Isolation and Characterization of Jack Bean $\beta$-Galactosidase*

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A simple procedure has been devised to isolate $\beta$ galactosidase from jack bean meal. The final preparation gives one major protein band in disc gel electrophoresis. The substrate specificity of this enzyme toward some natural oligosaccharides, glycoproteins, and sphingolipids has been examined in detail. Among three isomers of N-acetyllactosamine, Gal$\beta$1-6GlcNAc is most readily hydrolyzed, followed by Gal$\beta$1-4GlcNAc; while Gal$\beta$1-XGlcNAc was hydrolyzed very slowly. This property can be used to distinguish the galactose linkage in asialo-GM$_1$(Gal$\alpha$1-3Gal$\alpha$1-4Gal$\alpha$1-4Glc-Cer) and that in lacto-N-neotetraosylceramide (Gal$\beta$1-4GlcNAc$\beta$1-3Gal$\beta$1-4Glc-Cer). For hydrolyzing glycolipids, the effect of sodium taurodeoxycholate and sodium taurochenodeoxycholate on the rate of hydrolysis was carefully examined. This enzyme hydrolyzes lactosylceramide and asialo-GM$_1$ faster than G$_{M_1}$. These results suggest that in addition to the type and linkage of the penultimate sugar unit, the sugar unit at the distal position of the saccharide chain also affects the hydrolysis rate. It also readily liberates 80% of $\beta$-galactosyl units from asialo-$\alpha$, acid glycoprotein. Escherichia coli $\beta$-galactosidase on the other hand cannot hydrolyze asialo-$\alpha$, acid glycoprotein, lactosylceramide, G$_{M_1}$, asialo-G$_{M_1}$, and lacto-N-neotetraosylceramide.

The molecular weight of this enzyme is about 75,000 and the isoelectric point is pH 8.0. With $p$-nitrophenyl $\beta$-D-galactopyranoside as substrate, optimal activity occurs at pH 2.8 with glycine-HCl buffer and at pH 3.5 with citrate-phosphate buffer. With lactose as substrate, the pH optimum in these two buffers are 2.8 and 4.0, respectively. $K_m$ values for $p$-nitrophenyl $\beta$-D-galactopyranoside, $o$-nitrophenyl $\beta$-D-galactopyranoside and lactose are 0.51 mM, 0.63 mM, and 12.23 mM, respectively. Many inhibitors for this enzyme including inorganic ions, monosaccharides, and glycosides are investigated. In contrast to $E$. coli $\beta$-galactosidase, jack bean $\beta$-galactosidase is not inhibited by $p$-aminophenyl thio-$\beta$-$\beta$-$\beta$-D-galactopyranoside.

EXPERIMENTAL PROCEDURE

Materials—Jack bean meal was obtained from Nutritional Biochemical. $p$-Nitrophenyl $\beta$-D-galactopyranoside and neuraminidase from Clostridium perfringens were purchased from Sigma; isopropyl thio-$\beta$-$\beta$-$\beta$-D-galactopyranoside from Schwarz/Mann. G$_{M_1}$ and G$_{M_1}$ gangliosides were isolated from normal human brain (23). The asialo derivatives of G$_{M_1}$ and G$_{M_1}$ gangliosides were prepared by hydrolyzing the ganglioside with 1 M HCOOH for 1 hour at 100°C as previously described (8). Globoside, trihexosylceramide, and lactosylceramide were isolated from human red cell stroma (24). $\alpha$, Acid glycoprotein (orosomucoid) was isolated from human serum (25). The asialo derivative of $\alpha$, acid glycoprotein was prepared by treating the glycoprotein with neuraminidase (26).

