**G\textsubscript{M1} Ganglioside \(\beta\)-Galactosidase A**

PURIFICATION AND STUDIES OF THE ENZYME FROM HUMAN LIVER*

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**SUMMARY**

\(G_{M1}\)-ganglioside \(\beta\)-galactosidase A (EC 3.2.1.23) has been purified 17,000-fold from human liver. The enzyme appeared as a single band of protein on polyacrylamide gel electrophoresis. Double immunodiffusion of rabbit anti-\(\beta\)-galactosidase A antiserum against either \(G_{M1}\) \(\beta\)-galactosidase A or liver supernatant gave a single precipitin band. The apparent molecular weight was 65,000 to 75,000 by gel filtration of the native enzyme and 72,000 by sodium dodecyl sulfate gel electrophoresis of the denatured, reduced, and carboxymethylated enzyme. The purified enzyme liberated \(\beta\)-galactose from synthetic chromogenic and fluorogenic \(\beta\)-galactosides, as well as lactose, \(N\)-acetyllactosamine, and the glycoprotein asialofetuin. The same catalytic site(s) appeared to be responsible for the hydrolysis of \(G_{M1}\)-ganglioside and 4-methylumbelliferyl-\(\beta\)-D-galactopyranoside. The enzyme also cleaved \(\alpha\)-D-fucoside and \(\alpha\)-L-arabinoside linkages. Lactulose, galactosyl hydroxylysino, galactosyl-hydroxylysyl peptides, galactocerebroside, lactosylceramide, and monogalactosyl diglyceride were very poor substrates. Anti-\(G_{M1}\)-galactosidase A antiserum quantitatively precipitated both \(\beta\)-galactosidase A and \(\beta\)-galactosidase B. Neutral \(\beta\)-glucosidase ("nonspecific" \(\beta\)-galactosidase), galactocerebroside, \(\beta\)-galactosidase, and lactosylceramide \(\beta\)-galactosidase were not precipitated by anti-\(G_{M1}\)-galactosidase A antiserum.

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A large number of mammalian \(\beta\)-D-galactosidases (EC 3.2.1.23) has been detected in various tissues (see Refs. 1 and 2 for review). These have been implicated in the degradation of carbohydrates (3, 4), glycolipids (5, 6), glycoproteins (7), and glycosaminoglycans (8). Studies of the aglycone specificity of these enzymes are incomplete. Such studies are difficult due to the multiplicity of \(\beta\)-galactosidases present, each of which may hydrolyze a number of substrates. We have identified previously two \(G_{M1}\)-ganglioside\(^2\) \(\beta\)-galactosidases in human liver, a major form termed "A" (mol wt 60 to 70 \(\times\) 10^4) and a minor form "B" (mol wt 600 to 800 \(\times\) 10^4) (10). Ho et al. reported similar results at the same time (11). \(\beta\)-Galactosidases have been purified to varying degrees from rabbit brain (12), calf intestine (3), human liver (13, 14), and bovine testis (8). These enzymes have been defined usually by their activity on low molecular weight chroomogenic and fluorogenic \(\beta\)-galactosides. Reports of the activities of these preparations with natural substrates such as lactose (3, 4, 12), Gal-Cer (6, 13), Lac-Cer (12, 13), \(G_{M1}\)-ganglioside and its asialo-derivative \(G_{A1}\) (8, 10, 11, 13), and various glycoproteins (8) have differed.

Thus far no studies have appeared in which a human \(\beta\)-galactosidase has been purified and studied with respect to its kinetics, specificity, and structure. This information is necessary for a better understanding of those mutations which affect \(G_{M1}\) \(\beta\)-galactosidase activities in humans, the \(G_{M1}\)-gangliosidoses (15, 16). We present here the purification and kinetic, structural, and immunochemical studies of the major human liver \(G_{M1}\) ganglioside \(\beta\)-galactosidase.
Liquid scintillation counting and radioisans of thin layer chromatography plates were carried out as described previously (10). All of the operations were performed at 1-4°C unless otherwise stated. Protein was measured by the method of Lowry et al. (19) following base digestion in the case of homogenates. Bovine serum albumin was used as the protein standard. Sodium chloride concentrations in column eluents were determined by conductometric titrations of standard sodium chloride solutions. Solutions were concentrated by ultrafiltration using UM-10 membranes (Amicon) at 50 p.s.i.

