A Protein Activator for the Enzymic Hydrolysis of G\textsubscript{\text{m}}\textsubscript{2} Ganglioside*

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A protein activator (G\textsubscript{M\text{2}}-activator) specific for stimulating the hydrolysis of G\textsubscript{m\text{2}} ganglioside (GalNAc\textsubscript{b}1 → 4Gal\textsubscript{d}3 → 2NeuAcβ1 → 4Glcβ1 → 1' Cer) by β-hexosaminidase A has been purified over 10\textsuperscript{4}-fold with a high yield from human liver. The purification procedure includes the adjustment of the pH of liver extract to pH 4.3, followed by ammonium sulfate precipitation, Sephadex G-200 filtration, and column chromatographies on DEAE-Sephadex A-50, Matrex Gel Blue A, and octyl-Sepharose 4B. The physical properties of the G\textsubscript{M\text{2}}-activator are: (a) moderately heat-stable up to 50 °C in crude states, but very unstable in the purified form even at 37 °C; (b) molecular weight, about 23,500; and (c) isoelectric point about 4.8. Chemical analysis identifies this activator as a protein.

G\textsubscript{M\text{2}}-activator is very specific for stimulating the hydrolysis of G\textsubscript{M\text{2}} ganglioside, but only slightly effective in stimulating the hydrolysis of asialo-G\textsubscript{M\text{2}} or globotetraosylceramide catalyzed by β-hexosaminidase A. Unlike bile salts such as sodium taurodeoxycholate, this activator does not stimulate the hydrolysis of the above three glycosphingolipids catalyzed by β-hexosaminidase B. It does not stimulate the hydrolysis of synthetic substrates such as 4-methylumbelliferyl-β-GalNAc and p-nitrophenyl-β-GalNAc. The oligosaccharide derived from G\textsubscript{M\text{2}} ganglioside is not hydrolyzed by β-hexosaminidase A or B in the presence of either G\textsubscript{M\text{2}}-activator or sodium taurodeoxycholate. The rate of the hydrolysis of G\textsubscript{M\text{2}} ganglioside is affected by both the amount of G\textsubscript{M\text{2}}-activator and β-hexosaminidase A. The molar ratio of enzyme to G\textsubscript{M\text{2}}-activator for obtaining the maximal hydrolysis of G\textsubscript{M\text{2}} ganglioside is close to 1:1, while the molar ratio of G\textsubscript{M\text{2}} ganglioside to G\textsubscript{M\text{2}}-activator is about 300:1. These results suggest that G\textsubscript{M\text{2}}-activator interacts with β-hexosaminidase A rather than the lipid substrate.

From the relationship between the accumulation of glycosphingolipids and the deficiencies of enzymes in various sphingolipidoses, there is little doubt that most, if not all, glycosphingolipids are catabolized by the sequential hydrolyses of sugar units from the nonreducing end of the saccharide chains catalyzed by a series of exoglycosidases. Due to their lipophilic nature, the in vitro hydrolysis of glycosphingolipids by exoglycosidases requires the presence of bile salts such as sodium taurodeoxycholate. Since bile salts do not exist in tissues other than liver, it is conceivable that the enzymic hydrolysis of glycosphingolipids in vivo is assisted by substances other than bile salts. Recently, several protein activators isolated from various tissues have been found to replace bile salts in stimulating the in vitro enzymic hydrolyses of glycosphingolipids. The activators which stimulate the hydrolysis of galactoscerubrosides sulfatide (1–3), glucocerebroside (4–7), G\textsubscript{M\text{1}} ganglioside (8–11), and G\textsubscript{M\text{1}} ganglioside (12–14) have been isolated in varying degrees of purity. We have shown that the enzymic hydrolyses of G\textsubscript{M\text{1}} ganglioside and G\textsubscript{M\text{2}} ganglioside require two separate protein activators (15).

