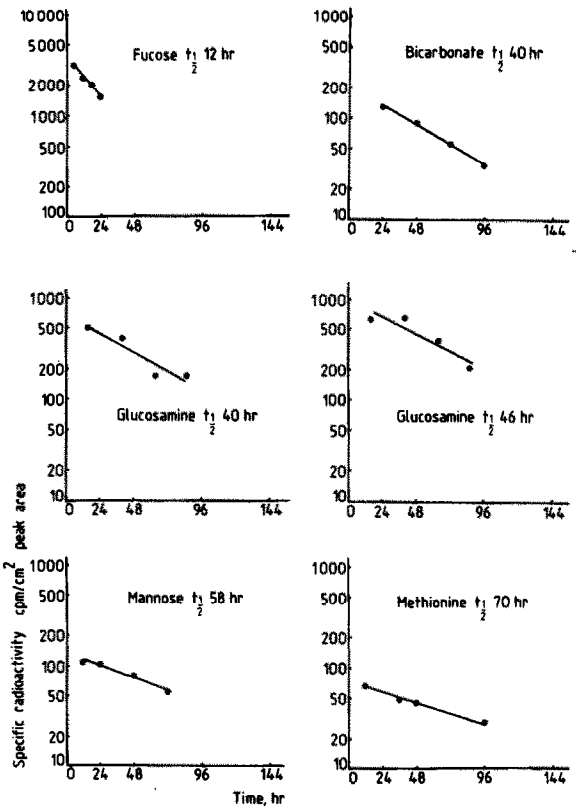

Fig.1. For the half-life determination of protein, 0.5 mCi of [^{35}S]-L-methionine (1040 Ci/mmol, 1 Ci = 3.7 × 10^{10} Becquerel)/100 g body wt was injected, followed by an injection with unlabelled L-methionine (10 mg) 2 h later, and then again at 12-h intervals. The animals were killed 12, 36, 48, 96 and 144 h after administration of the label as described in section 2. The correlation coefficient r was −0.99. Half-life of protein-bound L-fucose: Rats were injected i.p. with 0.55 [6-^{3}H]-L-fucose (15 mCi/mmol)/100 g body wt, followed by a chase of 50 mg unlabelled L-fucose/100 g body wt 2 and 12 h later, then at subsequent 12-h intervals. Animals were killed 6, 12, 18 and 24 h after the administration of label. The correlation coefficient r was −0.99. The injection of 0.14 mCi [1-^{14}C]-D-mannose (40 mCi/mmol)/100 g body wt was followed by a chase of 50 mg unlabelled D-mannose/100 g body wt, 2 and 12 h later, and then at subsequent 12-h intervals. Livers were removed 12, 24, 48 and 72 h after administration of the label. The correlation coefficient r was −0.985. For determination of the half-life of *N*-acetyl-D-glucosamine, rats were injected i.p. with 0.3 mCi [6-^{3}H]-D-glucosamine (28 mCi/mmol)/100 g body wt, followed 2 h later by a chase of unlabelled glucosamine (50 mg/100 g body wt). At 12-h intervals after injection of the label, unlabelled D-glucosamine and *N*-acetyl-D-mannosamine (50 mg/100 g body wt each) were injected; 2 h before the label was given 50 mg *N*-acetyl-D-mannosamine/100 g body wt were injected i.p. Livers were removed at 15, 40, 64 and 86 h after administration of the label. The half-life values of D-glucosamine were obtained from two separate experiments: (a) $t_{½}$ of 40 h (r = −0.94); (b) $t_{½}$ of 46 h (r = −0.92); 2.9 mCi $Na^{14}CO_3$ (20 mCi/mmol)/100 g body wt was injected i.p. and animals were killed 24, 48, 72 and 96 h later. The correlation coefficient r was −0.99.

neuraminic acid (33 h) (fig.1) [1]. Assuming that the carbohydrate units of DPP IV are complex *N*-glycosidic [3], a superposition of different half-lives of the inner core *N*-acetyl-D-glucosamine and the *N*-acetyl-D-glucosamine at the outer branches explains this intermediate half-life. Moreover, the *N*-acetylneuraminic acid of DPP IV may also be labelled, but to a relatively small degree due to the dilution of label in the pool of CMP-*N*-acetyl-neuraminic acid. From these data we conclude that in DPP IV terminal sugars turn over several times during the life span of this plasma membrane glycoprotein, whereas the core sugars behave more like the polypeptide chain. Such a turnover process could reflect a regulatory cycle for DPP IV. Alternatively, it could occur during recycling of this glycoprotein, as has been shown for some membrane receptor glycoproteins [11–14]: in the lysosomal compartment terminal carbohydrates may become more accessible to glycosidases; prior to reinsertion into the plasma membrane the glycoprotein is fully reglycosylated in the Golgi complex. This intramolecular heterogeneous turnover of the carbohydrates and the protein moiety has now been demonstrated for 5 other glycoproteins of liver plasma membrane [15]. A coordinated turnover of bound-D-mannose and the protein part has been found in two lysosomal enzymes of macrophages [16].

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