The following compounds were generous gifts: lacto-N-neotetraosylceramide, Dr. S. Basu, University of Notre Dame, Indiana; Gal$\beta$1-4Man$_2$, Dr. B. Lindberg, Stockholm University, Sweden; Gal$\beta$1-4Man$\alpha$1-4Glc$\alpha$1-1’ceramide; Gal$\beta$1-Gal$\beta$1-1’ceramide; 2-[6-(6-aminohexanamido)hexamido] ethyl - 1-thio - $\beta$ - D - galactopyranoside, and its Sepharose 4A derivative (28), Dr. Y. C. Lee, Johns Hopkins University, Maryland; Gal$\beta$1-3GlcNAc, Gal$\beta$1-4GlcNAc, Gal$\beta$1-6GlcNAc, Dr. A. Gauhe, Max-Planck Institute,

1 The abbreviation and trivial names used are:
G$_{M_1}$, Gal$\alpha$1-4Gal$\alpha$1-3Gal$\alpha$1-4(inu-Aac)2-3)
Gal$\beta$1-4Glc$\beta$1-1’ceramide;
G$_{M_1}$GalNAc$\beta$1-4(NeuAca)2-3Gal$\beta$1-4Glc$\beta$1-1’ceramide;
globoside, Gal$\beta$1-4Gal$\alpha$1-4Gal$\beta$1-4Glc$\beta$1-1’ceramide;
trihexosylceramide, Gal$\alpha$1-4Gal$\beta$1-4Glc$\beta$1-1’ceramide;
lactosylceramide, Gal$\beta$1-4Glc$\beta$1-1’ceramide;
Cer, ceramide.

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† Recipient of Research Career Development Award 1-K04-HD 50280 from the United States Public Health Service.
Enzyme Assays—During the enzyme isolation, β-galactosidase activity was routinely assayed at 37°C by using p-nitrophenyl β-D-galactopyranoside as substrate. An enzyme solution (1 to 50 μl) was added to 1 ml of 2 mM p-nitrophenyl β-D-galactopyranoside dissolved in 0.05 M glycine-HCl buffer, pH 7.5. After incubation for preset time, 3 ml of 0.2 M sodium borate buffer, pH 9.8, were added to stop the reaction; absorbance of the resultant solution was read at 405 nm. A continuously monitored spectrophotometric assay method (30) was used to determine the $K_m$. One unit of β-galactosidase is defined as the amount of enzyme which hydrolyzes 1 μmol of p-nitrophenyl β-D-galactopyranoside/min under the conditions described above. The specific activity of the enzyme was expressed as units per mg of protein. Protein was determined by the method of Lowry et al. (31) with crystalline bovine serum albumin as standard. When lactose was used as substrate the liberated free p-glucose was determined by glucose oxidase as previously described (32).

Analytical Methods—Analytical thin layer chromatography of gangliosides and neutral sphingolipids was performed according to the procedures previously described (8, 33). For quantitative estimation of glycolipids on thin layer plates, the intensities of the bands were compared visually with that of the known quantities of the glycolipid (5). The conversion of a given glycolipid to its derivative with one less sugar unit by glycosidases was determined by following the rates of disappearance of the original glycolipid and the appearance of the new glycolipid (5). When glycoprotein, oligosaccharide, or sphingolipid was used as substrate, free β-galactose in the enzyme digests was quantitatively determined by automated anion exchange chromatography (34). Isoelectrofocusing was performed on the LKB 110-ml column using ampholine of pH 6 to 10 according to the procedure described by the manufacturer. Polycrylamide gel electrophoresis was performed according to the procedure described by Davis (55).

RESULTS

Purification of β-Galactosidase from Jack Bean Meal

Unless otherwise indicated, all operations for the enzyme isolation were carried out at 0-4°C. Centrifugation was carried out in a Sorvall RC2-B refrigerated centrifuge. Samples were routinely centrifuged for 20 min at the following speeds: 7,000 rpm for GS-3 large capacity rotor; 9,000 rpm for GSA high speed rotor; 12,000 rpm for SS-34 superspeed rotor.

