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A NEW VARIANT OF TYPE-AB GM2-GANGLIOSIDOSIS

Su-Chen Li, Yoshio Hirabayashi and Yu-Teh Li

Department of Biochemistry and Delta Regional Primate Research Center, Tulane University, New Orleans, Louisiana 70112

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SUMMARY: A patient diagnosed as having Type-AB GM2-gangliosidosis was found to have a defect in β-hexosaminidase A, but not in the activator (GM2-activator) specific for the enzymic hydrolysis of GM2 ganglioside. β-Hexosaminidase A and B isolated from the brain of the patient showed normal activity toward synthetic substrates, but could not hydrolyze GM2 ganglioside in the presence of GM2-activator isolated from normal human liver or brain. The level of GM2-activator in the brain of this patient was three times higher than that found in the two control brains. The activator isolated from the brain of this patient was able to stimulate the hydrolysis of GM2 ganglioside catalyzed by human hepatic or brain β-hexosaminidase A but not by B.

Three biochemically distinct types of GM2-gangliosidoses have been described (1). Type-B (classical Tay-Sachs disease) has been established to be caused by the deficiency of β-hexosaminidase A (2-acetamido-2-deoxy-β-D-glucoside acetamidodeoxyglucohydrolase, EC 3.2.1.30), and Type-0 (Sandhoff's disease), by the deficiency of both β-hexosaminidases A and B. Type-AB (late infantile or juvenile GM2-gangliosidosis) is characterized by the presence of a normal or elevated level of both β-hexosaminidases A and B. Since there is no apparent enzyme deficiency, the accumulation of GM2 ganglioside in the tissues of Type-AB GM2-gangliosidoses cannot be easily explained. Recently, Conzelmann and Sandhoff (2) reported that the heated, crude extract derived from the kidney of a patient with Type-AB GM2-gangliosidosis was deficient in a factor which stimulates the hydrolysis of GM2 ganglioside catalyzed by human hepatic β-hexosaminidase A. Hechtman (3) has also reported the deficiency of a similar activator in the crude liver extract of a patient with Type-AB GM2-gangliosidosis. We herewith report a different variant of Type-AB GM2-gangliosidosis with a defective β-hexosaminidase A.

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EXPERIMENTAL PROCEDURE

Materials - Tritium labeled GM2 ganglioside, human hepatic β-hexosaminidases A and B, GM2-activator from human liver and brain were prepared according to the methods described previously (4).

Pathological Brain - Dr. K. Suzuki kindly supplied the brain tissue from a patient with type-AB GM2-gangliosidosis; this patient (a Puerto Rican boy who died at the age of 4.7 years) was the "Case 1" reported by Goldman et al. (5).

Enzyme Assays - Hydrolysis of GM2 ganglioside by β-hexosaminidases in the presence of GM2-activator or sodium taurodeoxycholate was assayed according to the method described previously (4,6). One unit of GM2-activator is defined as the amount of activator which stimulates the hydrolysis of 1 pmole GM2 ganglioside per h per unit of β-hexosaminidase A under the standard assay condition (4). The specific activity of the GM2-activator is expressed as the units of activator per mg protein. Assays with p-nitrophenyl-β-GlcNAc were carried out as previously described (7). One unit of β-hexosaminidase is defined as the amount of enzyme which hydrolyzes 1 μmole of p-nitrophenyl-β-GlcNAc per min at 37°C. Protein was determined by the method of Lowry et al. (8), using bovine serum albumin as standard.

