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Immunochemical and Biochemical Investigation of Hexosaminidase S

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INTRODUCTION

Hexosaminidase exists in human tissues in several isozymic forms of which the major ones are A and B [1, 2]. In addition, the following minor isozymes have been identified in normal or pathological tissues: HEX C [3–5], HEX P [6, 7], and HEX I_1 and I_2 [8]. One of these minor isozymes is the residual hexosaminidase present in patients of variant 0 of G_{M2} gangliosidosis [9] denoted HEX S [10, 11], which was first reported by Sandhoff et al. [12].

Recent studies on partially purified preparations of HEX S indicate that this isozyme is distinctly different from all other known isozymic forms of hexosaminidase, including HEX A and C [10]. In its immunological reactivity, it was shown to react only with antisera directed towards HEX A but not with anti-HEX B.

HEX A has recently been shown to undergo conversion to the B and S forms; these studies imply that HEX S is a homo-oligomer of α chains [13]. This information and the availability of the suggested molecular subunit structure model of hexosaminidases [14] prompted us to further investigate the immunochemical and biochemical properties of HEX S.

MATERIALS AND METHODS

Enzymatic Assay

Synthetic substrates. Enzymatic activity of hexosaminidase was determined using the synthetic substrates 4-methyl umbelliferyl-*N*-acetyl-β-D-glucosaminide (MUF GlcNAc) (Pierce, Rockland, Ill.) or *N*-acetyl galactosaminyl derivative (MUF GalNAc) (Koch-Light, Colnbrook England). The reaction mixture (0.3 ml) contained 0.04 M Na citrate buffer, pH 4.4, 1 mM substrate, 0.2 mg BSA (grade A, Calbiochem, San Diego, Calif.) and the appropriate concentration of enzyme. The enzymatic reaction took place at 37°C for 10 min. One unit of enzymatic activity is defined as the amount of enzyme which liberates 1 μmol 4-methyl umbelliferone with 1 min at pH 4.4 and 37°C from MUF GlcNAc (1 mM) under the reaction conditions specified above. For pH profile studies, the enzyme and substrate samples were diluted in a series of citrate-phosphate buffers (0.05 M) at pH range 3.13–7.54.

Degradation of glycosphingolipids G_{M2} and G_{A2}. The enzymatic degradation of the glycosphingolipids G_{M2} (GalNAc β1 → 4[3 ←2 αNeuAc] Gal β1 → 4 Glc β1 → 1 Cer) and

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G_{A2} (GalNac β1 → 4 Gal β1 → 4 Glc β1 → 1 Cer) was performed as described (Sandhoff et al., in preparation.)

Ganglioside G_{M2}, labeled by catalytic reduction of the double bond in its sphingosine moiety with tritium gas (16.2 μCi/μmol) [15] and solubilized in chloroform-methanol (2:1, v/v), was pipetted into the reaction vessel and dried under a stream of nitrogen. Citrate buffer, 1.25 μmol, pH 4.3, and 0.1 μmol of sodium taurodeoxycholate (Sigma, St. Louis, Mo.) were added, and the mixture was sonicated three times for 5 seconds each. Enzyme solution (0.07–1 U) was added to give an final volume of 50 μl. The mixture was vigorously shaken and incubated for 3–20 hr at 37°C.

An aliquot of 40 μl was analyzed by thin layer chromatography. Silica gel 60 plastic backed sheets (Merck, Darmstadt, Germany) were used as adsorbent; chloroform-methanol, 0.25% NaCl in H_2O (62:38:8 v/v), as solvent system. Labeled product and substrate spots were identified by a scanning apparatus (Berthold, Germany), cut out, and their radioactivity quantified in a liquid scintillation counter (Mark II, Nuclear Chicago).

The incubation mixture for the degradation of the glycosphingolipid G_{A2} was prepared by analogy to that given above and contained in a final volume of 200 μl:12.5 nmol G_{A2} (190 μCi/mol) [12], 25 μmol citrate buffer, pH 4.0; 0.5 μmol sodium taurodeoxycholate (Sigma) and 18 mU HEX A or B. The mixture was shaken for 1 hr at 37°C and analyzed by thin layer chromatography as given above with solvent system containing chloroform-methanol-water (14:6:1, v/v).

Heat Inactivation

Enzyme samples were incubated for various periods and temperatures (see Results), transferred to ice, and their enzymatic activity determined. To study the effect of antibody binding on the heat lability profile, HEX S (10 μl) was mixed with antiserum (40 μl) and citrate buffer containing 1 mg/ml BSA (50 μl) was added. The mixture was then incubated for 50 min at 50°C, transferred to ice, and the enzymatic activity was determined. All antisera were heated for 30 min at 56°C before use to destroy any endogenous hexosaminidase.

Chromatographic Procedures

Analytical gel filtration was performed on Sephadex G-200 (Pharmacia, Uppsala, Sweden) column (1.6 × 88 cm). Descending chromatography was carried out at 4°C at a flow rate of 10 ml/hr, and fractions of 1.5 ml were collected. Protein markers for molecular weight were bovine gamma globulin (Armour, Kankakee, Ill.), bovine serum albumin (grade A, Calbiochem) ovalbumin (Worthington, Freehold, N.J.) and pure HEX A and B [14]. Affinity chromatography is described under Results.