Step 1. Extraction and Ammonium Sulfate Precipitation—A 200-g portion of jack bean meal was suspended in 1.2 liters of water and stirred for 2 hours at room temperature. The suspension was strained through cheesecloth. The turbid filtrate was adjusted to pH 5.5 at room temperature with 1.5 M sodium citrate, pH 2.7, and centrifuged to obtain 900 ml of extract. Solid (NH₄)₂SO₄ was added to this extract to obtain 30% saturation. After standing for 2 hours, the precipitate was removed by centrifugation and was discarded. In some batches of jack bean meal, little or no precipitate occurred at this saturation. β-Galactosidase in the supernatant was precipitated by adding solid (NH₄)₂SO₄ to 60% saturation. After standing overnight, the precipitated protein containing β-galactosidase was collected by centrifugation and dissolved in 100 ml of 0.1 M sodium phosphate buffer, pH 7.0, to obtain an opaque solution.

Step 2. Gel filtration on Sephadex G-200—A 25-ml portion of the enzyme solution obtained at Step 1 was applied to a Sephadex G-200 column (5 x 90 cm) which had been equilibrated with 0.1 M sodium phosphate buffer, pH 7.0. The column was eluted with the same buffer at 25 ml/hour. The elution profile is shown in Fig. 1. The fractions containing β-galactosidase activity as indicated by the horizontal bar were pooled and precipitated by reverse dialysis against saturated (NH₄)₂SO₄ solution. The precipitate was dissolved in 10 ml of 0.02 M sodium phosphate buffer, pH 8.0, and dialyzed exhaustively against this buffer.

Step 3. Chromatography on DEAE-Sephadex A-50—A 5-ml aliquot of the enzyme solution obtained from Step 2 was applied to a DEAE-Sephadex A-50 (2.5 x 30 cm) column previously equilibrated with 0.02 M sodium phosphate buffer, pH 8.0. The column was eluted with the same buffer and β-galactosidase emerged at the void volume. There was a small protein peak devoid of enzyme activity eluted after the β-galactosidase peak. Under this condition, those glycosidases other than β-galactosidase remained firmly bound to the column. The fractions containing β-galactosidase activity were pooled and concentrated to 2 ml by ultrafiltration using an Amicon model A2 stirred cell with a UM10 membrane.

Step 4. Affinity Chromatography—One milliliter of the enzyme solution obtained at Step 3 was exhaustively dialyzed against 0.01 M sodium citrate buffer, pH 4.0, and applied to a similarly equilibrated affinity column (1 x 12 cm) of Sepharose 4B coupled with 2-[6-(6-aminohexanamido)-hexanamido]-ethyl-1-β-thio-D-galactopyranoside. The column was subsequently eluted with the same buffer at 5 ml/hour. The β-galactosidase activity was retarded relative to the main protein peak (Fig. 2). Those fractions containing β-galactosidase activity (tubes 11 to 19) were pooled and concentrated by ultrafiltration as described in Step 3. A summary of the specific activity and recovery during the purification procedure based on 200 g of jack bean meal is given in Table 1.

General Properties of Jack Bean β-Galactosidase

Purity—When examined by polyacrylamide gel electrophoresis at pH 9.0, the enzyme preparation obtained after affinity chromatography showed one major protein band (Fig. 3). In order to locate the enzyme activity, a parallel gel column was sliced into 2 mm sections and incubated with p-nitrophenyl β-D-galactopyranoside for 16 hours. The enzyme activity was found to coincide with the position of the major protein band on stained column. At pH 4.0, the enzyme migrated as one broad diffused band. For checking the cross-contamination of other glycosidases, 0.1 unit of the purified β-galactosidase was incubated separately with the following p-nitrophenyl glycosides: α-β-D-galactopyranoside, α- and β-D-mannopyranosides, α- and β-D-glucopyranosides and α- and β-2-acetamido-2-deoxy-β-D-glucopyranosides, according to the condition for assaying the β-galactosidase for 16 hours at 37°C. No hydrolysis of the above mentioned substrates was observed. The preparation is devoid of protease activity as measured by prolonged incubation with Azoceol.