In order to understand the normal catabolism as well as the pathological accumulation of glycosphingolipids in patients with sphingolipidoses, it is important to isolate these activators in pure form and characterize their mode of action. In this report, we describe the isolation and characterization of an activator (G\textsubscript{M\text{2}}-activator) which stimulates the hydrolysis of G\textsubscript{M\text{2}} ganglioside catalyzed by β-hexosaminidase A (2-acetamido-2-deoxy-β-D-glucoside acetalaminodeoxyglucosylceramide, EC 3.2.1.30). Some preliminary accounts of this work have been presented (15, 16). While this work was in progress, reports on similar activators isolated from human liver (8, 9) and kidney (11) have appeared.

**EXPERIMENTAL PROCEDURES**

**Materials**

G\textsubscript{M\text{2}} ganglioside was isolated from Tay-Sachs brain (17) and globotetraosylceramide (GbOseCer) from human erythrocytes (18). Radioactive G\textsubscript{M\text{2}} ganglioside and radioactive GbOseCer, tritium-labeled at the terminal GalNAc, were prepared by the galactose oxidase and NaB\textsubscript{3}H\textsubscript{4} reduction procedure as described by Radin (19) with slight modifications (20). Asialo-G\textsubscript{M\text{2}} was prepared from G\textsubscript{M\text{2}} ganglioside by mild acid hydrolysis (21). The oligosaccharide derived from G\textsubscript{M\text{2}} ganglioside was prepared by ozonolysis (18). The following were purchased from commercial sources: p-nitrophenyl-β-GlCNac, p-nitrophenyl-β-GalNAc and octyl-β-glucoside, Sigma; Matrex Gel Blue A, Amicon Corp.; octyl-Sepharose, Con A-Sepharose, and agarose IEF, Pharmacia; Ampholine, LKB; and methyl-α-D-mannoside, Calbiochem. The following were generous gifts: G\textsubscript{M\text{2}} ganglioside, Dr. Svennerholm, University of Goteborg, Sweden; purified β-hexosaminidase A, Dr. Svennerholm, University of Goteborg, Sweden; purified β-hexosaminidase B and A isolated from human placenta, Dr. Brady of the National Institutes of Health and Dr. Lowden, The Hospital for Sick Children, Toronto, Canada, respectively; oligosaccharide from G\textsubscript{M\text{2}} ganglioside, Dr. O'Brien, University of California, San Diego.

**Enzymes**

For convenience, p-nitrophenyl-β-GlCNac was used as a substrate for β-galactosidase.

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\footnotesize{The abbreviations and trivial names used are: G\textsubscript{M\text{2}}, Galβ1 → 3Galα1 → 4(Gluβ1 → 3Galβ1 → 1' Cer) 4(NeuAcα2 → 3Galβ1 → 1' Cer).}

\footnotesize{1 The abbreviations and trivial names used are: G\textsubscript{M\text{3}},Galβ1 → 3Galα1 → 4Glcβ1 → 1' Cer; αGalα1 → 3Galβ1 → 1' Cer; and αGalα1 → 3Galβ1 → 4Glcβ1 → 1' Cer.}

\footnotesize{2 For convenience, p-nitrophenyl-β-GlCNac was used as a substrate for β-galactosidase.}
to detect β-hexosaminidase activity during purification. One unit of β-hexosaminidase is defined as the amount of enzyme which hydrolyzes 1 μmol of p-nitrophenyl-β-GalNAc at 37 °C. The specific activity of the enzyme is expressed as units per mg of protein. Enzyme isolation was carried out at a temperature between 0 and 5 °C. β-Hexosaminidases were isolated from human liver simultaneously with the GM2-activator (see "Purification of GM2 Activator"). The separation of β-hexosaminidase A from β-hexosaminidase B was achieved by using a DEAE-Sephadex chromatography (15). The isozymes A and B were verified by Cellulose electrophoresis (22). The β-hexosaminidase A or B obtained above was further purified by adsorbing the enzyme on a Con A-Sepharose column (2 × 15 cm) (23) and by an affinity column packed with Sepharose-p-aminophenyl-β-GalNAc (24). The specific activity of β-hexosaminidase A or B obtained after affinity chromatography was about 150 units per mg of protein, which was about 90% pure as judged by polyacrylamide gel electrophoresis.