Liver tissues, obtained at autopsy from adult male patients who died from trauma were stored at -20°C for up to 6 months before use. These appeared disease-free on gross inspection. No alteration in the specific activity of 4-methylumbelliferyl β-galactosidase occurred during this time.

Buffer I was 500 mM sodium citrate-10 mM sodium chloride-1 mM EDTA, pH 4.00. Buffer II was 10 mM sodium phosphate-10 mM sodium chloride-1 mM EDTA, pH 7.00.

**Enzyme Assays**—4-Methylumbelliferyl β-galactosidase and neutral β-glucosidase were assayed as reported previously (20). 4-Methylumbelliferyl α-L-arabinosidase was assayed in the same way except that 4-methylumbelliferyl α-L-arabinosidase activity was measured in the same assay system as that for 4-methylumbelliferyl β-galactosidase except for the use of a spectrophotometric rather than fluorometric measurement. Absorbances were determined at the following wavelengths for the aglycones shown: phenyl, 285 nm; o- and m-nitrophenyl, 420 nm; p-nitrophenyl, 400 nm; 1-naphthyl, 322 nm; and 2-naphthyl, 330 nm. Extinction coefficients for these aglycones were determined under conditions of maximum fluorescence from disaccharide substrates (Gal-β-Hyl and Glc-β-Hyl peptides, incubations were carried out as for 4-methylumbelliferyl β-galactosidase (20). Galactose release was measured as follows: 0.5 ml of 0.25 M Tris chlorico, pH 8.0, was added, the tubes were immersed in a boiling water bath for 2 min and then cooled in an ice bath. One-half milliliter of 5 mM NAD and 50 μg per ml of galactose dehydrogenase was added. The mixture was incubated at 37°C for 15 min. NADH production was then measured fluorometrically as for 4-methylumbelliferyl β-galactosidase.

Asialofetuin β-galactosidase assays were carried out as described previously using (Gal-1→4)asialofetuin (21). GM₃-ganglioside β-galactosidase was measured as described previously (10), except that for some assays, the DEAE-cellulose microcolumn was reduced in size to 0.9 x 2.0 cm, the volume of 10 mM galactose washes was reduced to 50 μl and these were frozen at -20°C in tightly sealed capillary tubes prior to use. The enzyme did not lose activity in this state for at least 1 month.

Pooled fractions from the DEAE-cellulose column shown in Fig. 1 were concentrated to 5.0 ml and applied to a Sephadex G-150 column as described in Fig. 2. Pooled β-galactosidase A activity from the Sephadex G-150 chromatography was concentrated to 4.8 ml and applied to ECTEOLA-cellulose ("ultrapurified" as described by the manufacturer) as in Fig. 3. The β-galactosidase activity was pooled as in Fig. 3 and concentrated to 1.2 ml which was dialyzed overnight against 4 liters of Buffer II. Aliquots of 5 to 50 μl were then frozen at -20°C in tightly sealed capillary tubes prior to use. The enzyme did not lose activity in this state for at least 1 month.

Pooled β-galactosidase B activity from Sephadex G-150 chromatography (Fig. 2) was concentrated to 5.0 ml and applied to a column of Sepharose 6B as described previously for liver supernatant (20), except that the dialysis buffer included 10 mM NaCl. The pooled fractions of B enzyme were concentrated to 1.0 ml and stored at -20°C as for the A enzyme.

Polyacrylamide gel electrophoresis was carried out in the anionic system of Williams and Reisfeld, modified by the inclusion of 1 mM NaCl in the stacking gel buffer (23). To detect any enzymatic activity on the gels, they were sliced into 0.12 cm sections and the enzyme activity was assayed fluorometrically. Absorbances were determined at the following wavelengths for the aglycones shown: phenyl, 285 nm; o- and m-nitrophenyl, 420 nm; p-nitrophenyl, 400 nm; 1-naphthyl, 322 nm; and 2-naphthyl, 330 nm.