Molecular Weight and Isoelectric Point—The molecular weight of β-galactosidase, estimated from its chromatographic mobility in Sephadex G-100 column, is about 75,000 (36). The isoelectric point of β-galactosidase was found to be pH 8.0 by isoelectrofocusing using ampholine of pH 7 to 10 according to the procedure described by Vesterberg and Svensson (37).

Optimum pH—With p-nitrophenyl β-D-galactopyranoside as substrate, optimal activity of this enzyme occurred at pH 2.8 with glycine-HCl buffer, and at 3.5 with citrate phosphate buffer (38). When lactose was used, the pH optimum was 2.8.
Fraction Number

FIG. 1 (left). Sephadex G-200 filtration of β-galactosidase preparation obtained at Step 1. The enzyme solution (25 ml) containing 1.8 g of protein was applied to a Sephadex G-200 column (5 x 90 cm) which had been previously equilibrated with 0.1 M sodium phosphate buffer, pH 7.0. The column was eluted with the same buffer at a flow rate of 25 ml/hour. , absorption at 280 nm; , β-galactosidase activity expressed as the absorption at 400 nm produced by incubating 50-μl aliquots of each fraction with substrate for 10 min according to the assay conditions described in the text; 20-ml fractions were collected.

FIG. 2 (center). Affinity column chromatography of jack bean β-galactosidase. One milliliter of the enzyme solution obtained after Step 3, containing 2.8 mg of protein, was exhaustively dialyzed against 0.05 M sodium citrate buffer, pH 4.0, and applied to a column (1 x 8 cm) of 2-(6-[6-(6-amino-hexanamido)hexanamido]-ethyl-1-thio-β-D-galactopyranoside-linked Sepharose (3.3 μmol of ligands/ml of gel). The column was eluted with the same buffer. , absorption at 280 nm; , β-galactosidase activity expressed as the absorption at 400 nm produced by incubating 50-μl aliquots of each fraction with substrate for 30 min according to the assay conditions described in the text. One milliliter per fraction was collected.

FIG. 3 (right). Disc gel electrophoresis of jack bean β-galactosidase at pH 9.0. 1, crude extract (400 μg); 2, preparation obtained after Sephadex G-200 filtration (200 μg); 3, preparation obtained after DEAE-Sephadex A-50 chromatography (300 μg); 4, preparation obtained after affinity column chromatography (100 μg). The gels were stained with Amido black stain.

TABLE I

<table>
<thead>
<tr>
<th>Steps</th>
<th>Total units</th>
<th>Total proteins</th>
<th>Specific activity</th>
<th>Recovery</th>
</tr>
</thead>
<tbody>
<tr>
<td>Step 1. Extraction and (NH₄)₂SO₄ ppt</td>
<td>314</td>
<td>1010 mg</td>
<td>0.031</td>
<td>100</td>
</tr>
<tr>
<td>Step 2. Sephadex G-200 filtration</td>
<td>260</td>
<td>630 mg</td>
<td>0.41</td>
<td>83</td>
</tr>
<tr>
<td>Step 3. DEAE-Sephadex chromatography</td>
<td>140</td>
<td>18 mg</td>
<td>7.7</td>
<td>45</td>
</tr>
<tr>
<td>Step 4. Affinity chromatography</td>
<td>32</td>
<td>1.7 mg</td>
<td>18.6</td>
<td>10</td>
</tr>
</tbody>
</table>

with glycine-HCl buffer and was 4.0 with citrate-phosphate buffer. The apparent differences in the pH optimum might be attributed to difference in ionic strength of the buffers.

pH Stability and Heat Stability—The stability of β-galactosidase at various pH values was studied by placing the enzyme in 0.05 M citrate phosphate, sodium phosphate, and sodium borate buffers ranging in pH from 2 to 10 at room temperature for 15 hours. Then, the enzyme activity was assayed at 37°C as described under “Experimental Procedures.” The enzyme retained 80 to 100% activity from pH 3 to 10 but rapidly lost the activity below pH 3. At pH 2.2, it retained only 3% activity. The enzyme was stable at 60°C for 1 hour in 0.05 M sodium phosphate buffer, pH 7.0; however, at 65°C for 10 min, it lost more than 50% of activity.