Chemical and Physical Analyses

Amino acids and hexosamines were analyzed by the method of Moore and Stein (25). The isoelectric point and the purity of GM2-activator was determined by electrofocusing on a flat bed agarose gel isolation was carried out at a temperature between 0 and 5 °C. The specific activity of the enzyme on a Con A-Sepharose column (2 × 15 cm) (23) and by an affinity column packed with Sepharose-p-aminophenyl-β-GalNAc (24). The specific activity of β-hexosaminidase A or B obtained after affinity chromatography was about 150 units per mg of protein, which was about 90% pure as judged by polyacrylamide gel electrophoresis.

Assay of GM2 Activator

The standard assay mixture contained the following components in 0.2 ml: 3H-labeled GM2 ganglioside (1.5 × 10^6 cpm), 10 nmol, acetate buffer (10 mM), pH 4.6; β-hexosaminidase A or B, 0.5 unit in 3 to 5 μl which had been dialyzed against 10 mM acetate buffer, pH 4.6, and an appropriate amount of GM2-activator. The mixture was incubated at 37 °C for 1 to 17 h depending on the purity and the amount of GM2-activator. The reaction was terminated by heating the mixture at 100 °C for 3 min, and the liberated radioactive GalNAc was determined by the dialysis method (12). After dialysis, the dialysate was analyzed for the liberated radioactive GalNAc, and the retentate, for GM2 ganglioside by thin layer chromatography (12). When the oligosaccharide derived from GM2 ganglioside was used as substrate, the incubation mixture was directly analyzed by thin layer chromatography using chloroform: methanol: H2O (40:40:10) as a solvent.

When the highly purified β-hexosaminidase (specific activity, 150) and the highly purified GM2-activator (preparation after the second octyl-Sepharose chromatography) were used, 25 μg of ovalbumin was included in the incubation mixture to prevent the inactivation of the activator due to the excessive dilution of the proteins. In addition, the plastic tubes or the siliconized glass tubes were used to prevent the loss of the activator on glass surface.

One unit of GM2-activator is defined as the amount of activator which stimulates the hydrolysis of 1 pmoI of GM2 ganglioside per h per unit of β-hexosaminidase A under the standard assay condition. The specific activity of GM2-activator is the units of activator per mg of protein.

Purification of GM2 Activator

FIG. 1. Sephadex G-200 gel filtration of crude liver extract. The preparation obtained after (NH4)2SO4 precipitation (about 2.5 g of protein) was applied to a Sephadex G-200 column (5 × 80 cm) and eluted with 50 mM sodium phosphate buffer, pH 7.0. Fractions indicated by the horizontal bars were pooled and further purified.

Extraction and Sephadex G-200 Gel Filtration—Frozen normal human liver (1 kg portion) was diced and then homogenized with 5 liters of 10 mM phosphate buffer, pH 7.0, in a Waring Blender in 30-s intervals for a total of 2 min. The sample was cooled in ice between each interval. The homogenate was centrifuged at 9,000 × g to obtain a crude extract. The pH of the extract was adjusted to 4.3 with saturated citric acid solution. After standing overnight, the precipitate was removed by centrifugation. The supernatant was brought to 30% saturation with solid (NH4)2SO4, and allowed to stand overnight. The sample was centrifuged to remove the precipitate, and the supernatant was brought to 70% saturation with solid (NH4)2SO4. The precipitated protein was collected the next day by centrifugation and resuspended in 125 ml of 50 mM sodium phosphate buffer, pH 7.0. This solution which contained various glycosidases and activators was divided into 25-ml portions and applied to a Sephadex G-200...
column (5 x 80 cm) which had been equilibrated with the same buffer.
The column was eluted with the same buffer at a flow rate of 40 ml
per h and 20-ml fractions were collected. Fig. 1 shows the position of
β-galactosidase, β-hexosaminidase, and the activators eluted from
the column. The fractions containing activators were pooled. The
activators were precipitated by 70% (NH4)2SO4 saturation.

