VARIATION OF β-N-ACETYLHEXOSAMINIDASE-PATTERN IN TAY-SACHS DISEASE

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1. Introduction

Tay-Sachs disease is characterized by an accumulation of two types of glycosphingolipids, on the one hand ganglioside G_{M_2} (GalNAc-β-1,4-(NeuNAc-β-2,3)-Gal-β-1,4-Glc-β-1,1-(2-N-acyl) sphingosine), and on the other its asialo residue (GalNAc-β-1,4-Gal-β-1,4-Glc-β-1,1-(2-N-acyl)sphingosine) in nerve tissue [1–3]. The disease is assumed to involve a lack of the enzyme which catabolizes the stored substances, similar to that present in other sphingolipidoses [4,5]. In a special case of Tay-Sachs disease with visceral storage of kidney globoside (GalNAc-β-1,3-Gal-β-1,4-Gal-β-1,4-Glc-β-1,1 (2-N-acyl)sphingosine) a general lack of β-N-acetylhexosaminidase activity was found [6] (fig. 1d). The stored glycosphingolipids (ganglioside G_{M_2} and its asialo residue in nerve tissue, and kidney globoside in the visceral organs) in this case had in common a terminal β-glycosidic-bound N-acetylgalactosamine, being partially hydrolized by β-N-acetylhexosaminidase-preparations [6,7]. The same substances also accumulate in the various tissues of conventional Tay-Sachs cases, although the storage level of the asialo residue of ganglioside G_{M_2} and of the kidney globoside is found to be lower [6]. Accordingly, the lack of some particular β-N-acetylhexosaminidase was discussed as one of the possible causes for storage in cases of conventional Tay-Sachs disease [6]. In the present study the β-N-acetylhexosaminidase pattern of 4 cases of conventional Tay-Sachs disease is described. In three of the four cases a lack of the β-N-acetyl hexosaminidase fraction with an isoelectric point at pH 5 was found. Okada and O'Brien* have obtained similar results with basically different methods.

Abbreviations used: Gal, D-galactose; GalNAc, N-acetyl-D-galactosamine; Glc, D-glucose; NeuNAc, N-acetylneuraminic acid.

2. Materials and methods

2.1. *Materials*

Postmortal tissue of children 2 to 3 years of age was frozen several hours after death and stored at −20° until examination took place. The diagnosis of all the conventional Tay-Sachs cases including the variant without any demonstrable β-N-acetylhexosaminidase deficiency, was confirmed by the level of storage of glycosphingolipids in nerve tissue (quantitative evaluation according to ref. [8]). Ganglioside G_{M_2} constituted 12–18% of the total, whereas its asialo residue made up 3–6% of the total lipid extract. The special Tay-Sachs case where visceral storage of kidney globoside was found has already been described elsewhere [6].

2.2. *Preparation of the β-N-acetylhexosaminidases and their separation by isoelectric focusing*

The β-N-acetylhexosaminidases were prepared from 50 g brain tissue according to Frohwein and Gatt [9] and fractionated by isoelectric focusing using the method of Vesterberg and Svensson [13], as described earlier [10]. Convection-free electrolysis of a mixture of carrier ampholytes (LKB-Producter AB, Stockholm-Bromma, Sweden) in an appropriate electrolysis column

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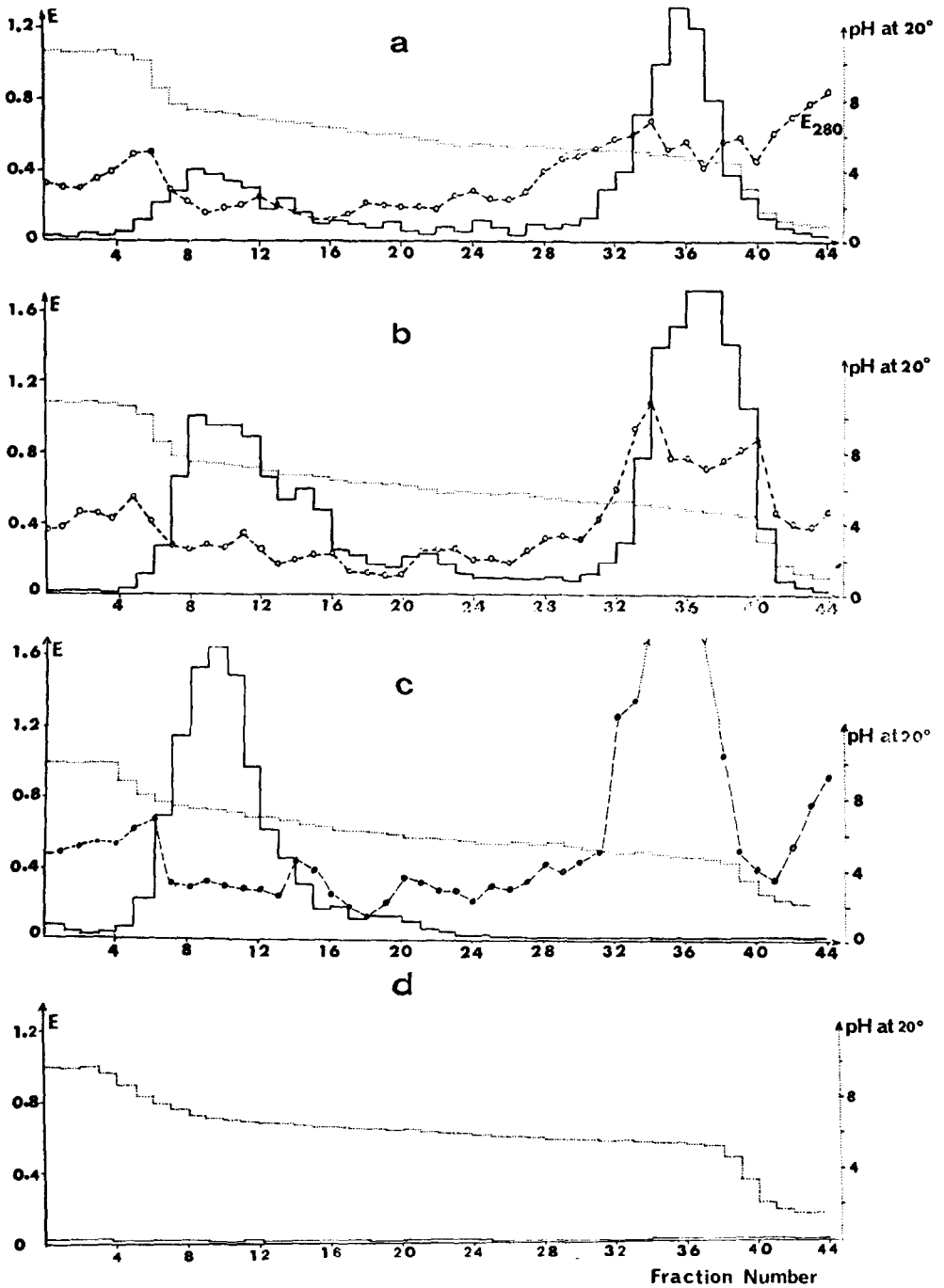