Inhibition Studies—The effect of metal ions on β-galactosidase activity was investigated by preincubation of the enzyme with the inhibitor for 30 min at room temperature before the addition of p-nitrophenyl β-D-galactopyranoside. The results are summarized in Table II. Of the various metal ions tested, Ag⁺, Hg²⁺, and Zn²⁺ to a lesser extent were potent inhibitors. β-Galactosidase was not inhibited by ethylenediaminetetraacetate. This table also includes the inhibition of β-galactosidase activity by several sugar derivatives. It is of interest to note that isopropyl thio-β-D-galactopyranoside but not p-aminophenyl thio-β-D-galactopyranoside exert significant inhibitory effect on the enzyme. As shown in the same table, 2-[6-[6-(6-amino-hexanamido)hexanamido]-ethyl-1-thio-β-D-galactoside is also a potent inhibitor for jack bean β-galactosidase. This inhibitor was, therefore, chosen to prepare an affinity column for this enzyme (see Fig. 2). The following K_i values were obtained by Dixon plots (39): p-aminophenyl thio-β-D-galactoside, 46 mM; isopropyl thio-β-D-galactopyranoside, 16 mM; 2-[6-[6-(6-amino-hexanamido)hexanamido]-ethyl-1-thio-β-D-galactopyranoside, 1.3 mM; β-D-galactono-1,4-lactone, 0.1 mM. All these compounds are competitive inhibitors.

Effect of Substrate Concentration—The effects of varying substrate concentration on the reaction rate for o- and p-nitrophenyl β-D-galactopyranosides and lactose was measured at 37°C using 0.05 M sodium citrate buffer, pH 4.0. The apparent Michaelis constant (K_m) for each substrate was determined from the Lineweaver-Burk plots to be: p-nitrophenyl β-D-galactopyranoside, 0.51 mM; o-nitrophenyl β-D-galactopyranoside, 0.63 mM; lactose, 12.3 mM.

Specificity of Jack Bean β-Galactosidase—The action of jack bean β-galactosidase on various saccharides, glycoproteins and glycolipids are summarized in Table III. As shown in this table, under the condition described Galβ1-8GlcNAc was com-
Galactopyranoside. Enzyme, inhibitors and substrates were dissolved in 0.05 M sodium citrate buffer, pH 4.0.

determined at 37°C by adding 0.5 ml of 4 mM p-nitrophenyl acetyllactosamine isomers and lactose was also studied:

<table>
<thead>
<tr>
<th>Inhibitors</th>
<th>Inhibitor concentration</th>
<th>Relative activity</th>
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</thead>
<tbody>
<tr>
<td>H₂OCl₂</td>
<td>1.0</td>
<td>1</td>
</tr>
<tr>
<td>AgNO₃</td>
<td>1.0</td>
<td>27</td>
</tr>
<tr>
<td>ZnSO₄</td>
<td>1.0</td>
<td>38</td>
</tr>
<tr>
<td>CuSO₄·Mo₃O₄·MoCl₂·DaCl₂·CaCl₂</td>
<td>1.0</td>
<td>94 98</td>
</tr>
<tr>
<td>EDTA</td>
<td>1.0</td>
<td>103</td>
</tr>
<tr>
<td>d-Galactono-1,4-lactone</td>
<td>2.0</td>
<td>27</td>
</tr>
<tr>
<td>d-Galactose</td>
<td>5.0</td>
<td>34</td>
</tr>
<tr>
<td>d-Glucose</td>
<td>5.0</td>
<td>100</td>
</tr>
<tr>
<td>μ-Aminophenylthio-β-D-galactopyranoside</td>
<td>5.0</td>
<td>100</td>
</tr>
<tr>
<td>Isopropylthio-β-D-galactopyranoside</td>
<td>5.0</td>
<td>70</td>
</tr>
<tr>
<td>AHA-AHA-AES-βGalα</td>
<td>5.0</td>
<td>70</td>
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</table>

* 2-[6-(Aminohexamidino)hexamidino]-ethyl-β-D-galactopyranoside.