Isolation of β-Hexosaminidases and GM2-Activator from the Brain of Type-AB GM2-Gangliosidosis - Since only 10 g of the pathological sample was available to us initially, we used 10 g of normal brain to develop the following procedure for the simultaneous isolation of the partially purified GM2-activator and β-hexosaminidases. Unless otherwise indicated, the isolation of the enzymes and the activator was carried out at a temperature between 0 and 5°C. Frozen brain (10 g) was thawed and homogenized in 40 ml of distilled water with a Polytron homogenizer (Brinkman Instruments) and lyophilized. This freeze-dried powder was homogenized with 50 ml of acetone at -60°C and filtered. The acetone treated powder was again homogenized with 40 ml of 50 mM phosphate buffer, pH 7.0 for 30 sec and centrifuged at 13,000 x g for 20 min to obtain a clear extract. The pH of the extract was adjusted to 4.3 with a saturated citric acid solution. After standing for 6 to 8 h, the precipitate was removed by centrifugation. The supernatant was then brought to 70% saturation with solid $(NH_4)_2SO_4$. The precipitated protein was collected the next day by centrifugation, dissolved in and dialyzed against 10 mM sodium acetate buffer, pH 4.6. This solution containing β-hexosaminidases and the activator was applied to an octyl-Sepharose column (0.5 x 4 cm) which had been equilibrated with distilled water. The column was eluted with distilled water. β-Hexosaminidases were eluted with the unadsorbed proteins. The GM2-activator retained by the column under this condition was then eluted with octyl-β-glucoside (20 mg/ml). The fractions containing GM2-activator were pooled and then dialyzed against 10 mM sodium acetate buffer, pH 4.6. The β-hexosaminidases fraction from the octyl-Sepharose column were further purified by using a Con A-Sepharose column (0.5 x 4 cm) (9). Since the amount of β-hexosaminidases obtained from 10 g of brain tissue was not enough for the further separation of the enzymes into isozymes A and B, another 69 g of the same pathological brain was used to prepare β-hexosaminidases A and B. In this case, β-hexosaminidases were prepared by Sephadex G-200 gel filtration and DEAE-Sephadex A-50 chromatography according to the procedure described previously (10). The isozymes A and B were identified by cellogel electrophoresis (11).

RESULTS

The Levels of β-Hexosaminidases and GM2-Activator in the Brain of a Patient with Type-AB GM2-Gangliosidosis - The levels of GM2-activator in the

TABLE I

The Levels of GM2-Activator and β-Hexosaminidases in the Brains of Two Normals and a Patient with Type-AB GM2-Gangliosidosis

	$N_{2.7}$	$N_{2.5}$	Patient
Tissue (g, wet weight)	10 10	10 10	10.27
Total β-hexosaminidase (unit)	5.4 5.2	5.0 4.8	7.9
GM2-activator at 70% $(NH_4)_2SO_4$ step (x 10^5 unit)	1.62 1.30	0.81 0.71	4.20
Protein extracted after 70% $(NH_4)_2SO_4$ (mg)	47.78 47.12	49.99 47.11	44.26
GM2-activator after octyl-Sepharose (x 10^5 unit)	1.50 1.16	0.82 0.96	3.37
Protein after octyl-Sepharose (mg)	1.96 2.18	2.18 2.52	3.34

$N_{2.7}$ and $N_{2.5}$ are normal controls of 2.7 and 2.5 years of age.

brain of a Type-AB GM2-Gangliosidosis and the two normal brains of 2.5 and 2.7 years of age is summarized in Table I. For the normal brains, duplicates of 10 g tissues were analyzed to check the reproducibility of the method. As shown in Table I, the total β-hexosaminidase activity in the patient's brain was slightly higher than the two controls, yet the activator level was three times higher than the controls. The amount of β-hexosaminidase A determined by heat-inactivation was reported to be 58% of the total β-hexosaminidase activity (5). By using DEAE-Sephadex A-50 chromatography (see below) the amount of β-hexosaminidase A was estimated to be about 43% of the total activity. From these results it is apparent that neither GM2-activator nor β-hexosaminidase A were deficient in the brain of this patient.

