CHARACTERIZATION OF β-D-N-ACETYLHEXOSAMINIDASES C AND S IN FIBROPLASTS FROM CONTROL INDIVIDUALS AND PATIENTS WITH TAY-SACHS DISEASE

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1. Introduction

Among the different isoenzymes of β-D-N-acetyl-hexoaminidase that occur in human tissues most attention has been paid to the major forms called Hex A and Hex B. A deficiency of Hex A was shown to be responsible for the metabolic defect in GM_2-gangliosidosis type B (Tay-Sachs disease) [1]. In Sandhoff-Jatzkewitz disease, another form of this lysosomal storage disease, both Hex A and Hex B are deficient [2]. It has been suggested that the simultaneous absence of Hex A $(\alpha\beta)_n$ and Hex B $(\beta\beta)_n$ activity is caused by a defect in the common β-subunit [3]. A third isoenzyme called Hex C was first described by Hooghwinkel et al [4]. In contrast to Hex A and Hex B it failed to hydrolyse 4-methylumbelliferyl-β-D-galactosaminide. Subsequent studies revealed other differences between Hex C and the lysosomal isoenzymes Hex A and Hex B such as a neutral pH-optimum, extralysosomal localization and different immunological properties [5–9]. Recently another isoenzyme of β-hexosaminidase has been characterized and was called Hex S [10–12]. It is the major component of β-hexosaminidase activity in Sandhoff-Jatzkewitz disease and is absent in tissues from patients with Tay-Sachs disease. At acid pH Hex S exhibits activity towards 4-methylbelliferyl-β-D-glucosaminide and 4-methylumbelliferyl-β-D-galacto-saminide. From immunological and biochemical studies it was concluded that Hex S shares the α-subunit with Hex A but not the β-subunit [11–13].

It has long been a question whether there is a structural relationship between Hex C and Hex A.

Ropers et al. [14] suggested a common subunit structure for Hex A and Hex C. This was based on studies that claimed a deficiency of Hex C in tissues from patients with Tay-Sachs disease [4,14]. The isoenzyme pattern of hexosaminidase in man–rodent hybrids seemed to support this hypothesis [15,16]. Other reports however described the presence of Hex C in Tay-Sachs disease [5–9]. To find a reason for this discrepancy we have investigated the electrophoretic pattern of β-hexosaminidase in fibroblasts from control subjects and patients with Tay-Sachs disease using different staining conditions. With reference to the recent characterization of Hex S special attention was given to the distinction of Hex C and Hex S.

Our findings suggest that there is no evidence for a structural relationship between Hex C and Hex A, and the results rather favor a different interpretation of the studies with man–rodent hybrids.

2. Materials and methods

Skin fibroblasts from three different control individuals and six unrelated patients with Tay-Sachs disease were cultured in Falcon Flasks in Ham's F10 medium supplemented with 15% fetal calf serum and antibiotics as described before [17]. Homogenates of 3×10^6 cells per 100 µl were prepared by sonication in distilled water. Electrophoresis was performed on cellulose acetate gels (Cellogel, Chemetron, Italy) in 50 mM potassium phosphate, pH 6.8, at 4°C, for 90 min at 200 V. 10 µl Cell homogenate was used per

lane and the gels were stained with either 4-methylum-belliferyl-2-acetamido-2-deoxy-β-D-galactopyranoside (MU-galactosaminide 1.5 mM) or 4-methylumbelli-feryl-2-acetamido-2-deoxy-β-D-glucopyranoside (MU-glucosaminide 1.5 mM) in phosphate (0.2 M) citrate (0.1 M) buffer, pH 4.0 or pH 7.0 (1 h 37°C). Photographs were taken after exposure of the gels to ammonia vapor. Preparation of antiserum against purified human liver Hex A has previously been described [18]. 10 μl cell homogenate was incubated overnight at 4°C with 10 μl antiserum. The 40 000 X g supernatant was applied to the gels.

3. Results

The isoenzyme pattern of β-hexosaminidase was studied using different staining conditions. When 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopuranoside (MU-glucosaminide) is used as sub-strate, at either pH 4 or pH 7, a band of activity shows up anodal to Hex A both in fibroblasts from control subjects and patients with Tay-Sachs disease (fig.1). We have indicated this band as C/S because activity of Hex S as well as Hex C might be expected at this position using MU-glucosaminide as substrate [11]. At pH 4 this band shows up more strongly in control fibroblasts than in those from the patient. It was further noticed that in fibroblasts from the patient the band exhibits a higher activity at pH 7

than at pH 4. This was presumably because only the neutral isoenzyme Hex C is present in fibroblasts from the patient with Tay-Sachs disease.

When 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-galactopyranoside (MU-galactosaminide) is used as substrate instead of MU-glucosaminide the activity of all isoenzymes is lower, but the most anodal band still shows up in normal fibroblasts both at acid and neutral pH (fig.2). At pH 4 the band is indicated as Hex S, the acid isoenzyme, because it does not show up under these conditions in fibroblasts from the patient. At pH 7 the neutral isoenzyme Hex C contributes to the activity of this band. This is indicated by the weak band of activity at the C/S position at this pH in fibroblasts from patients with Tay-Sachs disease.