Neutral β-glucosidase were assayed as reported previously (20). ρ-Galactosidase assays were carried out as described previously except for the use of a spectrophotometric rather than fluorometric measurement. Absorbances were determined at the following wavelengths for the aglycones shown: phenyl, 285 nm; o- and m-nitrophenyl, 420 nm; p-nitrophenyl, 400 nm; 1-naphthyl, 322 nm; and 2-naphthyl, 330 nm. Extinction coefficients for these aglycones were determined under conditions of maximum fluorescence from disaccharide substrates (Gal-β-Hyl and Glc-β-Hyl peptides, incubations were carried out as for 4-methylumbelliferyl β-galactosidase (20). Galactose release was measured as follows: 0.5 ml of 0.25 M Tris chlorico, pH 8.0, was added, the tubes were immersed in a boiling water bath for 2 min and then cooled in an ice bath. One-half milliliter of 5 mM NAD and 50 μg per ml of galactose dehydrogenase was added. The mixture was incubated at 37°C for 15 min. NADH production was then measured fluorometrically as for 4-methylumbelliferyl β-galactosidase.

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Pooled β-galactosidase B activity from Sephadex G-150 chromatography (Fig. 2) was concentrated to 5.0 ml and applied to a column of Sepharose 6B as described previously for liver supernatant (20), except that the dialysis buffer included 10 mM NaCl. The pooled fractions of B enzyme were concentrated to 1.0 ml and stored at -20°C as for the A enzyme.

**Immunological Methods**—Male Norwegian white rabbits weighing about 2 kg were given injections of 60 μg of 17,000-fold purified β-galactosidase A (35 μg in each footpad) in complete Freund's adjuvant on Day 0. On day 10, 21 μg in incomplete Freund's adjuvant on Day 30. Double immunodiffusion was performed at 1-4°C on agar plates (Hyland 085.073C) and read after 40 hours.

**Identification of Reaction Products**—To identify the lipid reaction products of the action of β-galactosidase A on GM₃, 87 μg of silicone enzyme were incubated for 15 min in 100 μl of the standard assay system (10). A control lacking enzyme was also incubated. Then, 0.4 ml of chloroform-methanol (2:1) n ammonia (4:1:0.05) and 0.25 ml of water were added to each tube. Samples were mixed and centrifuged at 500 x g for 5 min. The lower phase was dried, redissolved in 50 μl of chloroform-methanol (2:1) and 20 μl were applied to a silica thin layer chromatography plate (Brinkmann 5763) alongside standards of GM₃, GM₁, and sodium taurocholate. The thin layer chromatography plate was developed in n-propyl alcohol-water (7:3) and visualized with an orcinol spray (24).

**RESULTS**

**Purification**—The purification of β-galactosidase A is described under "Methods" and Figs. 1 to 3 and is summarized in Table 1. Unless otherwise stated, all of the results in this report, except those of Table 1, were obtained either with the most highly purified preparation of Table 1, or of a second preparation of enzyme purified by the same method which had a specific activity of 45.0 μmol per mg per min. Although β-galactosidase A and B co-elute from DEAE-cellulose, they are separable on DEAE-Sephadex A-25 by salt gradient elution with β-galactosidase B being bound more tightly than β-galactosidase A. When β-galactosidase A was subjected to gel filtration on Sepharose 6B or Sephadex G-100 or starch gel electrophoresis (10), no β-galactosidase B activity could be detected. Puriﬁed β-galactosidase A had the same electrophoretic mobility on gel as the A band in the initial liver supernatant.

**ACKNOWLEDGMENT**

FIG. 1. DEAE-cellulose chromatography of human liver \( \beta \)-galactosidase. Dialyzed supernatant (220 ml) from the pH 4.6 precipitation step was applied to a DEAE-cellulose column (2.5 × 20 cm) pre-equilibrated with Buffer II. After all of the supernatant had been applied, Buffer II alone was run on (indicated by begin wash). At the point marked begin NaCl gradient, a gradient of NaCl in Buffer II was begun. Tubes of \( \beta \)-galactosidase shown by the horizontal bar were pooled. The flow rate was 1.3 ml per min and 5.6-ml fractions were collected. 4-Methylumbelliferyl (4\text{MU}) \( \beta \)-galactosidase, \( \bullet \bullet \); protein (A\text{280}), \( \bigcirc \bigcirc \); NaCl gradient, \( \triangle \triangle \).