Properties of β-Hexosaminidases and GM2-Activator Isolated from the Pathological Brain - Since the level of GM2-activator was markedly elevated in this patient, and no deficiency of β-hexosaminidases was detected using synthetic substrates, we then examined the hydrolysis of GM2 ganglioside by

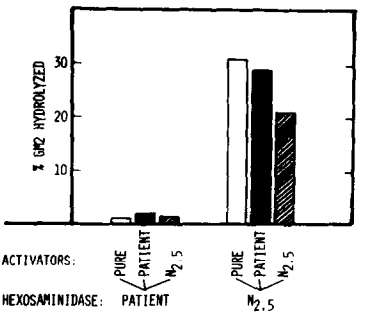

Fig. 1. Hydrolysis of GM2 ganglioside using β-hexosaminidases and activators derived from the pathological brain and a normal brain of 2.5 years of age ($N_{2.5}$). The incubation mixture contained [^{3}H]-labeled GM2 ganglioside (1.7×10^4 cpm), 10 nmole; acetate buffer (10 mM), pH 4.6; β-hexosaminidases, 0.4 unit; and approximately 2×10^3 units of GM2-activator. The sources for β-hexosaminidases and GM2-activators are indicated. The incubation was carried out at 37°C for 16 hours. The pure activator was isolated from normal human brain using the method described previously (4).

β-hexosaminidases prepared from the brain of this patient in the presence of pure GM2-activator isolated from normal human brain. As shown in Fig. 1, the β-hexosaminidases (containing isozymes A and B) isolated from the brain of the patient could not hydrolyze GM2 ganglioside in the presence of the pure GM2-activator. Under the same conditions, the β-hexosaminidases obtained from a normal control could hydrolyze GM2 ganglioside. The cross examination of the enzymes and the activators isolated from this case and from a normal control (Fig. 1) revealed that the β-hexosaminidases from this patient could not hydrolyze GM2 ganglioside in the presence of the partially purified activator isolated from the brain of either this patient or from the control brain. However, the β-hexosaminidase preparation isolated from the control brain could hydrolyze GM2 ganglioside in the presence of the partially purified activator isolated either from the patient's brain or from the control brain. These results further support the finding that this patient indeed had an abnormality in the enzymes, but not in the activator.

Properties of β-Hexosaminidases A and B Isolated from the Pathological Brain - The crude β-hexosaminidases obtained from the brain of this patient were separated into three peaks, designated as A_1, A_2 and B, by DEAE-Sephadex