Isoelectric Focusing

Isoelectric focusing (IEF) was carried out in a sucrose gradient using a preparative IEF instrument (LKB, 8100). Isoelectrophoretic runs were carried out with 1% (final concentration) of carrier ampholine with pH ranges of 3.5–10 or 2.5–6.0 (LKB, Bromma, Sweden). The latter was prepared by mixing equal volumes of ampholine solutions 2.5–4.0 and 4.0–6.0.

The runs proceeded at 4°C for 66 hr with a constant voltage (500 V). Samples of 1 ml were collected, their pH measured (at 0°C), and their enzymatic activity assayed after an appropriate dilution in citrate buffer.

Immunochemical Methods

Antisera. Preparation of antibodies to HEX A and B in goats and the preparation of specific anti-HEX A, which react exclusively with this isozyme and not with HEX B, have been described previously [17]. Antibodies to HEX B from Tay-Sachs liver were prepared in goats by two weekly injections containing 2 mg of partially purified HEX B from Tay-Sachs liver extract in complete Freund's adjuvant (Difco, Detroit, Mich.). Attempts were made to immunize with partially purified HEX S (A_{280} = 7.0, 32 mU/ml) by injecting rabbits intradermally with 1 ml of

enzyme solution emulsified in complete Freund's adjuvant. After three weekly injections, the animals were bled and serum separated.

Immunological assays. Radial immunodiffusion technique and radioimmunoassay as developed for the specific quantitative determination of HEX A and B have been described previously [17]. For enzyme binding assay, enzyme sample was diluted in phosphate-buffered saline (PBS) to about 10-fold the desired concentration for enzymatic reaction. The diluted enzyme (50 μl) was mixed with 50 μl of various concentrations of goat antisera (diluted in PBS containing 5% nonimmune serum) and incubated for 30 min at 37°C. Rabbit anti-goat IgG in an amount sufficient for the precipitation of all the goat IgG (usually 300 μl) was added, and the mixture was maintained for 30 min at 37°C and overnight at 4°C, after which the tubes were centrifuged and the enzymatic activity determined in both the supernatant and the precipitate (after dilution in 0.1 M citrate buffer pH 4.4). The results are expressed in percent binding of the total enzymatic activity.

RESULTS

Isolation of HEX S

Partially purified HEX S from the liver of an O variant of infantile G_{M2} gangliosidosis was used in this study. A specimen of liver (21 g) frozen at −20°C was homogenized in 80 ml of 10 mM Na-phosphate buffer, pH 6.0 (at 0°C), in an Omni-mixer (Sorvall, Newtown, Conn.) at top speed for 3 min. The specific activity of hexosaminidase in the homogenate was 0.168 mU/mg protein, which is about 0.2% of the enzyme levels found in normal liver tissue. After 30 min the homogenate was centrifuged (16,700 *g*, 30 min), and the supernatant was applied to a Sepharose-bound ϵ-aminocaproyl-*N*-acetyl glucosaminide (Seph-CNAG; 1.3 × 25 cm) which was prepared as described previously [16]. The column on which the enzymatic activity had been adsorbed was washed with 10 mM each of phosphate buffers of pH 6.2, 7.1, and 8.5 and subsequently eluted with a stronger ionic strength (0.1 M Na phosphate, pH 8.4) which released the enzyme from the column. The full enzymatic activity was recovered in this step with a 12-fold purification.

For the cleavage of glycosphingolipids G_{M2} and G_{A2}, further purification was carried out: supernatant (700 ml) of the homogenate obtained from 190 g of O variant liver, containing 2.6 U and prepared as above, was applied to a Con-A-Sepharose column (Pharmacia) [70 ml] at 4°C which was equilibrated with 10 mM phosphate buffer, pH 6.0. The column was washed with the same buffer, eluting 1.2 U of unbound enzyme, and subsequently another 0.8 U were eluted at 20°C with 10% α-methylglucoside (Sigma) in the same buffer. Both enzyme fractions, dialyzed against 10 mM phosphate buffer, pH 6.0, were subjected to chromatography on Sepharose-bound (6-amino-hexyl)-1-thio-2-acetamido-2-deoxy-β-D-galactopyranoside (Sandhoff et al., in preparation) equilibrated with the same buffer. Each enzyme fraction was loaded onto a 10 ml column which was then washed with 10 mM citrate buffer, pH 4.0. The enzyme was subsequently eluted with 0.1 mM *N*-acetylglucosamino-lactone in the same buffer followed by 0.1 M phosphate buffer, pH 8.0. The active fractions were pooled (1.8 U), dialyzed against 10 mM phosphate buffer, pH 6.0, and applied to a 10 ml DEAE-cellulose equilibrated with the same buffer. This column on which the enzymatic activity had been adsorbed was washed with 10 mM phosphate buffer, pH 6.0, followed by the same buffer containing 0.1 M and 0.15 M NaCl. The enzyme was

released from the column by 0.3 M NaCl in the same buffer in a sharp peak containing 1.1 U. The eluted enzyme solution was concentrated first by ultracentrifugation (370,000 g × 42 hr) and then by vacuum dialysis in collodion bags (SM 13,200, Sartorius, Göttingen, Germany) against 50 mM citrate buffer, pH 4.4. The amount of enzyme recovered was 0.5 U with a specific activity of 2.38 U/mg protein and an overall purification factor of more than 10,000.