Fig. 1. Variation of β-N-acetylhexosaminidase pattern in the brain tissue of cases with Tay-Sachs disease. a) Normal human pattern; b) Unaltered pattern in a conventional Tay-Sachs case; c) Partially defective pattern in a conventional Tay-Sachs case; d) Defective pattern in the special Tay-Sachs case with visceral storage of kidney globoside [6]. Curves were obtained by isoelectric focusing as described in section 2. —— β-N-Acetylhexosaminidase activity measured at 410 nm; -o-o-o-o-o-o Extinction at 280 nm; . . . . . pH values.

gives a stable pH gradient, in which the multiple β-N-acetylhexosaminidases can be focused at their respective isoelectric points [14]. A mixture containing 50 μl of each fraction taken from the electrolysis column, 1 μMol *p*-nitrophenyl-N-acetyl-β-D-glucosaminide and 950 μl 0.1 m citrate-phosphate buffer (pH 4.5; 10^{-3} M EDTA) was incubated for 30 min at a temperature of 37°. The incubated mixture was then processed according to Findlay et al. [11], whereby the amount of the liberated *p*-nitrophenol was determined at a wavelength of 410 nm.

3. Results and discussion

Fig. 1a demonstrates the normal human brain pattern of β-N-acetylhexosaminidases of children 2 to 3 years of age. The two main enzyme fractions have their isoelectric points at pH 7.3 and 5, respectively [10]. The analogous β-N-acetylhexosaminidase pattern of the special case of Tay-Sachs disease with visceral storage of kidney globoside is shown in fig. 1d. A total lack of both β-N-acetylhexosaminidase fractions was found. Fig. 1c shows a partial lack of β-N-acetylhexosaminidase activity in brain tissue from a case of conventional Tay-Sachs disease. The β-N-acetylhexosaminidase fraction with its isoelectric point at pH 5 is completely absent. Rather than the expected enzyme fraction, a very large amount of enzymatic inactive material was found (fig. 1c). This enzyme defect, however, could not be established in brain tissue of a further case of conventional Tay-Sachs disease (fig. 1b). Here, the pattern of β-N-acetylhexosaminidases appeared to be normal qualitatively, though not quantitatively. All remaining enzyme fractions of these two conventional Tay-Sachs cases showed β-N-acetylglucosaminidase as well as β-N-acetylgalactosaminidase activity and hydrolyzed the asialo residue of ganglioside G_{M_2}.

More recently, the occurrence of two additional cases of conventional Tay-Sachs disease enabled us to confirm the lack of the β-N-acetylhexosaminidase fraction with an isoelectric point at pH 5 in liver and brain, similar to the case presented in fig. 1c. The enzyme fraction with an isoelectric point at pH 7.3 showed a 3- to 4-fold increase over normal activity in brain tissue of all the conventional Tay-Sachs cases hitherto studied (fig. 1b,c and both additional cases).

Since the deficiency of the enzyme fraction with an isoelectric point at pH 5 does not appear in one of the cases of conventional Tay-Sachs disease cited above (fig. 1b), the relationship between this enzyme defect and the storage of glycosphingolipids still remains an open question. According to an investigation of purified enzyme preparations as yet unpublished, both β-N-acetylhexosaminidase fractions have a very similar substrate specificity. A possible genetic connection might be suspected, since both enzyme fractions were lacking in the tissues of the particular case of Tay-Sachs disease with visceral storage of kidney globoside. Both enzyme fractions have been localized in the lysosomes by studies performed with human spleen tissue [12]. Further studies will have to elucidate to which extent the two fractions of β-N-acetylhexosaminidase – despite of their apparent similarities – have separate physiological functions.

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