**Table II**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Incubation time</th>
<th>% Hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galβ1–3GlcNAc</td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Galβ1–4GlcNAc</td>
<td>10</td>
<td>75</td>
</tr>
<tr>
<td>Galβ1–2GlcNAc</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Galβ1–4Glc</td>
<td>10</td>
<td>42</td>
</tr>
<tr>
<td>Galβ1–6Man</td>
<td>10</td>
<td>19</td>
</tr>
<tr>
<td>Galβ1–6ManNac</td>
<td>10</td>
<td>10</td>
</tr>
<tr>
<td>Asialoα,β-acid glycoprotein</td>
<td>190</td>
<td>79</td>
</tr>
<tr>
<td>Lactosylceramide</td>
<td>120</td>
<td>100</td>
</tr>
<tr>
<td>G₃₃</td>
<td>120</td>
<td>9</td>
</tr>
<tr>
<td>Asialo-G₃₃</td>
<td>120</td>
<td>82</td>
</tr>
</tbody>
</table>

* Not determined.

Jack bean β-galactosidase efficiently cleaves β-D-galactosyl residues from asialo-α,β-acid glycoprotein. Approximately 80% of the total bound β-galactose was liberated within 2 hours of incubation. Prolonged incubation did not liberate more β-galactose from the glycoprotein suggesting that about 20% of the total β-galactose in this glycoprotein is resistant to jack bean β-galactosidase. This result is analogous to that of Hughes and Jeanloz (26), who found that β-galactosidase of *Diplococcus pneumoniae* released 80% of β-galactose from the asialo-α,β-acid glycoprotein. Distler and Jourdain (40) also found that β-galactosidase of bovine testis liberated 87% of β-galactose from asialo-α,β-acid glycoprotein. In agreement with Hughes and Jeanloz (26), we also found that *E. coli* β-galactosidase was not able to hydrolyze β-galactosyl units from α,β-acid glycoprotein. D-Galactosyl units in freezing point depression glycoprotein were found to be resistant to jack bean β-galactosidase. Although this glycoprotein was reported to contain terminal β-galactosyl units linked 1→4 to α-acetyl-galactosamine (29), only 3% of the D-galactose was liberated after 24 hours of incubation.

We also have examined the liberation of β-galactosyl units from a number of sphingoglycolipids by jack bean β-galactosidase. This enzyme requires the addition of a detergent such as the crude sodium taurocholate isolated from canine bile for the hydrolysis of lipid substrates. Although crude sodium taurocholate has been widely used to stimulate the enzyme hydrolysis of sphingoglycolipids, this preparation is an ill defined mixture of bile salts containing more than 10 components which often interfere with the analysis of thin layer chromatography. The stimulating effect of the crude bile salts is also inconsistent from lot to lot. We, therefore, examined various pure bile salts obtained from Calbiochem for their ability to stimulate the hydrolysis of β-galactosyl units from sphingoglycolipids. Among the sodium salts of taurocholedocholate, taurocholate, taurolithocholate, and taurodihydrocholate, we found that sodium taurochola
docholate and sodium taurodeoxycholate were most active in stimulating jack bean β-galactosidase to hydrolyze Glc₃₃, gangli
gloside and lactosylceramide. In general, sodium taurochola
docholate is better than sodium taurodeoxycholate. With 0.8 unit of the enzyme and 50 μM substrate concentration, the stimulation of Glc₃₃, gangli
gloside and lactosylceramide by sodium taurochola
docholate reached the maximum at the concentration of 300 μg/0.4 ml (1.43 mM). Rapid decrease in hydrolysis rate occurred when the detergent concentration was greater than 400 μg/0.4 ml (1.91 mM). Under the same condition, stimulation of Glc₃₃, hydrolysis by sodium taurochola
docholate reached the maximum at the concentrations of 500 μg/0.4 ml (2.39 mM). Greater concentration of taurochola
docholate did not cause significant decrease in hydrolysis rate. For the hydrolysis of lactosylcera
mide (50 μM), less detergent is required for maximal rate of hydrolysis: 200 μg/0.4 ml (0.85 mM) for sodium taurochola
docholate and 400 μg/0.4 ml (1.91 mM) for sodium taurochola
docholate. Therefore, at the substrate concentration of 50 μM, we chose 1.43 mM (300 μg/0.4 ml) sodium taurodeoxycholate for