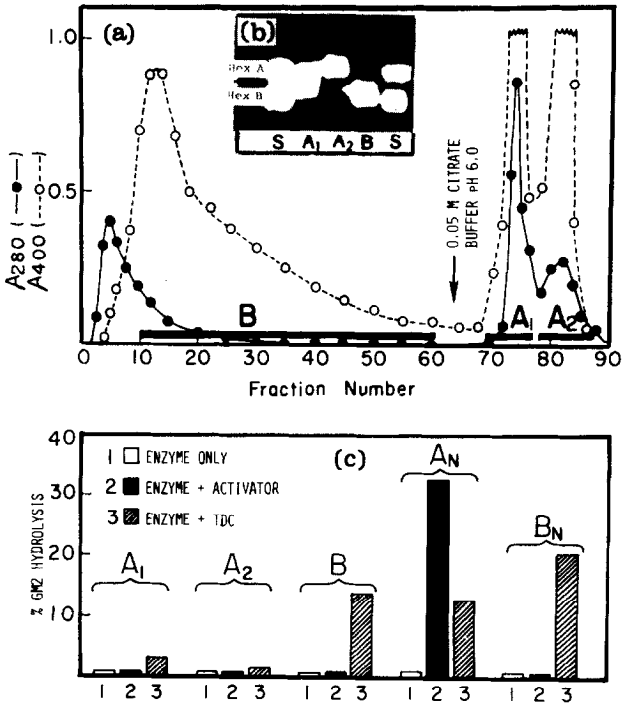

Fig. 2. (a), DEAE-Sephadex A-50 chromatography of β-hexosaminidases prepared from the pathological brain. From 69 g of pathological brain, 38 units (in 5.5 ml) of β-hexosaminidase preparation was obtained by $(NH_4)_2SO_4$ precipitation and Sephadex G-200 filtration (10). This preparation was dialyzed against 50 mM sodium phosphate buffer, pH 7.0 and applied to a DEAE-Sephadex A-50 column (1 x 25 cm) which had been equilibrated with the same buffer. After washing off the unadsorbed β-hexosaminidase B with the same buffer, β-hexosaminidase retained by the column was eluted with 50 mM sodium citrate buffer, pH 6.0. The β-hexosaminidase A was split into two peaks (A_1 and A_2). —●—, absorbance at 280 nm for protein. ---○---, absorbance at 400 nm for β-hexosaminidase activity using p-nitrophenyl-β-GlcNAc as substrate. Fractions indicated by the horizontal bars were pooled, concentrated by ultrafiltration using Amicon PM-10 membrane and dialyzed against 10 mM sodium acetate buffer, pH 4.6. (b), Cellogel electrophoresis of the fractions indicated in (a). (c), Hydrolysis of GM2 ganglioside by the fractions indicated in (a) in the presence of GM2-activator (4 x 10^3 units) or sodium taurodeoxycholate (TDC, 200 μg). For each assay 0.3 units of β-hexosaminidase was used. A_N and B_N are β-hexosaminidases A and B isolated from normal brain.

A-50 chromatography (Fig. 2a). The distribution of these activities toward p-nitrophenyl-β-GlcNAc are A_1, 17.6%; A_2, 25.3% and B, 57.3%. The electrophoresis on cellogel (Fig. 2b) revealed that the enzymes A_2 and B had mobilities similar to those of the pure β-hexosaminidases A and B isolated from normal human liver. The enzyme A_1 migrated between the enzymes A_2 and B. The ability of these enzymes to hydrolyze GM2 ganglioside was examined in the

presence of either the GM2-activator isolated from normal brain or sodium taurodeoxycholate. As shown in Fig. 2c, neither isozyme A_1 nor A_2 isolated from the pathological brain could hydrolyze GM2 ganglioside in the presence of GM2-activator. β-Hexosaminidase B from normal brain and from the pathological brain appeared similar in that neither could hydrolyze GM2 ganglioside in the presence of GM2-activator but both could hydrolyze GM2 ganglioside slowly in the presence of sodium taurodeoxycholate. On the other hand, the hydrolysis of GM2 ganglioside by A_1 and A_2 in the presence of sodium taurodeoxycholate is much less than that of isozyme B.

DISCUSSION

We have devised a simple and effective way of partially purifying the GM2-activator and β-hexosaminidases simultaneously from 10 g of brain tissue. This enabled us to examine the nature of both GM2-activator and β-hexosaminidases from the brain of a patient with Type-AB GM2-gangliosidosis. It is important to note that sodium taurodeoxycholate can stimulate the hydrolysis of GM2 ganglioside carried out by β-hexosaminidases A and B (4,12,13). The GM2-activator, on the other hand, only stimulates β-hexosaminidase A, but not B in carrying out the hydrolysis of GM2 ganglioside (4). If only sodium taurodeoxycholate was used to examine the ability of the β-hexosaminidases from this pathological brain to hydrolyze GM2 ganglioside, the abnormality of this β-hexosaminidase preparation would not have been detected. The possible existence of factors which might inhibit the hydrolysis of GM2 ganglioside in the brains of this patient has been carefully considered. Neither the activator nor β-hexosaminidase A isolated from the pathological brain had any inhibitory activity toward the GM2 hydrolysis carried out by pure β-hexosaminidase A.

The results presented in this report suggest that there may be reasons other than the activator deficiency (2,3) which cause the accumulation of GM2 ganglioside in Type-AB GM2-gangliosidosis. In the present case, the mutation may have occurred in the structural gene for β-hexosaminidase A in such a

manner that the enzyme could no longer hydrolyze GM2-ganglioside but still retained activity toward synthetic substrates. This is the first description of a defective β-hexosaminidase in the brain of a patient with Type-AB GM2-gangliosidosis.

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