FIG. 2. Sephadex G-150 chromatography of human liver \( \beta \)-galactosidases. Five milliliters of the concentrated pool of activity from DEAE-cellulose was applied to a Sephadex G-150 bed (2.5 × 98 cm) pre-equilibrated with Buffer II and eluted with the same buffer. \( \beta \)-Galactosidases A and B were pooled separately as shown by the horizontal bars. The flow rate was 0.4 ml per min and 2.7-ml fractions were collected. 4-Methylumbelliferyl (4\text{MU}) \( \beta \)-galactosidase, \( \bullet \bullet \); protein (A\text{280}), \( \triangle \triangle \).

FIG. 3. ECTEOLA-cellulose chromatography of human liver \( \beta \)-galactosidase A. The concentrated \( \beta \)-galactosidase A activity (4.8 ml) from Sephadex G-150 was applied to a ECTEOLA-cellulose bed (0.9 × 98 cm) pre-equilibrated with Buffer II and washed with the same buffer up to the point indicated by begin NaCl gradient. A gradient of NaCl in Buffer II was applied. Tubes shown by the horizontal bar were pooled. The flow rate was 0.3 ml per min and fractions of 3.0 ml were collected. 4-Methylumbelliferyl (4\text{MU}) \( \beta \)-galactosidase, \( \bullet \bullet \), NaCl gradient, \( \bigcirc \bigcirc \).
enzymes. The proportion of the two is estimated to be 85% A and 15% B from gel filtration of the supernatant on Sepharose 6B as described previously (10).

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Volume</th>
<th>Activity</th>
<th>Protein</th>
<th>Specific activity</th>
<th>Purification</th>
<th>Recovery</th>
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<tr>
<td>Homogenate</td>
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<td>833</td>
<td>315,000</td>
<td>0.0025</td>
<td>1</td>
<td>100</td>
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<tr>
<td>Supernatant</td>
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<td>774</td>
<td>152,000</td>
<td>0.0051</td>
<td>2.0</td>
<td>93</td>
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<td>Concanavalin A-Sepharose</td>
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<td>444</td>
<td>1,920</td>
<td>0.23</td>
<td>T</td>
<td>54</td>
</tr>
<tr>
<td>pH 4.6 Supernatant</td>
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<td>315</td>
<td>352</td>
<td>0.89</td>
<td>342</td>
<td>38</td>
</tr>
<tr>
<td>DEAE-cellulose</td>
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<td>64</td>
<td>2.5</td>
<td>970</td>
<td>19</td>
</tr>
<tr>
<td>Sephadex G-150</td>
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<td>10.0</td>
<td>9.5</td>
<td>3,660</td>
<td>12</td>
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<tr>
<td>ECTEOLA-cellulose</td>
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<td>1.0</td>
<td>44.5</td>
<td>17,100</td>
<td>5.4</td>
</tr>
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</table>

* α-Galactosidases A and B are separated on this column. Activities prior to this step therefore refer to a mixture of the A and B enzymes. The proportion of the two is estimated to be 85% A and 15% B from gel filtration of the supernatant on Sepharose 6B as described previously (10).

**Table I**

**Purification of β-galactosidase A**

- α-Galactosidases A and B are separated on this column. Activities prior to this step therefore refer to a mixture of the A and B enzymes. The proportion of the two is estimated to be 85% A and 15% B from gel filtration of the supernatant on Sepharose 6B as described previously (10).

**Fig. 4 (left).** Polyacrylamide gel of human liver β-galactosidase A in the presence of sodium dodecyl sulfate, stained with Coomassie blue. β-Galactosidase A (25 μg) was denatured, reduced, and carboxymethylated and subjected to electrophoresis in a 5% polyacrylamide gel by the method of Weber et al. (26). The cathode and anode are uppermost. The gels were 7 cm long prior to staining.