To establish that the activity at the C/S position only represents Hex S when the gels are stained at pH 4 with MU-galactosaminide we treated the homogenates with anti-Hex A antiserum before electrophoresis. Figure 3 shows the isoenzyme pattern in fibroblasts from control individuals and from patients with Tay-Sachs disease both with and without antiserum treatment. The band of activity that shows in control fibroblasts at the C/S position using these staining conditions is removed completely by the antiserum. This indicates that the activity was due to Hex S only. The activity that is left at the Hex A position and the origin after antiserum treatment might be caused by unprecipitated immune complexes.
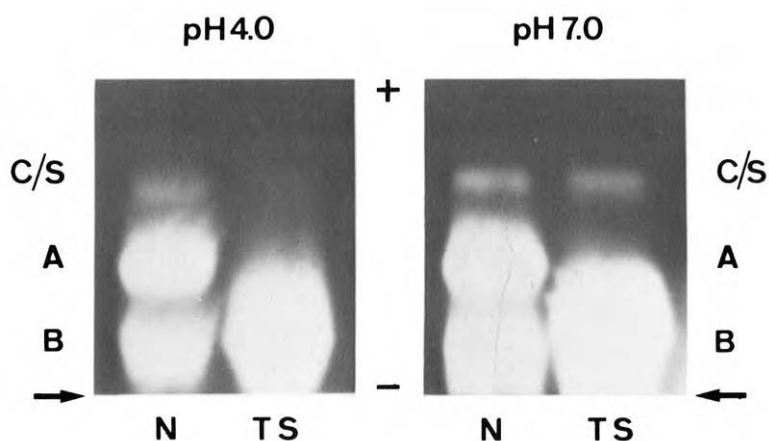

Fig.1. Isoenzyme pattern of β-hexosaminidase in fibroblasts using MU-glucosaminide as substrate. N = control subject. TS = patient with Tay-Sachs disease.
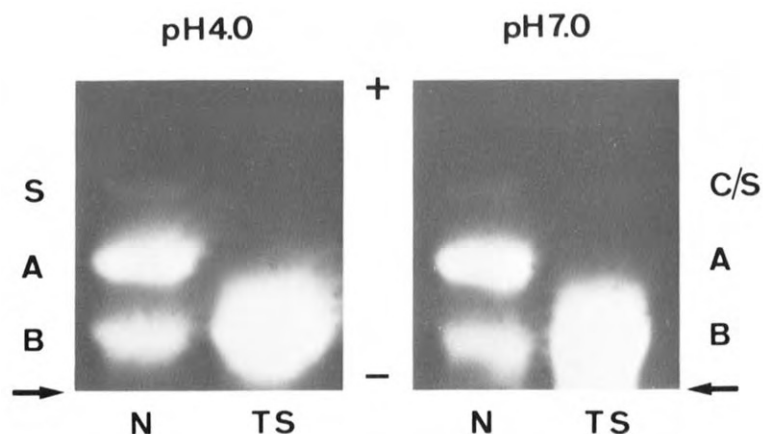
Fig.2. Isoenzyme pattern of β-hexosaminidase in fibroblasts using MU-galactosaminide as substrate. N = control subject. TS = patient with Tay-Sachs disease.

As described above there is no activity at the C/S position in Tay-Sachs fibroblasts under these conditions. Antiserum treatment results in loss of Hex B activity. Hex C can be demonstrated in normal fibroplasts and those from the patient after precipitation of Hex S using MU-glucosaminide at pH 7 (fig.4). With this substrate Hex C activity was also found at pH 4 after antiserum treatment although it was much less.

Three different control fibroblasts strains and six cell strains from unrelated patients with Tay-Sachs disease were investigated in this way and similar patterns were observed. In all three control strains Hex C and Hex S activity could be demonstrated, whereas in none of the fibroblasts strains from the patients was Hex S activity detected. Hex C activity could always be demonstrated.
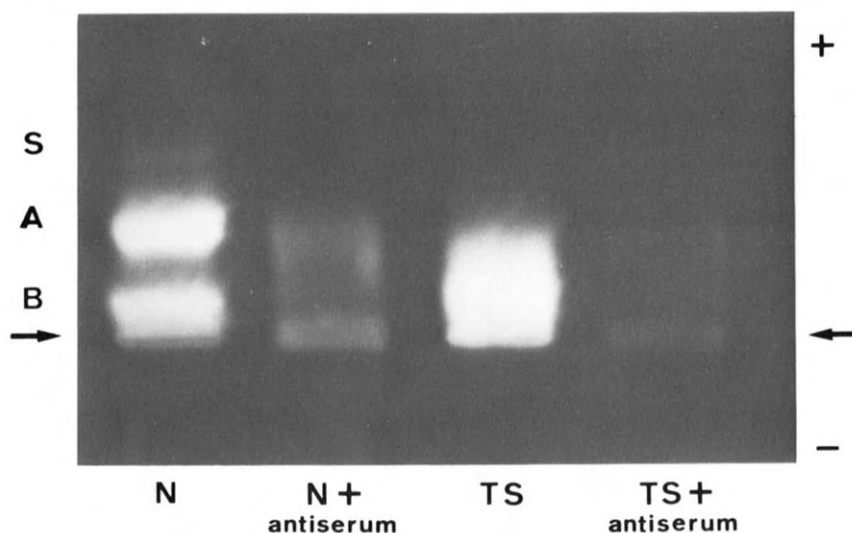