General Biochemical Properties of HEX S

The pH optimum for hydrolysis of synthetic substrate. The pH optimum profiles for HEX A, B, and S, expressed as percent activity of the maximal values obtained are shown in figure 1. Unlike HEX A and B which give similar curves, a basic shift of about 0.4–0.5 pH U was exhibited by HEX S.

Substrate specificity. Kinetic studies have been performed using MUF GlcNAc and MUF GalNAc as substrates. The K_m values and the relative maximal velocities were calculated from the Lineweaver-Burk [18] double reciprocal plot (fig. 2). The K_m value for the *N*-acetylgalactosaminyl and the *N*-acetylglucosaminyl substrates were 0.33–0.4 mM and 0.9 mM, respectively, values which are higher than the values reported previously for HEX A and B [15, 16].

To study the specificity of HEX S against glycosphingolipid substrates in comparison to HEX A and B, a highly purified preparation of HEX S was used (specific activity 2.38 U/mg protein). The neutral glycosphingolipid G_{A2} was cleaved by all three hexosaminidases A, B, and S, with HEX B being the most active and HEX S having the least activity (table 1). On the other hand, the acidic ganglioside G_{M2} was cleaved more rapidly by HEX A than by the HEX B and S under the conditions used (i.e., in the presence of 2 mM sodium taurodeoxycholate).

Heat stability of HEX S. The heat stability of HEX S was compared to that of other hexosaminidases. The results given in figure 3 demonstrate that all three isozymes are inactivated within 10 min of incubation at 60°C. Heat treatment at 50°C, affects the activity of HEX A and S in contrast to the marked stability of HEX B. All three isozymes are stable to incubation at 40°C; under these conditions HEX S and B were

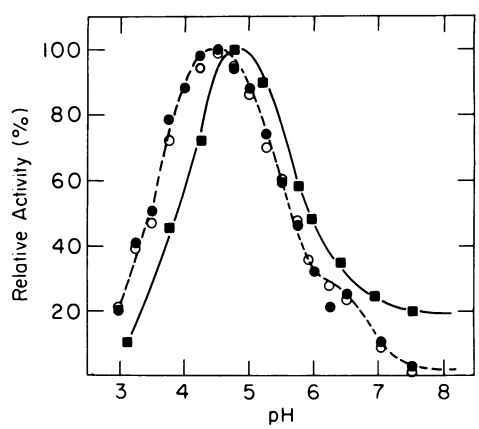

FIG. 1.—pH profile of HEX A (●), B (○), and S (■). Maximal activity values were considered 100%.

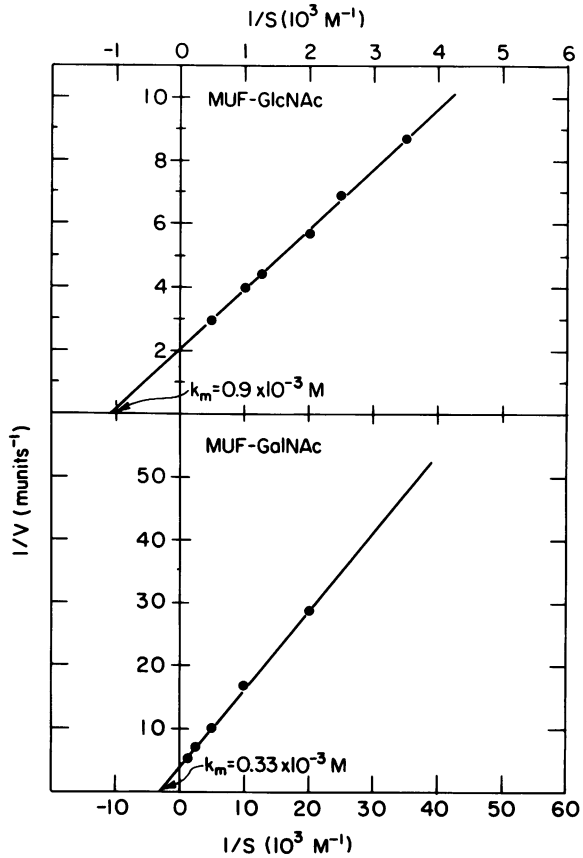

FIG. 2. — Double reciprocal plot [18] for the activity of HEX S using MUF GlcNAc (*top*) and the parallel galactosaminide derivate (*bottom*).

not affected at all, whereas a decrease of about 10% in activity was observed with HEX A.

Physicochemical Properties: Molecular Weight and Isoelectric Point

The molecular weight of HEX S was determined by gel filtration on Sephadex G-200 at 4°C. According to the elution volumes (fig. 4), the apparent molecular weights of HEX A, B, and S are 100,000, 108,000 and 103,000, respectively.

Isoelectric focusing in the pH range 3.5–10.0 indicated that the enzymatic activity is concentrated mainly in one acidic peak. Further resolution of this peak in pH gradient 2.5–6 has shown that most of the enzymatic activity resides in a peak of protein focused at pH 4.2 with a small shoulder at about pH 4.0 (fig. 5). The minor acidic peak of enzymatic activity appeared in only some experiments and its origin is not clear.