**Fig. 5 (right).** Double immunodiffusion (Ouchterlony) plate of β-galactosidase A and liver supernatant with anti-β-galactosidase A antiserum (center well). The procedure is described under "Methods." Contents of the wells were: 1, β-galactosidase A (25 ng); 2, β-galactosidase A (60 ng); 3, liver supernatant diluted to 0.044 μmol per ml per min of 4-methylumbelliferyl β-galactosidase activity; 4, β-galactosidase A (20 ng); 5, liver supernatant, 0.44 μmol per ml per min of 4-methylumbelliferyl β-galactosidase activity. Liver supernatant was prepared by Method B of Ref. 10 except for the use of a 1:1 (w/v) homogenate. Dilutions were in 10 mM sodium phosphate-10 mM NaCl, pH 7.0. Each well contained 5 μl. No precipitin bands were seen either when control or preimmunization sera were used or when β-galactosidase A was replaced by 10 mM sodium phosphate-10 mM NaCl, pH 7.0.

portions, of the A or B enzymes could be found at the following NaCl concentrations: 5 mM, 10 mM, 100 mM, and 500 mM or 10 mM NaCl containing 1 mM dithiothreitol.

Gel electrophoresis of denatured β-galactosidase A was carried out to detect the presence of protein subunits (Fig. 4). The enzyme was either denatured in guanidine, reduced, and carboxymethylated (26), or heated with sodium dodecyl sulfate and mercaptoethanol prior to electrophoresis (26). Migration relative to bromphenol blue was the same whether denaturation was accompanied by carboxymethylation (0.59 and 0.60 for two samples) or was not (0.60 and 0.61 for two samples). When Buffer II alone was carried through either of the denaturation protocols and subjected to electrophoresis, no staining of the gels was observed. Migration relative to polypeptides of known molecular weights (Fig. 6) corresponded to a molecular weight of 72,000. This is in good agreement with that found above by gel filtration of native β-galactosidase A and also that found previously for A activity in liver supernatant (10), (mol wt 60,000 to 70,000). These results suggest that β-galactosidase A consists of a single polypeptide chain of molecular weight 72,000.

**Comparison with Other β-Galactosidases**—Previous work by us (10) and Ho et al. (11) has indicated the identity of 4-methylumbelliferyl β-galactosidase A and GM 2, β-galactosidase A. Our present results further confirm this finding, showing that both activities co-purify (Table II). In contrast, β-galactosidase activities against Gal-Cer, Lac-Cer, and monogalactosyldipalmitin do not co-purify with GM 2, β-galactosidase, indicating that these activities are due to other enzymes.

**Gm 2 β-Galactosidase Activity**—Using the purified enzyme, activity was linear with respect to time and quantity of the enzyme protein over the ranges shown in Fig. 7. Galactose was the only radioactive product of the reaction (Fig. 8). Examination of the lipid products of the reaction by thin layer chromatography showed Gm 2 to be the only nonradioactive product.

**Michaelis-Menten Constants**—A study of the apparent Michaelis-Menten parameters of purified β-galactosidase A with 18 substrates indicated that the structural requirements for the aglycone are not stringent (Table III). However, the enzyme did not cleave lactulose, Gal-Hyl, or Gal-Hyl peptides.

**Competition between Gm 2-Ganglioside and 4-Methylumbelliferyl β-Galactoside**—The hydrolysis of an equimolar mixture of Gm 2-ganglioside and 4-methylumbelliferyl β-galactoside was studied over the range 0.0125 to 0.2 mM (individual substrate concentrations). Mixtures were assayed for the production of [1H]galactose and 4-methylumbelliferyl. The rate of formation of the
Table II
Specific activities of β-galactosidases during purification
Purification shown here is not the same one as that in Table I.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>β-Galactoside substrates</th>
<th>4-methylumbelliferyl galactoside</th>
<th>Gm1-ganglioside</th>
<th>Gm1-ceramide</th>
<th>Monogalactosyldi-palmitin</th>
<th>monogalactosylceramide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>µmol/mg min</td>
<td>nmol/mg min</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Supernatant</td>
<td>0.0084</td>
<td>0.0011</td>
<td>0.046</td>
<td>0.012</td>
<td>0.0046</td>
<td></td>
</tr>
<tr>
<td>Concanavalin A-Sepharose</td>
<td>0.28</td>
<td>0.032</td>
<td>1.15</td>
<td>0.31</td>
<td>0.64</td>
<td></td>
</tr>
<tr>
<td>ECTEOLA-cellulose</td>
<td>39.5</td>
<td>6.4</td>
<td>20.3</td>
<td>0.458</td>
<td>0.66</td>
<td></td>
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</tbody>
</table>