DEAE-Sephadex A 50 Chromatography—The activator fraction
obtained from Sephadex G-200 filtration as shown in Fig. 1 was
subsequently applied to a DEAE-Sephadex A 50 column to separate
Gm2-activator from Gm1-activator as described previously (15). The
Gm2-activator fraction from the DEAE-Sephadex column was lyoph-
лизирован и further purified.

Matrex Gel Blue A Chromatography—Gm2-activator (400 mg in
protein derived from 1 kg of liver) obtained after DEAE-Sephadex A-50
chromatography was dissolved in, and dialyzed against 10 mM
acetate buffer, pH 4.6, and applied to a column (2.5 X 20 cm) of
Matrex Gel Blue A which had been equilibrated with the same buffer.
After washing with 2 bed volumes of 10 mM acetate buffer, pH 4.6,
the column was eluted with a linear gradient of NaCl generated by
150 ml of 10 mM acetate buffer, pH 4.6, in the mixing chamber, and
150 ml of 25 mM phosphate buffer, pH 7.0, containing 100 mM NaCl
in the reservoir. Fractions of 6 ml were collected. Gm1-activator was
eluted between pH 4.6 and 5.5 as indicated in Fig. 2A. These fractions
were pooled and freeze-dried. Gm2-activator based on 3 kg of human
liver was subsequently applied to a DEAE-Sephadex A 50 column to separate
Gm2-activator from Gm1-activator. Gm2-activator was subsequently separated from the glycosidases by Sephadex G-200 filtration (Fig. 1). Gm2-activator and Gm1-activator were subsequently separated from each other by using DEAE-
Sephadex A 50 column chromatography (15). Figs. 2, A, B, and C show the elution profiles for the purification of Gm2-activator by Matrex Gel Blue A and octyl-Sepharose columns. These columns were very effective in purifying Gm2-activator with a high yield. The rechromatography of Gm2-activator in an octyl-Sepharose column resulted in additional 10-fold pu-
rification with very little loss of the activity. At a protein concentration of 50 μg/ml, the activator could be kept in a
freezer (−20 °C) for 6 months without any loss of activity.

Electrophoretic Analysis—The purity of Gm2-activator ob-
tained from the second octyl-Sepharose column was analyzed by subjecting 10 μg of the activator protein to polyacrylamide
gel electrophoresis at pH 8.3 and by isoelectrofocusing in
polyacrylamide gel using Ampholine of pH 3–6. In both cases,
trials to stain the gel with either Amido black or Coomas-
sie blue and to recover the stimulatory activity from the gels
were unsuccessful. The same amount (10 μg) of bovine albu-
min was always included as a control to monitor the electro-
phoresis and the staining solutions. Only 5% of the stimulatory
activity was recovered in the stacking gel, suggesting that
Gm2-activator might be inactivated or denatured by the chemical
agents used for polymerizing the gel or, due to some
unknown reason, Gm2-activator was not able to penetrate the
polyacrylamide gel. Pre-electrophoresis of the gel prior to
the application of the sample did not change the results.
We subsequently found that the activator could penetrate the
agarose gel and the stimulatory activity could be recovered from the agarose gel after electrophoresis. Fig. 3 shows that by a flat bed agarose gel isoelectrofocusing Gm2-activator (10 μg) could be stained by both Amido black and Coomassie blue. The densitometric scanning of the electrophocusing pattern (Fig. 3, upper panel B) showed one major band (75% of
the total protein) at pH 4.75 and two minor bands at pH 4.9 (15% protein) and 5.0 (8% protein), respectively. A parallel gel
was sliced into 2-mm sections and checked for the stimulatory
activity. The activity was found to be associated with the major protein band at pH 4.75 and the minor protein band
with pH 4.9. The smallest band at pH 5.0 did not have the stimulatory activity.

