STUDY OF HEXOSAMINIDASE ISOZYME RECONSTRUCTION DURING HYBRIDIZATION OF FIBROBLASTS FROM PATIENTS WITH TAY-SACHS' AND SANDHOFF'S DISEASES

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Development of the G_{M_2}-gangliosidoses, including Tay-Sachs' disease (TSD) and Sandhoff's disease (SD), is known to be the result of gene mutations in the 15th and 5th chromosomes, coding the formation of α- and β-subunits of hexosaminidase (Hex) respectively [2-4, 13].

The diagnosis of G_{M_2}-gangliosidoses at the subunit level can be made by the method of genetic complementation, which has begun to be developed in recent years. The essence of this method as applied to G_{M_2}-gangliosidoses is as follows. Fibroblasts obtained from patients with TSD, with Hex A insufficiency (αβ-heteropolymer) and with the presence of Hex B (ββ-homopolymer) are hybridized by means of different agents (Sendai virus, polyethylene-glycol – PEG, etc.) with fibroblasts from patients with SD, in which there is a deficiency of Hex A and Hex B, but which contain Hex S (αα-homopolymer), which is absent in normal human cells. As a result of this hybridization a heterokaryon is formed, in which genetic complementation takes place, i.e., the formation of normal forms of Hex A and Hex B from active α- and β-subunits coded by genomes of the original cells.

The aim of this investigation was to use the genetic complementation method to study processes of reconstruction of Hex isozymes and also to confirm the biochemical diagnosis of TSD and SD.

EXPERIMENTAL METHOD

Cultures of fibroblasts from healthy human skin (control) and the skin of children with TSD and SD, diagnosed previously in East Germany by determination of enzyme activity in the fibroblasts and leukocytes and results of electrophoretic analysis, were used as the test objects.

Hybridization was carried out by a modified method [8] in the presence of PEG. The PEG concentration during treatment of the cells was reduced gradually from 65 to 15%. The duration of treatment of the cells with PEG was from 2 to 3.5 min. For biochemical investigations the cells were cultured for several days in Eagle's medium with 20% bovine serum. To determine the hybridization index, some of the cells treated with PEG were diluted in the ratio of 1:3 or 1:4 and transferred to culture on coverslips. After culture for 24 h at 37°C the cells were washed to remove culture medium, stained by the Romanovsky–Giemsa method for 4-5 min, dried, and mounted in balsam. The hybridization index was determined as the ratio between the number of nuclei in confluent cells and the total number of nuclei in confluent and single cells, multiplied by 100.

Protein was determined by the methods of Lowry [12] and Bradford [6]. The composition of the Hex isoforms was studied by isoelectric focusing (IEF) in a thin layer of polyacrylamide gel on plates from LKB (Sweden), in a pH gradient 3.5-9.5, according to recommendations

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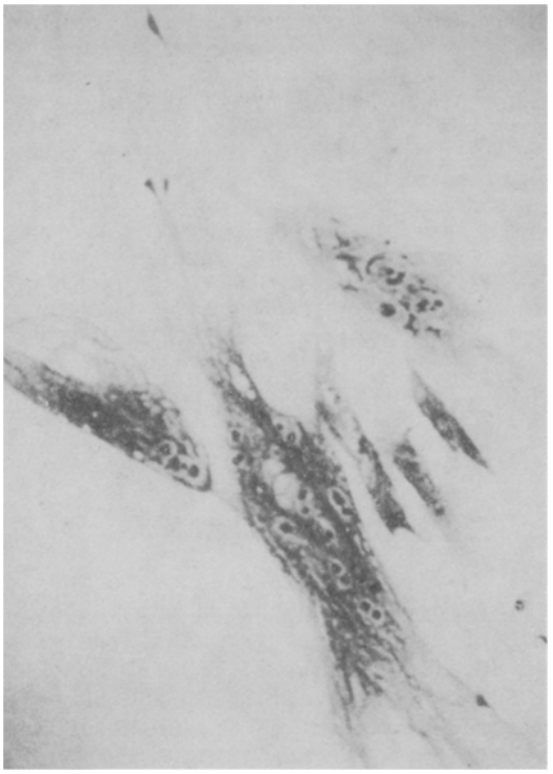

Fig. 1. Mononuclear cells obtained during confluence of human skin fibroblasts (900 ×).

in [14]. Multiple forms of Hex were identified by the method described previously [5], using 4-methylumbelliferyl-2-acetamido-2-deoxy-β-D-glucopyranoside from Koch-Light (England) as the substrate.

EXPERIMENTAL RESULTS

Homokarya of normal fibroblasts, fibroblasts of children with TSD and SD, and heterokarya of fibroblasts of children with TSD and SD were obtained by the hybridization method. The resulting hybrid fibroblasts contained from two to 10 nuclei or more (Fig. 1). During combined culture of cells from individuals with TSD and SD (without preliminary treatment with PEG) the hybridization index ranged from 0.5 to 5.6%.

It will be clear from Fig. 2 that two forms of Hex are present in normal fibroblasts, namely A and B, whereas only one form of the enzyme is found in hybrid TSD/TSD cells, namely Hex B. Neither form of Hex is present in SD/SD hybrid cells, but there is a third form of the enzyme, evidently Hex S, which is absent, as was pointed out above, in normal cells. Hybridization of fibroblasts from children with TSD and SD leads to the formation of Hex A, which is not formed during ordinary combined culture of these cells (Figs. 2 and 3). The fact will be noted that Hex A reconstructed during hybridization is less abundant than normally. Quantities of both forms of Hex close to normal can evidently be obtained only in the case of 100% hybridization with the formation of TSD/SD heterokarya only. According to data in the literature, however, the percentage of formation of heterokarya by fusion of two different types of fibroblasts is low [7]. These results, and also the comparatively low hybridization index (40-50%) in the present experiments can evidently also explain the small quantity of reconstructed Hex A observed on IEF. An increase in the hybridization index to 60-70% in the later experiments led to more abundant manifestation of Hex A, according to IEF data.