The migration of protein and bromophenol blue on polyacrylamide gels is shown in Fig. 6 (left). Comparison of the mobilities of denatured, reduced, and carboxymethylated β-galactosidase A and molecular weight standards on polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulfate. Five per cent acrylamide gels were used as described by Weber et al. (26). Standards (purchased from Sigma Chemical Co.) were: phosphorylase a from rabbit muscle; bovine serum albumin; ovalbumin (grade V), and bovine pancreatic α-chymotrypsinogen A. Standards were heated in sodium dodecyl sulfate and mercaptoethanol prior to electrophoresis (26). About 10 µg of each were applied to a gel.

![Diagram of protein migration](http://www.jbc.org/)

Fig. 7 (right). a, effect of time of incubation on galactose release from Gm1-ganglioside by β-galactosidase A. β-Galactosidase A, 0.43 ng, was incubated for the times shown in the assay system described under “Methods.” b, effect of enzyme protein incubated on galactose release from Gm1 by β-galactosidase A. Incubations were for 15 min in the assay system described under “Methods.” All of the incubations were in duplicate.

![Diagram of galactose release](http://www.jbc.org/)

Fig. 8. Examination of the radioactive products of reaction of β-galactosidase A and Gm1-ganglioside. Gm1 was incubated either with β-galactosidase A (123 ng) (●) or without (○) for 15 min in the standard Gm1, β-galactosidase A assay system. The reactions were then cooled in ice and 10 µl were applied to a cellulose thin layer chromatography plate (Eastman 6064) which was developed in n-propyl alcohol-water (7:3) and then radioautographed as described previously (10). Recovery of radioactivity from each sample exceeded 98%.
The following physical and kinetic properties of the purified enzyme were described (Table III). Analysis of the products of the reaction with β-galactosidase A, [Gal-βH]-GM₃, and 0.25 mM glucose present revealed that galactose release was inhibited by 55% of the level found in the absence of glucose. Approximately 10% of the radioactivity released appeared as compounds migrating slower than galactose. None of these compounds co-migrated with lactose and they had mobilities relative to lactose of 0.06, 0.67, and 1.35. Further characterization of these products was not attempted.

Inhibitors of GM₃ β-Galactosidase A—A number of galactose analogs, glycosides, and lipids inhibited the activity of GM₃ β-galactosidase A (Table IV). Inhibition by lipid did not necessarily relate to a structural resemblance to the galactosyl moiety; L-α-lecithin and GM₃-ganglioside were the most potent lipid inhibitors.

**DISCUSSION**

Reports on the substrate specificities of β-galactosidases which act on synthetic substrates, disaccharides, and galactolipids have presented a confusing picture. In part, this may be due to the deductions made from the use of impure preparations. The preparation of highly purified β-galactosidase A has allowed more precise characterization of the specificity of this enzyme.

The following physical and kinetic properties of the purified and crude β-galactosidase A (10) showed no significant differences: electrophoretic mobility on starch gels; apparent molecular weight on gel filtration; and apparent Kₘ with either GM₃-gan-
Galactosidase B. <0.5 0.7
Galactosidase A. 3.1 0.7

zymatic activity which was not precipitated after incubation with control antisera.

Figure gives the percentage of enzyme activity which was not precipitated after incubation with anti-β-galactosidase A antisera compared to control antisera.

<table>
<thead>
<tr>
<th>Enzyme source</th>
<th>GM1-ganglioside</th>
<th>4MU β-galactoside</th>
<th>Gal-Cer</th>
<th>Lac-Cer</th>
<th>Neutral 4MU β-glucoside</th>
</tr>
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<tbody>
<tr>
<td>β-Galactosidase A . . . .</td>
<td>3.1</td>
<td>0.7</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>β-Galactosidase B . . . .</td>
<td>&lt;0.5</td>
<td>0.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Liver supernatantb . . . .</td>
<td>&lt;0.5</td>
<td>13.7</td>
<td>112</td>
<td>86</td>
<td>88</td>
</tr>
</tbody>
</table>

* 4MU represents 4-methylumbelliferyl.
* Supernatant prepared by Method B of Ref. 10.