Amino Acids and Sugar Compositions—Table II summa-
izes the amino acid composition of the purified Gm2-activator. The
recovery of the weight in terms of the amino acids was
105%. Based on the moles of amino acid per mol of Gm2-
activator, the minimum calculated molecular weight of Gm2-
activator was about 23,000. This value corresponds to the

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**Table 1**

<table>
<thead>
<tr>
<th>Steps</th>
<th>Total activity</th>
<th>Yield</th>
<th>Specific activity</th>
<th>Purification</th>
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<tr>
<td></td>
<td>units x 10³ mg</td>
<td></td>
<td>units/h/mg/unit</td>
<td></td>
</tr>
<tr>
<td>Supernatant after pH</td>
<td>1.04</td>
<td>100</td>
<td>83,170</td>
<td>125</td>
</tr>
<tr>
<td>4.3 treatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(NH₄)₂SO₄, 30-70% ppt</td>
<td>2.12</td>
<td>204</td>
<td>22,721</td>
<td>932</td>
</tr>
<tr>
<td>Sephadex G-200</td>
<td>1.24</td>
<td>119</td>
<td>10,090</td>
<td>1,228</td>
</tr>
<tr>
<td>DEAE-Sephadex A-50</td>
<td>1.08</td>
<td>104</td>
<td>1,233</td>
<td>8,860</td>
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<tr>
<td>Matrex Gel Blue A</td>
<td>0.94</td>
<td>90.8</td>
<td>268</td>
<td>35,224</td>
</tr>
<tr>
<td>Octyl-Sepharose</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>1.09</td>
<td>104.6</td>
<td>6.56</td>
<td>1.66 x 10⁶</td>
</tr>
<tr>
<td>2nd</td>
<td>0.75</td>
<td>72.0</td>
<td>0.56</td>
<td>1.47 x 10⁷</td>
</tr>
</tbody>
</table>
A Protein Activator for GM₂-Hydrolysis

protected by the addition of ovalbumin or bovine serum albumin at a concentration of 25 μg per 200 μl of the incubation mixture.

Effect of Nonspecific Proteins on the Hydrolysis of GM₂ Ganglioside—The hydrolysis of GM₂ ganglioside was greatly affected by the purity of β-hexosaminidase A as well as the purity of GM₂-activator. Fig. 4A shows that the highly purified GM₂-activator obtained from the second octyl-Sepharose column was not effective in stimulating the hydrolysis of GM₂ ganglioside carried out by the highly purified β-hexosaminidase A obtained from the affinity column (specific activity, 150), while the same GM₂-activator, except at very low concentrations, could stimulate the partially purified β-hexosaminidase A obtained from the Con A-Sepharose column (specific activity, 30) to hydrolyze GM₂ ganglioside. If a nonspecific protein such as ovalbumin or bovine serum albumin was included in the incubation mixture, the highly purified β-hexosaminidase A became able to hydrolyze GM₂ ganglioside.

TABLE II
Amino acid composition of GM₂-activator

<table>
<thead>
<tr>
<th>Amino acids</th>
<th>Mol/mol protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aspartic acid</td>
<td>25.0</td>
</tr>
<tr>
<td>Threonine</td>
<td>16.7</td>
</tr>
<tr>
<td>Serine</td>
<td>31.0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>35.7</td>
</tr>
<tr>
<td>Proline</td>
<td>14.3</td>
</tr>
<tr>
<td>Glycine</td>
<td>25.0</td>
</tr>
<tr>
<td>Alanine</td>
<td>15.7</td>
</tr>
<tr>
<td>Cysteine</td>
<td>3.7</td>
</tr>
<tr>
<td>Valine</td>
<td>15.0</td>
</tr>
<tr>
<td>Methionine</td>
<td>3.7</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>11.3</td>
</tr>
<tr>
<td>Leucine</td>
<td>19.7</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>6.0</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>7.3</td>
</tr>
<tr>
<td>Histidine</td>
<td>2.0</td>
</tr>
<tr>
<td>Lysine</td>
<td>12.0</td>
</tr>
<tr>
<td>Arginine</td>
<td>5.3</td>
</tr>
<tr>
<td>Total</td>
<td>249.4 residues</td>
</tr>
</tbody>
</table>

molecular weight of 23,500 which was estimated by Sephadex G-75 gel filtration. GM₂-activator was found to contain 25% of amino acids in aspartic and glutamic acids. The amount of amide associated with these two amino acids was not determined. Threonine, serine, glycine, and proline make up another 35% of the molecules, while the content of basic amino acids is very low. No hexosamine was detected in GM₂-activator by the amino acid analyzer.