**Table III**

Specificity of jack bean and *Escherichia coli* β-galactosidase

For the hydrolysis of N-acetyllactosamine isomers and various disaccharides, incubation mixture contained the following components in 100 μl: substrate, 0.125 μmol; 4 μmol of sodium citrate buffer, pH 4.0; enzyme, 0.75 unit.

<table>
<thead>
<tr>
<th>Substrate</th>
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<th>% Hydrolysis</th>
</tr>
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<tbody>
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<td>Galβ1–3GlcNAc</td>
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<td>Galβ1–4GlcNAc</td>
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</tr>
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<td>Galβ1–4Glc</td>
<td>10</td>
<td>42</td>
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<tr>
<td>Galβ1–6Man</td>
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<td>Galβ1–6ManNac</td>
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</tr>
<tr>
<td>Asialo-G₃₃</td>
<td>120</td>
<td>82</td>
</tr>
</tbody>
</table>

* Not determined.

completely hydrolyzed in 10 min, while 75% hydrolysis of Galβ1–4GlcNAc and virtually no hydrolysis of Galβ1–3GlcNAc were detected. The effect of aglycon moiety on the rate of hydrolysis was examined by replacing the GlcNAc residue with Glc, Man, or ManNAc. The results show a reduction of the hydrolysis because of different aglycons. For comparison, specificity of *Escherichia coli* β-galactosidase towards N-acetyllactosamine isomers and lactose was also studied:

their specificities toward Galβ1–3GlcNAc are very different. In contrast to jack bean β-galactosidase, the enzyme from *E. coli* hydrolyzes 1→3-linked isomer faster than 1→4-linked isomer. It also hydrolyzes lactose faster than jack bean β-galactosidase.
the hydrolysis of lactosylceramide and G₃M, ganglioside. As shown in Table III, jack bean β-galactosidase hydrolyzed lactosylceramide much faster than G₃M, ganglioside. Lactosylceramide was almost completely hydrolyzed in 2 hours, while only 40% of G₃M, ganglioside was hydrolyzed after 24 hours of incubation. In contrast to G₃M, ganglioside, asialo-G₃G₁ was readily hydrolyzed by jack bean β-galactosidase. E. coli β-galactosidase was not able to hydrolyze either G₃M, asialo G₃M, or lactosylceramide under a variety of conditions.

### DISCUSSION

In order for a glycosidase to be useful for the structural analysis of complex saccharide chains, it is imperative that the enzyme free from other glycosidases can be easily isolated and that detailed information about its substrate specificity is available. By using the simple procedure described above, β-galactosidase is completely separated from other glycosidases. For practical purposes, β-galactosidase isolated by DEAE-Sephadex can be used to study the structure of complex carbohydrate chains.

It is surprising that p-aminophenyl thio-β-D-galactopyranoside, an effective competitive inhibitor of E. coli β-galactosidase (41), does not inhibit the hydrolysis of p-nitrophenyl β-D-galactopyranoside by jack bean β-galactosidase even at 20 mM concentration (Table II). This lack of inhibition by aryl thio-β-D-galactopyranoside is reflected in the lack of affinity of this β-galactosidase to the affinity column prepared by succinylaminokyl group attached to phenyl thio-β-D-galactopyranoside (42). However, we found that thio-β-D-galactopyranoside attached to an alkyl aglycon exhibited competitive inhibition to this β-galactosidase.