Immunochemical Studies

Liver samples of three normal individuals, three with the classical form of

TABLE 1

COMPARISON OF REACTION VELOCITIES OF HEX A, B, AND S ON DIFFERENT SUBSTRATES

SUBSTRATE (CONCENTRATION)	ENZYMATIC ACTIVITY (UNITS)* HEX S (2.38 U/mg)	HEX A (90 U/mg)	HEX B (280 U/mg)
MUF GlcNAc (1 mM)	1.0	1.0	1.0
MUF GalNAc (0.8 mM)	0.162	0.160	0.170
Glycolypid G_{A2} (62.5 μM)	0.48×10^{-3}	0.85×10^{-3}	2.83×10^{-3}
Ganglioside G_{M2} (120 μM)	$< 0.5 \times 10^{-5}$†	4.6×10^{-5}	0.58×10^{-5}

* Enzymatic activity unit is defined as the amount of enzyme degrading 1 μmol of substrate per min under the specific reaction conditions. All the numbers in the table are related to 1 U of enzyme activity as determined on MUF GlcNAc. The specific activity of each preparation towards MUF GlcNAc is given in parenthesis.

† Calculated from 20 hr incubation.

Tay-Sachs disease (variant B), and two with infantile G_{M2} gangliosidosis (variant O) were prepared as follows: centrifuged homogenates were diluted with 10 mM Na phosphate buffer, pH 6.0, to yield identical values of hexosaminidase activity in all cases. An enzyme binding test was performed with these samples using either goat anti-human HEX B which reacts with both HEX A and B or the antibodies specific to HEX A. The result of binding as a function of serum dilutions are shown in figure 6. Anti-HEX B was shown to completely bind the enzyme from normal as well as from variant B (Tay-Sachs disease) livers, whereas the enzyme of variant O liver was only slightly bound to it. Antibodies specific to HEX A did not bind the enzyme present in Tay Sachs disease (variant B) liver, but bound up to 60% of the enzyme from normal tissue. The two preparations of variant O livers bound to the specific anti-A serum with maximal values of 40%–60% of their total enzymatic activity.

Purified HEX S, as well as pure HEX A and B, exhibiting the same enzymatic activity, were subjected to a similar enzyme binding assay. The results are depicted in

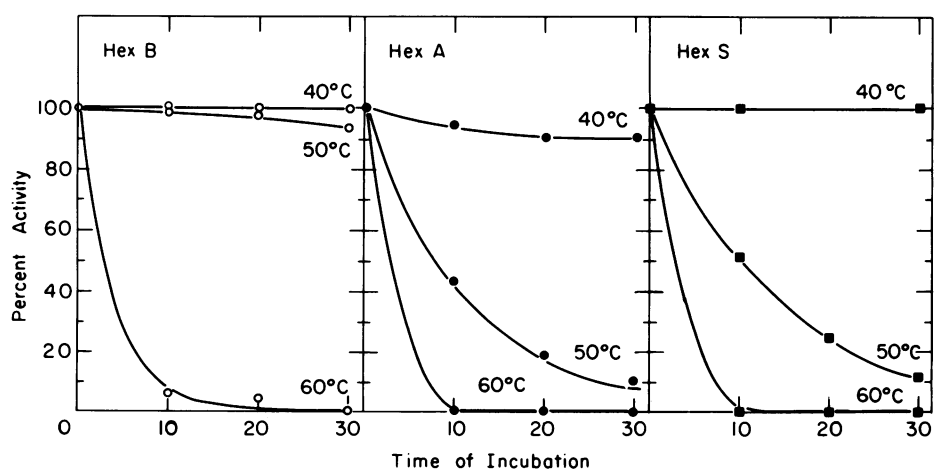

FIG. 3.—Heat stability of HEX A (•), B (○), and S (■) at 40°C, 50°C and 60°C. Assays were carried out in 0.04 M citrate buffer, pH 4.4, containing 1 mg/ml BSA.

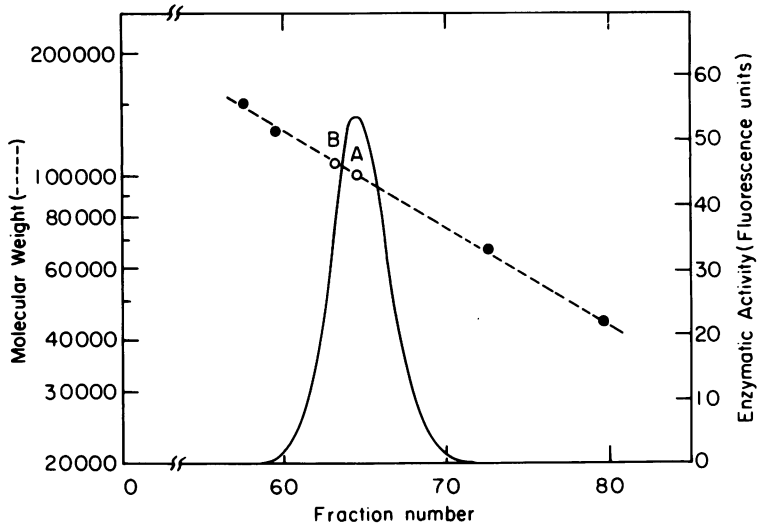

FIG. 4.—Gel filtration of HEX S on Sephadex G-200. Markers for the determination of molecular weight were, in increasing order, ovalbumin, BSA, HEX A and B (determined by enzymatic-activity), BSA-dimer, and bovine γ-globulin.