Heat stability—GM₂-activator is moderately heat-stable up to 50 °C in the crude states such as the preparations obtained at Sephadex G-200 gel filtration and DEAE-Sephadex A-50 chromatography. However, it became very heat-unstable after being highly purified. The GM₂-activator preparation obtained at the second octyl-Sepharose step lost 40–50% of its activity at 37 °C in 30 min. This inactivation could be partially

FIG. 3. Agarose isoelectric focusing of the highly purified GM₂-activator. The upper panel shows the isoelectric focusing pattern of 10 μg of bovine serum albumin (A) and 10 μg of GM₂-activator obtained from the second octyl-Sepharose column (B). The pH gradient was formed by using Ampholine of pH 3–6. The gel was stained with Coomassie blue. The lower panel shows the stimulatory activity for GM₂ ganglioside hydrolysis and the pH gradient in a parallel gel.

FIG. 4. Effect of ovalbumin and β-hexosaminidase A on the hydrolysis of GM₂ ganglioside. (A), effect of the purity of β-hexosaminidase A on the hydrolysis of GM₂ ganglioside; ——, the highly purified β-hexosaminidase A (specific activity, 150) obtained from the affinity column; ——, the partially purified β-hexosaminidase A (specific activity, 30) obtained from a Con A-Sepharose column; ——, the highly purified β-hexosaminidase A in the presence of 25 μg of ovalbumin. (B), the effect of the concentration of ovalbumin on the hydrolysis of GM₂ ganglioside. Each point in (A) and (B) was analyzed under standard assay condition using 0.5 unit of the highly purified β-hexosaminidase A and 0.8 μg of the highly purified activator.
to the same extent as the partially purified enzyme. This must be due to the fact that the partially purified β-hexosaminidase A or Gm2-activator preparations contained a protein (or proteins) which could stabilize the Gm2-activator. Fig. 4B shows the effect of ovalbumin on the hydrolysis of Gm2 ganglioside by the highly purified β-hexosaminidase A in the presence of Gm2-activator. Twenty-five μg of ovalbumin or bovine serum albumin was sufficient to stabilize the system to give the maximal hydrolysis. Table III summarizes the effect of various substances on the hydrolysis of Gm2 ganglioside carried out by the highly purified activator and β-hexosaminidase A. Ovalbumin (50 μg), Gm2-activator (12 μg), and sodium taurodeoxycholate (200 μg) were the most effective, followed by asialo-α1-acid glycoprotein (50 μg) and bovine serum albumin (50 μg). Interestingly, the intact α1-acid glycoprotein (50 μg) was not effective at all. Except for sodium taurodeoxycholate, none of the substances listed in Table III could exert a significant stimulation on the hydrolysis of Gm2 ganglioside in the absence of the activator. The hydrolysis of Gm2 ganglioside in the presence of sodium taurodeoxycholate alone, without the activator, was only less than one-half of that in the presence of both sodium taurodeoxycholate and the activator.

**Effect of Gm2-Activator on the Hydrolysis of Gm2 Ganglioside**—Under our standard assay condition, using the best grade of β-hexosaminidase A and Gm2-activator in the presence of 25 μg of ovalbumin, the hydrolysis of Gm2 ganglioside was proportional to the amount of Gm2-activator up to 0.4 μg of the activator per 0.2 ml of incubation mixture (Fig. 4A). Above the amount, the rate of Gm2 ganglioside hydrolysis started to taper off. At a substrate concentration of 10 nmol/200 μl, the rate of hydrolysis of Gm2 ganglioside, expressed as nanomoles of Gm2 ganglioside hydrolyzed per h, depends on both the amount of β-hexosaminidase A and Gm2-activator as expressed by a three-dimensional stoichiometric relationship shown in Fig. 5. When only 0.1 unit of β-hexosaminidase A was used, the maximal hydrolysis of Gm2 ganglioside was only 1.8 nmol; the increase in the amount of Gm2-activator could not bring the corresponding increase in the hydrolysis of Gm2 ganglioside. Similarly, when only 0.1 μg of Gm2-activator was used, the maximal hydrolysis of Gm2 ganglioside hydrolysis was 0.9 nmol; the increase of β-hexosaminidase A could not bring about the proportional increase in the hydrolysis of Gm2 ganglioside. Using 0.8 μg of Gm2-activator and 0.5 unit of β-hexosaminidase A, the maximal hydrolysis of Gm2 ganglioside was 5.5 nmol. The maximal rate of Gm2 ganglioside hydrolysis was obtained when the molar ratio of β-hexosaminidase A to Gm2-activator was close to 1:1. From the results presented in Table III, it is evident that the rate of Gm2 ganglioside hydrolysis is influenced by the amount of Gm2-activator as well as the amount of hexosaminidase A.

