ANTIBODY AGAINST PURIFIED HUMAN HEXOSAMINIDASE B CROSS-REACTING WITH HUMAN HEXOSAMINIDASE A

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Summary

Human hexosaminidase B has been purified to virtually homogeneous state from placenta. An anti-serum has been prepared in rabbits against the purified preparation. The serum reacted equally with human hexosaminidase B (free of hexosaminidase A) and with human hexosaminidase A (free of hexosaminidase B) as shown by immunodiffusion and by precipitation of enzyme activity from solution.

Introduction

Tay-Sachs disease and Sandhoff's disease are glycolipid storage disorders characterized by the accumulation of GM_2 ganglioside. In Tay-Sachs disease, which occurs predominantly in Jewish populations, the electrophoretically rapid, thermolabile hexosaminidase (Hex), designated hexosaminidase A (Hex-A), is absent (1,2, 3,4). In Sandhoff's disease, which occurs in non-Jewish populations, both Hex-A and a thermostable, electrophoretically distinct Hex, designated hexosaminidase B (Hex B), are missing (2,5).

The biochemical and genetic relationship between these two enzymes has heretofore been unclear. Hex-B may lack the capacity to hydrolyze GM_2 ganglioside, since muscle preparations from patients with Tay-Sachs disease were unable to hydrolyze the natural substrate (4). Treatment with neuraminidase has been reported to convert Hex-A into a form with the same electrophoretic mobility

as Hex-B (6,7); it has been suggested that the basic defect in Tay-Sachs disease might be in the conversion of Hex-B to Hex-A, while patients with Sandhoff's disease may lack the capacity to synthesize Hex-B, and therefore have neither enzyme (7,8). Sandhoff (9) has recently reported purification of Hex-A and B from human liver. No data were given regarding either the homogeneity or antigenicity of his preparations.

We now report the purification of human Hex-B to an essentially homogeneous state and the production of an antiserum against this enzyme. We show that the same antibodies react approximately equally with both Hex-A and B.

Material and Methods

The final assay mixture contained 30 mM citrate-phosphate buffer, pH 4.4; 0.5 mM 4-methylumbelliferyl-N-acetyl-β-D-glucosaminide; 0.02% bovine serum albumin; and enzyme in a total volume of 100 µl. After incubation for 10 min. at 37° the reaction was stopped and fluorescence was measured as previously described (10).

The steps used for separation of Hex-A and B and for purification of Hex-B from human placenta are summarized in figure 1. The Sephadex-G-100 fraction was subjected to disc electrophoresis on polyacrylamide at pH 8.3 (11). Virtually all of the enzyme activity was present in an area corresponding to the one major protein band. An extremely faint additional band, probably comprising less than 2% of the total protein, was also observed. Acrylamide electrophoresis could not be repeated on the ECTEOLA unabsorbed fraction, since only about 80 µg protein remained.

The purified Hex-B was concentrated to approximately 0.3 ml and mixed with 0.3 ml Freund's adjuvant. One-half of this material was injected into the foot pad of a Royal Dutch rabbit immedi