figure 7. HEX A was almost completely bound by the two antibody preparations, while HEX B was bound by anti-HEX B only. HEX S, on the other hand, was totally bound by the specific anti-HEX A, even with higher efficiency than the A isozyme. A low, but highly significant binding of HEX S was obtained by anti-HEX B antiserum. The concentrations of this antiserum required for a 50% binding were eight times higher for HEX S than for the A and B isozymes, indicating that only a small population of antibodies in the anti-HEX B antiserum recognized determinants on HEX S. The same phenomenon, namely binding of HEX S by anti-HEX B, was also confirmed when antiserum to HEX B of Tay-Sachs disease (variant B) was employed (not shown in the figure).

To investigate the possibility of the existence of an enzymatically inactive, antigenically reactive enzyme, unfractionated liver homogenates (50–60 mg protein/ml) were subjected to a radioimmunoassay using the two antisera mentioned above and radioactive (^{125}I-labeled) HEX A (specific activity 40 mCi/mg). The results are given in table 2. The normal tissues exhibited antigenic reactivity with both antisera, whereas the sample of variant B liver reacted with anti-HEX B only. The extent of inhibition was proportional to the enzymatic activity in each preparation. Variant O, on the contrary, did not show any significant reaction with either antibody preparations, although a low inhibition was consistently observed with the specific anti-HEX A, when variant O liver preparation was tested without dilution. These results indicate that in variant O liver no extractable antigenically reactive material is present, which is not accounted for by the low enzymatic activity of HEX S in the preparation.

An alternative method used for a quantitative measure of the antigenic cross-reactivity was radial immunodiffusion using agarose gel plates containing either anti-HEX B or specific anti-HEX A. In the plates containing anti-HEX B, rings of

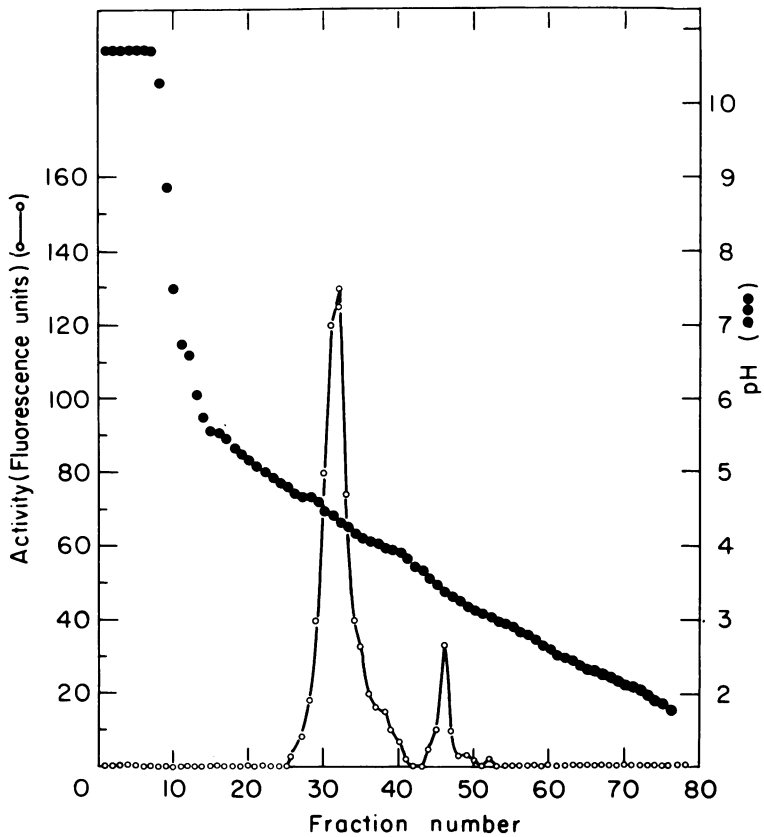

FIG. 5.—Isoelectric focusing of HEX S in the pH range 2.5–6.0.

enzyme activity were obtained with the normal and variant B liver samples, as well as with pure HEX A and B (fig. 8). HEX S did not yield visible activity rings in this gel. In the gel containing the specific serum to HEX A, the isolated B enzyme and the liver sample from the B variant of Tay-Sachs disease gave no activity rings, whereas the A and S isozymes or preparation of normal liver showed a clear-cut reaction. Quantitative evaluation of the antigenic specificity of each sample was achieved by plotting the net area of the activity rings vs. the dilution of the tested enzyme solution. The slope of such plots, expressed as the extrapolated ring area per 10 μl of the original enzyme solution, is therefore a measure for the antigenic activity of hexosaminidase in the sample. The slope was taken in the linear region of the plot which was between net ring areas of 10–50 mm^2. The results are shown in table 3.

