DEMONSTRATION AND PARTIAL CHARACTERIZATION OF ENDO-*N*-ACETYL-β-D-GLUCOSAMINIDASE IN HUMAN TISSUES

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1. Introduction

It has been postulated that the biological degradation of the oligosaccharide part of the serum type glycoproteins would be initiated by the splitting of the di-*N*-acetyl chitobiose moiety by an endo-*N*-acetyl-β-D-glucosaminidase (EC 3.2.1.96 endoglucosaminidase) [1,2]. This hypothesis is sustained by the finding of oligosaccharide chains of various lengths and compositions in urine and tissues of patients, suffering from lysosomal storage disorders as in sialidosis [3]. These oligosaccharides share a single *N*-acetylglucosamine residue at the reducing terminus. At the other end of the chain the sugar is present for which the hydrolysis is blocked due to the enzyme deficiency.

Endoglucosaminidase activity towards oligomannosidic glycans was reported in rat and pig tissues [2]. This finding was confirmed [4], localizing this type of enzymatic activity in the cytosolic fraction of rat liver and kidney, with an oligomannosidic substrate derived from ovalbumin. In the same tissue activity was also shown towards a glycopeptide isolated from asialotransferrin, a substrate of the *N*-acetyl-lactosaminic type [5]. To obtain support for the hypothesis [1,2], it was of interest to see whether endoglucosaminidase activity could be detected in human tissues. Here, the presence of enzyme activity towards oligosaccharides of the oligomannosidic and of the *N*-acetyl-lactosaminic type is demonstrated in human tissues. Furthermore, some properties of the enzyme (pH optimum, influence of thiol reagents and stability) in human kidney are described.

2. Materials and methods

Adult human tissues were homogenized in water (15%, w/v) using a Potter-Elvehjem homogenizer with a tight-fitting pestle at 0°C. The glycoasparagines used as substrates in this study were derived from ovalbumin (GP-IIIA, [6]) and the asialoglycoasparagine from human serotransferrin (ASTF) isolated as in [7]. They were radiolabeled as in [8]. The assay mixtures were as follows: 10 μl homogenate; 10 μl substrate, dissolved in water (200 μM); 10 μl 0.1 M sodium phosphate buffer of an appropriate pH, that contained 0.06% NaN_3 (w/v) and varying concentrations of DTT (0–150 mM).

The liberated GlcNAc-[^{14}C]Asn was separated from undigested substrate by high-voltage paper electrophoresis in a Savant apparatus. Electrophoresis (1 h at 4000 V) was performed in 0.1 M pyridine–acetate buffer (pH 5.4). The paper strip (Whatman paper, 70 × 5 cm) was cut into 1 cm pieces which were immersed in plastic counting vials in 1 ml water. Scintillation fluid (8 ml) was added and the radioactivity was measured in a Berthold BF-8000 liquid scintillation counter.

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Protein was determined following the method in [9], with bovine serum albumin as a standard.

Dithiothreitol (DDT) and 2-acetamido-1-*N*-(4-L-aspartyl)-2-deoxy-β-D-glucopyranosylamine (GlcNAc-Asn) were purchased from Sigma (St Louis MO). Pico-Fluor 30 scintillation fluid was from N.V. Packard Instruments (Brussels). [^{14}C]Acetic anhydride was from the Centre d'Energie Atomique (Gif-sur-Yvette).

3. Results

The results of preliminary experiments with different tissue homogenates indicated that kidney would be a suitable tissue to characterize the endoglucosaminidase activity towards the substrates used here. The pH optimum of the enzymatic activity towards both substrates was 6.5 ± 0.2, as is shown in fig.1. The reaction appeared to be linear for ⩾15 h at that pH value (fig.2). Even after 24 h the values obtained were 65–75% of the theoretical ones. Thiol reagents like dithiothreitol (DDT) activate the enzyme substantially. A concentration of 10 mM gave optimal activation; with higher values the activity decreased (fig.3).

At the optimal conditions for the kidney enzyme we measured the activity in homogenates of liver, kidney, spleen and brain. It appeared that the ratio of

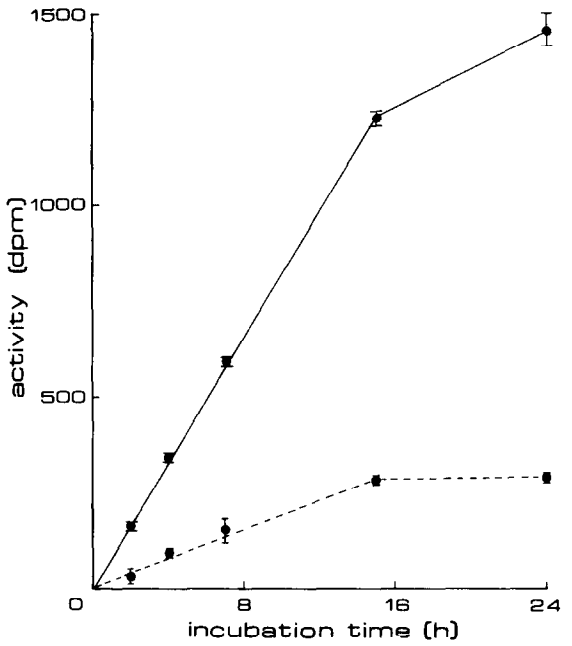

Fig.2. Effect of the incubation time at 37°C on the endoglucosaminidase activity in human kidney homogenate towards the glycoasparagine from ovalbumin (GP-IIIA, ——) and the asialoglycoasparagine from serotransferrin (ASTF, – – –).