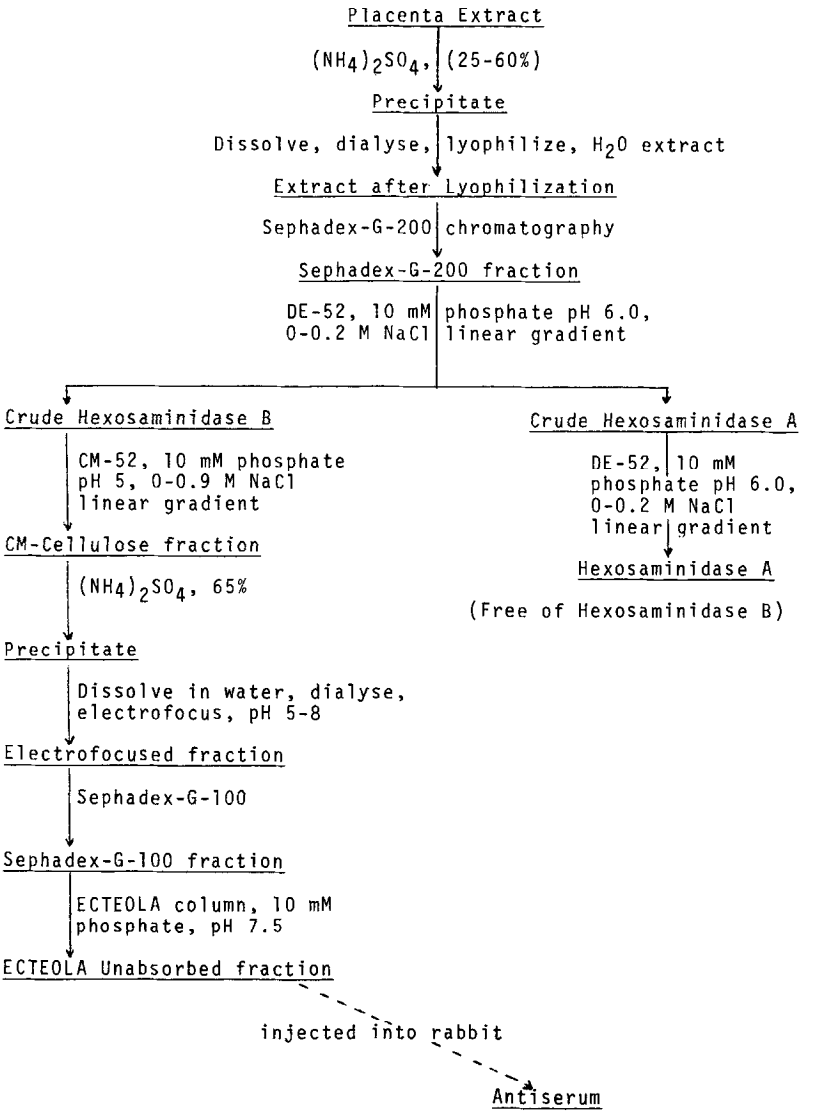

Fig. 1. Purification of Hexosaminidases from human placenta.

ately and the remainder was injected 21 days later. After 15 days the rabbit was bled. The serum was heated for 30 minutes at 56°, centrifuged at 105,000 g for one hour, filtered through a 0.22μ millipore filter and stored under sterile conditions. Control serum was obtained in identical fashion from a rabbit injected with Freund's adjuvant mixed with an equal volume of 0.01 M phosphate buffer, pH 6.0.

Results

Twenty microliter aliquots of Hex-B (CM-cellulose fraction) free of hexosaminidase A activity, and of hexosaminidase A, free of hexosaminidase B activity, were incubated with 10 µl of various dilutions of antiserum in 0.9% NaCl solution, 30 µl water and 40 µl citrate-phosphate buffer, 0.14 M, pH 4.4. The mixture was allowed to stand for 20 minutes at room temperature and for 10 minutes at 4°. Forty microliters of water, 80 µl of citrate-phosphate buffer and 80 µl of 0.1% albumin solution was added and the tubes were centrifuged at 105,000 g for one hour at 4°. The supernatant fluid was assayed for Hex activity. As shown in figure 2, both Hex-A and B were inactivated by the antiserum, while control serum had no effect on either enzyme. In the absence of centrifugation, there was no loss of activity, indicating that the antibody did not interfere with the activity of the enzyme, but merely resulted in its precipitation. The slope of the inactiva-

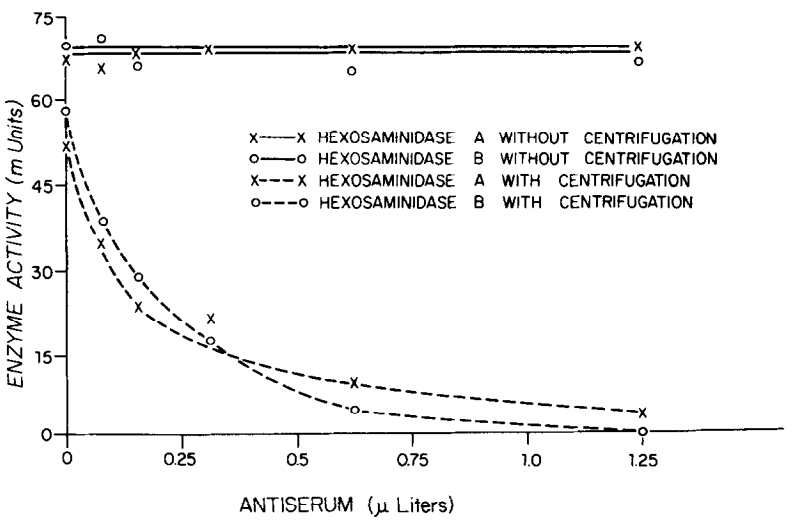

Fig. 2. Precipitation of hexosaminidase A (free of Hex-B) and hexosaminidase B (free of Hex-A) by antiserum. The experimental conditions are described in the text.

tion curve was the same with Hex-A and B: the same number of units of B and of A enzyme were precipitated by a given amount of antiserum. In control studies, partially purified α-galactosidase A and B (12) were treated with antiserum in the same manner, without any loss of activity.