To further investigate the antigenic properties of HEX S, heat inactivation was performed in the presence of anti-HEX B, anti-Tay-Sachs HEX B, specific anti-HEX A or nonimmune serum. The assays were carried out at 50°C for 30 min. The results expressed as residual activity vs. amount of added serum is shown in figure 9. Nonimmune serum had no effect on heat inactivation, whereas all the antisera gave significant protection (in amounts up to 25 μl). Specific anti-HEX A gave the highest

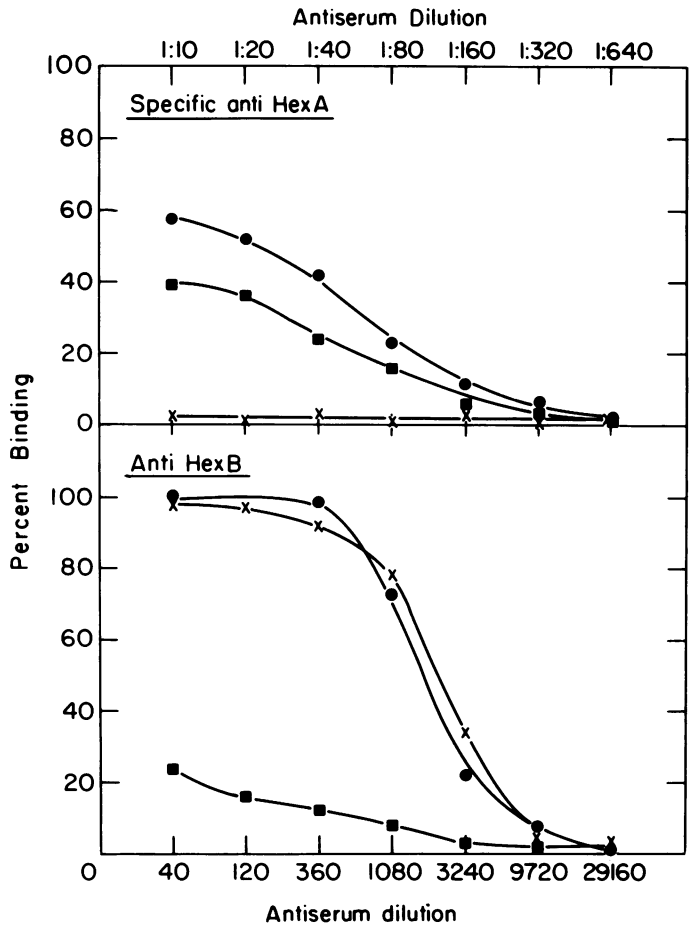

FIG. 6.—Enzyme binding assays performed with crude unfractionated liver homogenates of normal (•), Tay-Sachs variant B (x) and variant O (■) individuals. The antisera used for binding were goat anti-HEX B (*bottom*) and specific anti-HEX A (*top*). Percent binding is of the total enzymatic activity used in the assay.

effect, but a significant stabilization was definitely brought about by anti-HEX B antisera.

DISCUSSION

The HEX S used throughout this study was a partially purified preparation, isolated from variant O liver by affinity chromatography on a specific ligand column [16]. In this procedure, a 12-fold purification was obtained. In later experiments, it has been observed that further 10-fold purification can be achieved by applying more concentrated buffer for washing the columns before elution. Such material or even more purified samples were used only in the part dealing with degradation of the natural substrates G_{M2} and G_{A2}.

In comparing the kinetic parameters of the specific catalytic activity of the various

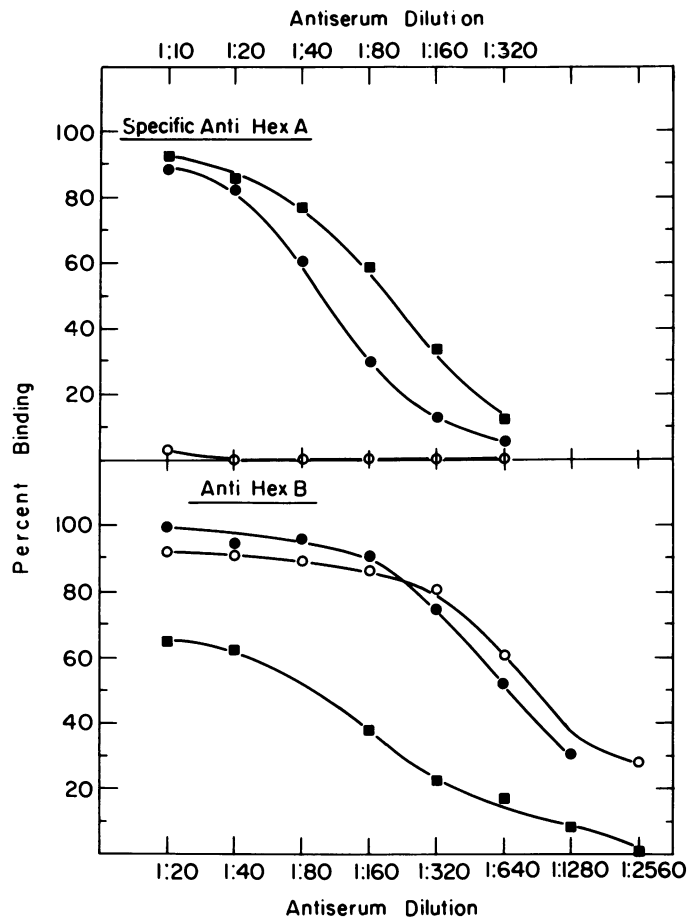

FIG. 7.—Enzyme binding assays performed with purified HEX A (•), B (○), and S (■). Details are as in figure 6.

isozymes towards *N*-acetylglucosaminyl and *N*-acetylgalactosaminyl substrates, comparable K_m values were obtained for the A, B, and S enzymatic forms. Moreover, the ratio between the maximal velocity of hydrolysis of MUF GlcNAc and MUF GalNAc by HEX S was 8.75, which is within the range obtained for HEX A and B [15].