Jack bean β-galactosidase hydrolyzes three isomers of N-acetyllactosamine at different rates. The 1→6-linked N-acetyllactosamine is most readily hydrolyzed, followed by 1→4 linked isomer. The enzyme hydrolyzes 1→3-linked N-acetyllactosamine very slowly (Table III). This property can be used to distinguish the nature of β-galactosyl linkage in asialo-G₃M (Galβ1→3GalNAcβ1→4Galβ1→4Glc--Cer), which is slowly hydrolyzed, and that in lacto-N-neotetrasaccharide (Galβ1→4GlcNAcβ1→3Galβ1→4Glc--Cer), which is rapidly hydrolyzed. Jack bean β-galactosidase is also able to distinguish the linkage of terminal β-galactosyl units in lacto-N-tetraose (Galβ1→3GlcNAcβ1→3Galβ1→4Glc) and lacto-N-neotetraose (Galβ1→4GlcNAcβ1→3Galβ1→4Glc) (10, 19). The nature of the aglycon moiety also affected the rate of hydrolysis. As shown in Table III, Galβ1→4GlcNAc was hydrolyzed faster than Galβ1→4Glc or Galβ1→4ManNAc. In contrast to jack bean β-galactosidase, bovine testes β-galactosidase hydrolyzes 1→3-linked Gal--GlcNAc faster than the 1→4 isomer, and the 1→6-linked isomer is hydrolyzed at the lowest rate. By using two β-galactosidases isolated from bovine testes and jack bean, it should be possible to distinguish the Galβ1→3GlcNAc, Galβ1→4GlcNAc, and Galβ1→6GlcNAc linkages. The specificity of E. coli β-galactosidase toward N-acetyllactosamines on the other hand, is quite different from the β-galactosidase of jack bean or bovine testes.

Jack bean β-galactosidase can readily hydrolyze β-galactosyl units from asialo-α₂-acid glycoprotein or asialo glycopeptide prepared from human α₂-protease inhibitor. However, it hydrolyzes the freezing point depression glycoprotein very slowly. The freezing point depression glycoprotein has been reported to contain Galβ1→4GalNAc--Thr sequence (29). The explanation for the resistance may lie in the β-galactosyl unit being linked to GalNAc instead of GlcNAc. Alternatively, the proximity of this disaccharide unit to the polypeptide moiety may result in a steric hindrance, unfavorable for the approach of the β-galactosidase.

When a glycosidase is used to hydrolyze the saccharide units in glycolipids, careful consideration should be given to the amount and the nature of the detergent used. As shown in Fig. 4, different detergents activate at different optimal concentrations. It seems reasonable to postulate that the situation is even more complex, namely the optimal micelle organization and architecture for presentation of substrate to enzyme may depend on the concentration of substrate and detergent and furthermore that the optimal ratio may vary with different substrate.

Jack bean β-galactosidase hydrolyzes about 40% of the intact G₃M, ganglioside in 24 hours, while under the same condition, it completely hydrolyzes the asialo-G₃M in about 3 hours. The result suggests that, in addition to the type and linkage of the penultimate sugar unit, the sugar unit at the distal position of the saccharide chain also affect the hydrolysis rate.

From the study presented in this report, it is clear that specific glycosidases are useful for the determination of both the anomeric configuration and the sequential arrangement of saccharide units in glycoproteins and glycolipids. It should be emphasized, however, that the specificities of glycosidases are rather complex. The same categories of glycosidases isolated from different sources often vary considerably in their substrate specificities. One should, therefore, interpret the results of enzyme hydrolysis with extreme caution. When used with...
care, combination of glycosidases with methylation analysis probably offers the most powerful tool available for the elucidation of the complete structure of heterosaccharide chains.

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REFERENCES

3. Li, Y.-T., and Li, S.-C. (1972) Methods Enzymol. 28, 702-713
37. McIlvaine, T. C. (1921) J. Biol. Chem. 49, 183-186
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