When a sufficient quantity of Hex-A or B was added to diluted antiserum so that approximately one-half of the enzyme was precipitated, the supernatant fluid after centrifugation still maintained the capacity to precipitate a small amount of Hex-A or B activity. The capacity of the supernatant to precipitate either activity was the same regardless of whether original treatment had been with A or B enzyme, indicating that the same group of antibodies was active against both enzymes.

Immunodiffusion studies were carried out by placing undiluted antiserum in the center well of an agar gel plate. Partially purified Hex-A and B, each free of the other enzyme were placed

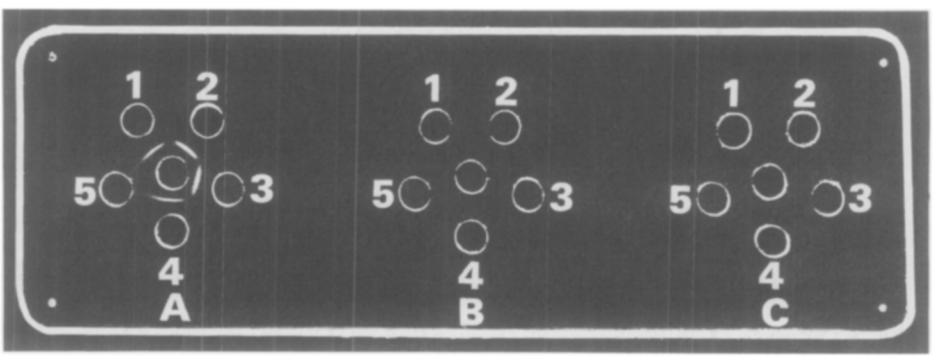

Fig. 3. Immunodiffusion studies using anti-hexosaminidase rabbit serum. The center wells in A and C contain antiserum and in B control serum.

A and B: 1 and 4 = Hex A; 2 = Hex B; 3 = Hex A + Hex B; 5 = empty.

C: 1, 3, and 5 = α-galactosidase A; 2 and 4 = α-galactosidase B.

in the outer wells. Precipitin lines were found both against A and B enzymes, but no lines were found against α-galactosidase A or B or when control serum was placed in the inner well (fig. 3).

Discussion

The injection of essentially homogeneous human Hex-B into a rabbit resulted in formation of antibodies reactive against both Hex-A and Hex-B. The possibility that the injected Hex-B was converted by the rabbit to Hex-A, resulting in the formation of a separate group of anti-Hex-A antibodies was ruled out by antibody absorption tests: treatment of serum with Hex-A resulted in loss of reactivity against Hex-B and vice-versa. The initial slope of the curve relating residual Hex activity to the amount of antiserum added, as shown in figure 2, suggests that the avidity of the antiserum for both forms of Hex is approximately the same. These studies indicate that the two forms of Hex are, indeed, very closely related. Our findings are consistent with the concept that the B enzyme may be the precursor of the A enzyme differing, perhaps, only by virtue of attachment of carbohydrate or other small molecules.

The availability of an antibody against these enzymes will make possible further examination of two alternate hypotheses regarding basis of Hex-A deficiency in Tay-Sachs disease. If Tay-Sachs disease is due to a defect in a structural locus for Hex-A, then an immunologically reactive but catalytically inactive protein might be found in this disease, as is the case, for example, in hemophilia, galactosemia, and glucose-6-P dehydrogenase deficiency. If the defect is in the conversion of Hex-B to A, because of deficiency of a specific sialotransferase, for example, no immunologically reactive catalytically inactive protein corresponding to Hex-A would be expected. In preliminary studies, we

have compared the precipitation of Hex activity from normal human liver homogenate with that of the liver of a patient with Tay-Sachs disease. The slope of the curve was the same in both cases, suggesting that no catalytically inactive but immunologically cross-reacting protein was present in Tay-Sachs disease.

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