The failure of Suzuki and Suzuki (40) to find a stimulation of β-galactosidase activity by anionic detergents is surprising and is in contrast to the findings of Ho et al. (11) and our own previous report (10). We reported previously at least a 100-fold stimulation of GM1 β-galactosidase activity by anionic and cationic detergents. The specific activities Suzuki and Suzuki report for the enzyme are consequently more than 2 orders of magnitude lower. For this reason, their conclusions relating to the properties of GM1 β-galactosidase should be treated cautiously. Miyatake and Suzuki (41), however, have reported that sodium taurocholate was required for the activity of rat brain GM1β-galactosidase.

The finding that β-galactosidase A hydrolyzed 4-methylumbelliferyl α-L-arabinoside and p-nitrophenyl-β-d-fucoside in addition to β-d-galactosides is similar to that of the *Escherichia coli* β-galactosidase (1). Substitution of Ca or Caive hydrolysis of lactosamine by hydrogen does not prevent hydrolysis, although the apparent Km increases. The recent reports that both 4-methylumbelliferyl β-d-fucosidase (42) and α-L-arabinosidase activities (16) are severely deficient in the GM1 gangliosidoses are in keeping with these properties of the enzyme.

It has recently been reported that the only form of β-fucosidase that could be detected in pig kidney hydrolyzed p-nitrophenyl-β-d-galactoside but not lactose (43).

Of the eight lipids examined, every one, except oleic acid, inhibited GM1 β-galactosidase activity. Inhibition occurred despite minimal structural resemblance of some of the lipids examined (lecithin, cholesterol, ceramide) to GM1-ganglioside. The simplest explanation is that these lipids change the structure of the micelles which are critical for optimal activity (10). Some of the effects of inhibitors reported by Ho et al. (11) are rather different to those found here, but because these workers used a different assay system and crude preparations of enzymes, a comparison with their work is difficult.

The molecular weight of the native enzyme was very similar to that found under the conditions which strongly favor subunit dissociation. This result suggests that there is only a single polypeptide chain in β-galactosidase A.

A structural relationship between β-galactosidases A and B is suggested by the following findings. Both enzyme activities are concurrently absent in the GM1-gangliosidoses (10). Both forms hydrolyze 4-methylumbelliferyl β-galactoside, GM1-ganglioside, and asialofetuin.β-β-Galactosidase B is quantitatively precipitated by anti-β-galactosidase A antisera. The failure to find precipitin bands with the B enzyme in gel diffusion experiments might be accounted for by severely restricted diffusion of the B enzyme, reflecting its high molecular weight.

Many of the pleiotropic effects of the mutation(s) of the GM1-
gangliosidoses are clarified by the substrate specificity of $\beta$-galactosidase A. The accumulation of gangliosides, oligosaccharides derived from glycoproteins, and glycopeptides is to be expected. Lac-Cer $\beta$-galactosidase and Gal-Cer $\beta$-galactosidase are not expected to be primary enzyme deficiencies in these disorders. Suzuki and Suzuki have, however, recently reported a deficiency of Lac-Cer $\beta$-galactosidase activity in the G\textsubscript{M1}\-gangliosidoses (44). In contrast, Wenger et al. (45) have found normal levels of Lac-Cer $\beta$-galactosidase in G\textsubscript{M1}-gangliosidoses type I and have recently confirmed this finding in a further six G\textsubscript{M1}-gangliosidoses livers.\(^4\) In human cerebral gray matter, Brady et al. (46) have reported increased Lac-Cer $\beta$-galactosidase. As expected from the negligible hydrolysis of Gal-Cer by $\beta$-galactosidase A, Gal-Cer $\beta$-galactosidase activity has been found to be normal or elevated in the G\textsubscript{M1}-gangliosidoses (44, 45).

The antigenic cross-reactivity of $\beta$-galactosidases A and B suggests that they possess polypeptide sequences in common and is consistent with the deficiency of both of these enzymes in the gangliosidoses. Suzuki and Suzuki have, however, recently reported a disorder in these disorders which retains cross-reactivity with fibroblasts derived from glycoproteins, and glycopeptides is to be enzygmatically inactive material in these disorders which retains cross-reactivity with $\beta$-galactosidase A.

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