Fig.3. Effect of anti Hex A antiserum on the pattern of β-hexosaminidase in fibroblasts. Gels were stained with MU-galactosaminide at pH 4.0. N = control subject. TS = patient with Tay-Sachs disease.
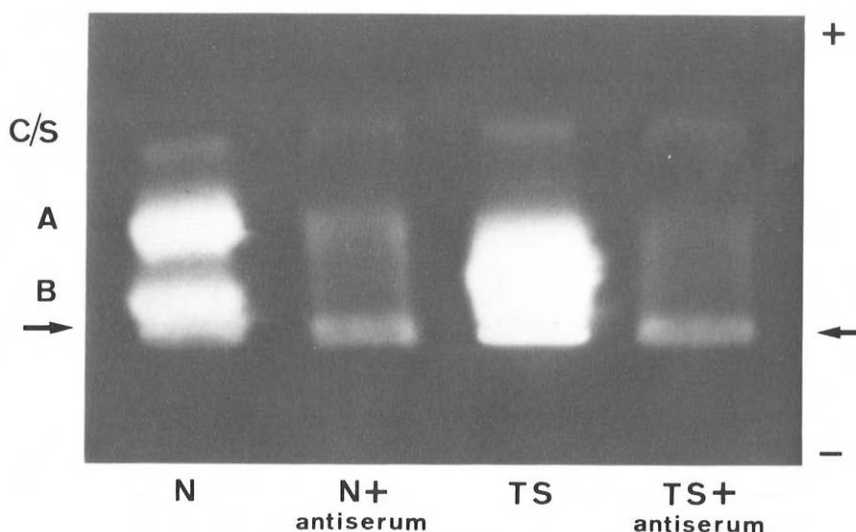
Fig.4. Effect of anti-Hex A antiserum on the pattern of β-hexosaminidase in fibroblasts. Gels were stained with MU-glucosaminide at pH 7.0. N = control subject. TS = patient with Tay-Sachs disease.

4. Discussion

Hex C activity has been demonstrated by several authors in tissues from patients with Tay-Sachs disease [5–9,20]. Others however did not succeed in detecting Hex C in Tay-Sachs disease [4,14,15,16,19]. These conflicting results seem to be explained by the recent characterization of Hex S by Ikonne et al. [11] and Beutler et al. [12] and by the fact that Hex S and Hex C are difficult to seperate in many electrophoretic systems. Also, in the system we used Hex S and Hex C have the same mobility.

Using MU-galactosaminide as substrate at pH 4 conditions were found for the specific staining of Hex S. With MU- glucosaminide no proper distinction could be made between Hex S and Hex C, but Hex C could be demonstrated with this substrate after precipitation of Hex S with anti-Hex A antiserum. At low pH the band of activity at the C/S position might be missed in tissues from patients with Tay-Sachs disease because it only represents the neutral isoenzyme Hex C. In tissues from control subjects Hex S contributes to the activity of this band which makes it easier to detect at low pH.

Regarding this we suggest a different interpretation of results obtained by Van Cong et al. [15,16] with

man–rodent hybrids. An isoenzyme was detected that was not present in either of the parental strains. It was called 'Hex A fast' and was thought to be composed of human α-subunits and rodent β-like subunits. It was demonstrated that the formation of this heteropolymeric molecule was dependent on the presence of Hex C. However, taking into account the substrate and the low pH that was used to stain the gels, it may well be that Hex S was scored instead of Hex C. The results would then have to be interpreted as synteny of Hex S and Hex A. This would be compatible with the proposed $(\alpha\alpha)_n$ subunit structure of Hex S [11,12] and the different immunological and biochemical properties of Hex C [5–9,20] in comparison with the other isoenzymes of β-hexosaminidase.

We have recently identified a hexosaminidase isoenzyme in man–chinese hamster hybrids as a heteropolymeric molecule [18]. It has the immunological properties of the α-subunit of human Hex A and chinese hamster hexosaminidase and it segregates with markers of human chromosome 15. One therefore might expect that Hex S is localized on this chromosome unless Hex S has subunits different from α. The present data suggest another locus for Hex C, but this has not been confirmed by gene localization studies. The method described in this

paper and the procedures described by Swallow et al. [20] to discriminate between Hex S and Hex C might facilitate these studies.

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