In enzymatic assays with natural substrates, it was found that HEX S is capable of cleaving the glycosphingolipid G_{A2} but less efficiently than HEX A and B, the latter being the most active (table 1). The activity of HEX S toward the acidic ganglioside G_{M2} could not be measured exactly due to an insufficient amount of the highly concentrated enzyme preparation. However, the result obtained suggests a small but significant activity of HEX S toward the ganglioside, which is clearly less than the activity of HEX A under the conditions used.

Since it was impossible for technical reasons to obtain enough highly purified preparation of HEX S for a detailed biochemical analysis, most of the structural

TABLE 2

RADIOIMMUNOASSAY OF LIVER HOMOGENATES FROM NORMAL, TAY-SACHS, AND VARIANT O INDIVIDUALS

TISSUE	DILUTION	PERCENT INHIBITION	
		Anti-HEX B*	Specific Anti-HEX A*
Normal liver†	1:4	100.0	100.0
No. 22	1:16	95.0	96.2
	1:64	91.1	83.1
	1:256	81.7	26.6
Normal liver	1:4	88.0	90.8
No. 23	1:16	73.5	44.4
	1:64	45.6	19.9
	1:256	31.8	9.9
Tay-Sachs liver	1:4	89.4	4.2
No. 32	1:16	72.1	6.4
	1:64	50.0	0.7
	1:256	29.3	6.2
Variant O liver	direct	6.4	13.5
	1:4	8.2	2.2
	1:16	7.8	4.7
	1:64	3.8	0
	1:256	3.6	0

* Goat anti-HEX B (1:160) or goat specific anti-HEX A (1:20) were tested.
† Enzymatic activity in this preparation was four times that of liver no. 23.

information of this investigation has been derived from immunochemical studies. HEX S reacts strongly with the antiserum which reacts exclusively with HEX A and also, but to a lower extent, with anti-HEX B. This finding differs from the observations reported by Ikonne et al. [10] and Beutler et al. [11], who could not show any reactivity of the S form with anti-B, which could be due to the relatively low percentage of cross-reactive antibody in the anti-B serum. This may also account for the difference observed here between the results of the enzyme binding assay and radial immunodiffusion; in the latter, the low concentration of antibodies present in the gel may not have permitted the formation of a visible ring.

The fact that only 40%–60% of the total enzymatic activity of the crude homogenate of variant O liver could be bound to either anti-HEX A or anti-HEX B, whereas the remainder of the activity did not react with either antiserum, suggests that part of the hexosaminidase in the preparation comprises the isozyme HEX C. This material was probably eliminated during the affinity chromatography which yielded an isozyme S preparation fully reactive with the anti-HEX A sera.

An interesting comparison of the different isozymes is the quantitative ratio between their enzymatic activities and their antigenic reactivities with a given antiserum, as performed for HEX A and S (table 3). In this particular experiment, a ratio of 4.65 was obtained for purified HEX A and S while the ratios obtained with 10 μl of the respective enzyme solutions was only 2.52. This discrepancy between enzymatic and antigenic activities indicates that the relative content of "A-specific" determinants per activity unit is higher, almost by a factor of two in HEX S as compared to HEX A.

The molecular model suggested previously for HEX S was a homo-oligomer of α

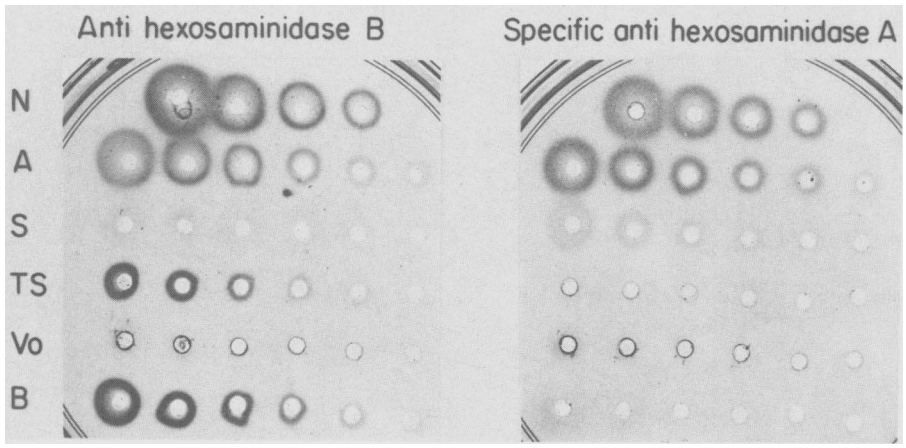

FIG. 8.—Radial immunodiffusion in 1.5% agarose gels containing anti-HEX B, 1 = 500 (*left*), or specific anti-HEX A, 1 = 100 (*right*) with samples of normal liver extract (N), pure HEX A (A), purified HEX S (S), Tay-Sachs liver extract (TS), variant O liver extract (VO), and pure HEX B (B). Each sample was tested in several 1:2 serial dilutions. The gels were stained for enzymatic activity after extensive rinsing.

chains [10, 11]. In accordance with the subunit structure that we suggested for HEX A and B [14], it appears that the S isozyme is composed of four α chains in two subunits, namely $\alpha_2\alpha_2$.