### Table III

**Effect of nonspecific substances on the hydrolysis of Gm2 ganglioside**

The rate of the hydrolysis of Gm2 ganglioside was determined under the standard assay condition using 0.5 unit of the highly purified β-hexosaminidase A from affinity column and 0.2 μg of the highly purified Gm2-activator from the second octyl-Sepharose column. The mixture was incubated at 37 °C for 3 h.

<table>
<thead>
<tr>
<th>Standard assay condition with the addition of Gm2 ganglioside hydrolyzed</th>
<th>Without Gm2-activator</th>
<th>With Gm2-activator</th>
<th>nmol/h/unit β-hexosaminidase A</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0.04</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td>Ovalbumin, 50 μg</td>
<td>0.53</td>
<td>2.51</td>
<td></td>
</tr>
<tr>
<td>Albumin, 50 μg</td>
<td>0.04</td>
<td>1.66</td>
<td></td>
</tr>
<tr>
<td>α1-Acid glycoprotein, 50 μg</td>
<td>0.04</td>
<td>0.32</td>
<td></td>
</tr>
<tr>
<td>Asialo-α1-acid glycoprotein, 50 μg</td>
<td>0.13</td>
<td>1.79</td>
<td></td>
</tr>
<tr>
<td>Gm2-activator (12), 20 μg</td>
<td>0.30</td>
<td>2.53</td>
<td></td>
</tr>
<tr>
<td>Sodium taurodeoxycholate, 200 μg</td>
<td>1.06</td>
<td>2.71</td>
<td></td>
</tr>
</tbody>
</table>

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**Specificities of Gm2-activator and sodium taurodeoxycholate**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>β-Hexosaminidase A</th>
<th>Gm2</th>
<th>Asialo-Gm2</th>
<th>GbOse4Cer</th>
<th>Oligosaccharide</th>
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<tbody>
<tr>
<td>Activity control (unit)</td>
<td>1.00</td>
<td>0.80</td>
<td>0.72</td>
<td>0.00</td>
<td>0.00</td>
</tr>
<tr>
<td>Activity with sodium taurodeoxycholate (unit)</td>
<td>1.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*The control experiment represents the hydrolysis of Gm2 ganglioside by 0.5 unit of β-hexosaminidase A in the presence of 25 μg of ovalbumin at 37 °C for the indicated time in the parentheses. The amount of activator added was 0.8 μg and sodium taurodeoxycholate, 200 μg.*

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**Effect of α1-Acid Glycoprotein on the Hydrolysis of Gm2 Ganglioside**—Under our standard assay condition, the best grade of β-hexosaminidase A and Gm2-activator in the presence of 25 μg of α1-acid glycoprotein, the hydrolysis of Gm2 ganglioside was proportional to the amount of Gm2-activator up to 0.4 μg of the activator per 0.2 ml of incubation mixture (Fig. 4A).

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A Protein Activator for GM$_3$-Hydrolysis

The hydrolysis of Gm$_3$ ganglioside by placental $\beta$-hexosaminidase A and B was our unpublished observation.
enzymic hydrolysis of glycosphingolipids is one of the important developments in the field of glycolipid catabolism. Complete delineation of the biological roles of protein activators will lead to a better understanding of the catabolism of glycosphingolipids as well as the pathogenesis of sphingolipidoses.

REFERENCES