The formation of Hex A and Hex B in heterokarya, it will be noted, depends to a certain extent on the duration of culture. For instance, one day after fusion, only very small quantities of both types of enzyme could be found by IEF in the heterokarya. After 5-7 days of culture their content in the heterokarya increased considerably; the ratio between Hex A and Hex B isozymes, according to the IEF data, remained more or less constant, in agree-

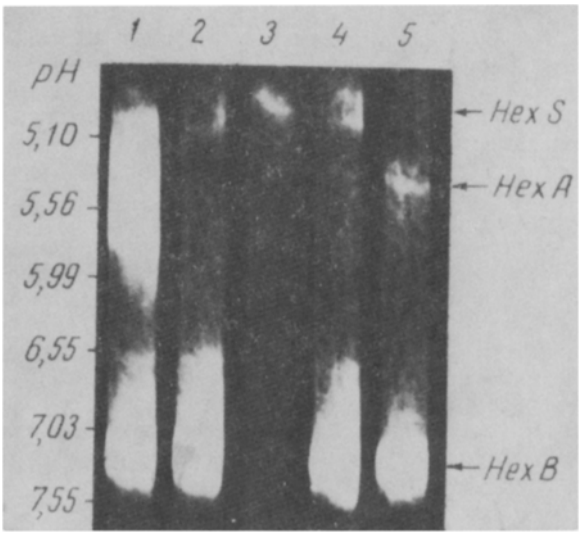

Fig. 2. Isoelectric focusing of Hex of cell homogenates obtained after hybridization of fibroblasts from children with TSD and SD. 1) Normal, 2) TSD/TSD homokarya, 3) SD/SD homokarya, 4) TSD and SD cells cultured together, 5) TSD/SD heterokarya (hybridization index 48%).

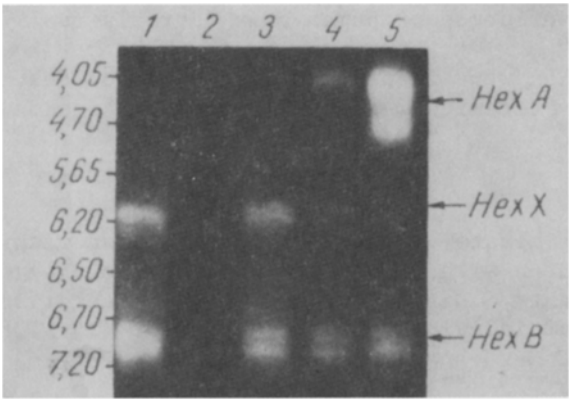

Fig. 3. Isoelectric focusing of Hex of TSD/TSD homokarya (1), SD/SD homokarya (2), TSD and SD cells cultured together (3), TSD/SD heterokarya (4), and normal fibroblasts (5).

ment with data in the literature [9]. The causes of this fact are not yet clear. It can be tentatively suggested that for effective functioning of the genomes in heterokarya, a certain period of time is necessary, after which the content of the reconstructed forms of the enzyme becomes constant. The possibility likewise cannot be ruled out that association of α- and β-subunits in normal fibroblasts and in hybrid TSD/SD cells can take place with different efficiency. It can also be postulated that α- and β-subunits, present in the original fibroblasts of children with SD and TSD respectively, are completely unable to interact with one another in fused cells, and reconstruction of the Hex forms is the result of combined functioning of the genomes in the heterokarya.

It will be clear from Fig. 3 that in some cases an unidentified form of Hex (Hex X), characterized by an intermediate mobility during IEF between that of Hex A and Hex B, was present in fibroblast homogenates. A definite connection could be found between this form of the enzyme and Hex B. For instance, in the absence or the presence of a reduced quantity

of Hex B, Hex X also was absent or present in reduced quantity. These data suggest that Hex X also consists of β-subunits, the number and nature of which differ from those in the Hex B molecule. We know, for example, that the β-chain of Hex A, unlike the β-chain of Hex B, contains an additional sialized peptide [11]. However, the causes of the appearance and the nature of Hex X still remain unexplained.

The results of IEF of Hex from different types of multinuclear cells point to the heterogeneity of both Hex A and Hex B (Fig. 3). Heterogeneity of both forms of Hex also was postulated by the writers on the basis of a study of isoforms of this enzyme in the fetal part of the placenta and various organs of the human fetus [1]. The presence of heterogeneity of Hex B, due to the existence of qualtitative differences between β-subunits, which was mentioned above, also was demonstrated previously for the enzyme from human blood serum and placenta [11]. The observed heterogeneity of Hex A may evidently be the result of different versions of assembly of α- and β-subunits during formation of the enzyme.

To conclude, the method of genetic complementation, which has recently found ever-widening application in the diagnosis of various types of inborn errors of metabolism [10], can be used to confirm the diagnosis of investigated types of G_{M_2}-gangliosidoses and for the further study of their heterogeneity. The genetic complementation method may also evidently be of great importance to the study of reconstruction of enzymes on account of different combinations during subunit assembly, and also the examination of other problems to do with the functioning of enzymes in the cell consisting of subunits, coded by different genetic loci, which have so far received little study.

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