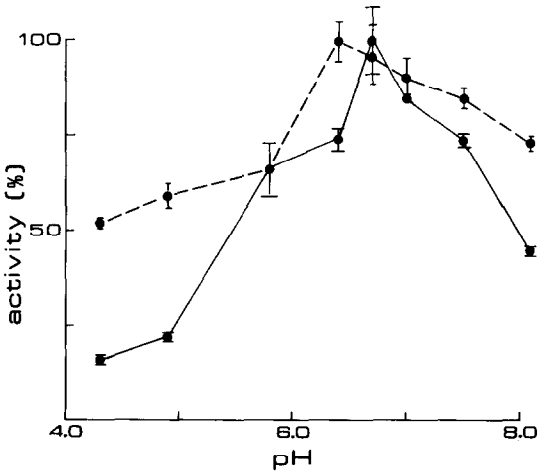

Fig.1. pH dependence of the endoglucosaminidase activity in human kidney homogenate towards the glycoasparagine from ovalbumin (GP-IIIA, ——) and the asialoglycoasparagine from serotransferrin (ASTF, – – –).

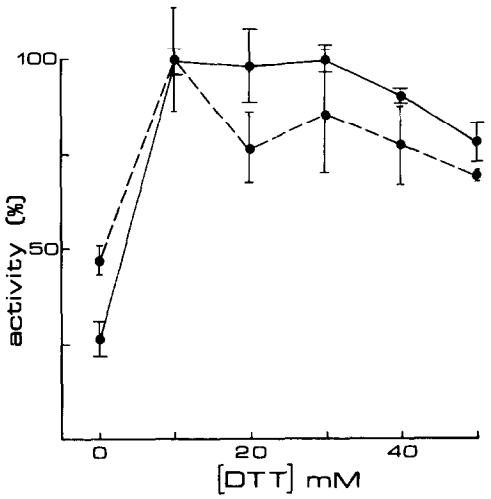

Fig.3. Effect of dithiothreitol (DDT) on the endoglucosaminidase activity in human kidney homogenate towards the glycoasparagine from ovalbumin (GP-IIIA, ——) and the asialoglycoasparagine from serotransferrin (ASTF, – – –).

these activities differed for each individual. In a typical case we found a value for spleen (16.2 pmol GlcNAc-[^{14}C]Asn . h^{-1} . mg $protein^{-1}$) that was higher than the value for kidney (12.4), whereas that of liver and brain were 2.3 and 2.5 pmol . h^{-1} . mg $protein^{-1}$, respectively. With ASTF the following values were obtained: kidney 2.8, spleen 12.9, liver 1.0. The ratios that could be calculated for the activities towards both substrates also varied from one tissue and/or individual to the other.

4. Discussion

The hypothesis that the action of an endo-*N*-acetyl-β-D-glucosaminidase is the first step in the degradation of the oligosaccharide chain of glycoproteins [1,2] required the demonstration of such an enzyme activity in human tissue. The human endo-*N*-acetyl-β-D-glucosaminidase activity found had a similar pH optimum to those described in rat tissues [2,4,5]. Furthermore, the ratios of the two activities (towards GP-IIIA and ASTF) differed in the various tissues of the same individual, indicating the presence of two separate enzyme proteins, as was suggested for the rat [5]. The stabilities of the human enzymes in solution are substantially higher than those found in rat tissues, although the dependence of the activities on the presence of thiol reagents is similar (R. J. P., G. S., J. M., unpublished). No conclusion can be drawn as yet on the subcellular location of the human endo-*N*-acetyl-β-D-glucosaminidase activities. Nevertheless, no lysosomal-like activity was found with an acidic pH optimum.

In [10] endoglucosaminidase activity in human liver was reported. That finding was based upon the fact that a human liver enzyme preparation, containing α-fucosidase and β-hexosaminidase, liberated an oligosaccharide chain from human parotid saliva glycoproteins. The carbohydrate composition and the presence of *N*-acetylglucosamine at the reducing terminus of this chain could be explained by the action of an endoglucosaminidase. However, prior to the incubation with the above enzyme preparation from human liver, the authors employed β-galactosidase and β-hexosaminidase from Jack Bean meal for the partial digestion of their glycopeptide substrate. Jack Bean meal also contains endoglucosaminidase activity (Bouquelet, S., personal communication). In addition, the report [10] failed to demonstrate whether the presence of *N*-acetylglucosamine at the reducing terminus of the oligosaccharide was due to an aspartamidohydrolase activity, also present in human liver [11], the activity of which was favoured by the reaction conditions used (pH 5.0, 4 days incubation).

The hypothesis in [1,2] demands the prior activity of an endo-*N*-acetyl-β-D-glucosaminidase to exoglycosidase digestion of the oligosaccharide chains of glycoproteins. Since, however, the only activity as yet described in rat and human tissue has the characteristics of a soluble fraction enzyme, it may be that the activity expressed in patients with congenital deficiencies in lysosomal hydrolases is induced in such cases and does not represent a normal metabolic pathway (Strecker, G., personal communication).

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