The mutation responsible for the variant O form of the disease is probably manifested in a deficiency in the production of β chains of hexosaminidase, allowing $\alpha_2\alpha_2$ structure as the only possibility for an active tetramer. The samples investigated in this study are indeed deficient in β chains and do not contain a defective form, as no appreciable amount of a corresponding antigenically cross reacting material can be demonstrated in them. The discrepancy between this finding and that reported by Srivastava and Beutler [19] can be explained by the possible variability among different patients, carrying unrelated mutations expressed in a similar phenotypic effect.

TABLE 3

ANALYSIS OF RADIAL IMMUNODIFFUSION DATA

SAMPLE	ENZYMATIC ACTIVITY (mU/ml)	RING AREA PER 10 μl SAMPLE*		AREA RATIO WITH SPECIFIC ANTI-HEX A VS. ANTI-HEX B
		Anti-HEX B (1:500)	Specific anti-HEX A (1:100)	
Pure HEX A	512	46	48	1.04
Pure HEX B	239	22	...	...
Variant B, liver homogenate	84	8	...	...
Normal liver homogenate	1,910	172	112	0.65
Purified HEX S	110	...	19	...

* Extrapolated value.

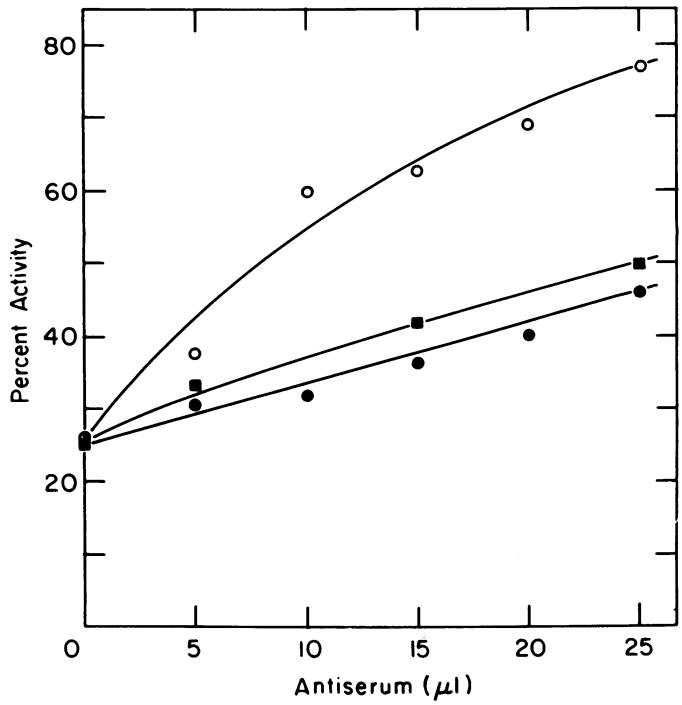

FIG. 9.—Stabilization of HEX S towards heat inactivation by anti-HEX B (•), anti-Tay-Sachs HEX B (■), and specific anti-HEX A (○). Percent activity is calculated from the enzymatic activity of untreated controls.

The suggested molecular model is corroborated by the following points of evidence. (1) HEX S reacts strongly with specific anti-HEX A serum and only poorly with anti-HEX B. (2) Repeated freezing and thawing of HEX A (consisting of $\alpha_2\beta_2$ structure) was shown by Beutler and Kuhl [13] to result in an interconversion to HEX B ($\beta_2\beta_2$) and S ($\alpha_2\alpha_2$). (3) A phenotypic complementation and de novo formation of HEX A occurred in somatic cell hybrids of cells from O and B forms of G_{M2} gangliosidosis; neither of the parental cell types initially exhibited HEX A activity [20–22]. (4) Obligatory heterozygotes to the O variant contain relatively higher values of HEX A [23, 24], suggesting that they manifest partial deficiency of β chain production.

Assuming that the above suggested model for subunit structure of HEX S is valid, it emerges that there is some immunological cross-reaction between α_2 and β_2 subunits in their native state. This finding suggests partial homology between the α and the β chains of hexosaminidase.

SUMMARY

Hexosaminidase S (HEX S), the residual isozyme found in tissues and body fluids of children with the O variant of G_{M2} gangliosidosis, was purified from tissues of variant individuals and biochemically and immunochemically characterized. This enzyme has an apparent molecular weight of 103,000 with an isoelectric point of 4.2, is heat labile

to the same extent as HEX A, and loses most of its activity following heating for 30 min at 50°C. HEX S reacts immunologically with the antisera against either HEX A or B, but the reaction is considerably stronger with the anti-A serum or with antibody preparations which react exclusively with the A isozyme. Results obtained by a radioimmunoassay using the various antisera indicated that there is no antigenically cross reacting material which lacks enzymatic activity in the variant tissues. These findings are in accord with a suggested molecular structure of two subunits, each composed of two α chains ($\alpha_2\alpha_2$) for HEX S; it also implies that α and β chains have some structural similarity which is manifested in antigenic cross-reactivity.

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