Blood Group Substance-degrading Enzymes
Obtained from *Streptomyces* sp.

**Part II. Purification and Characteristics of α-Galactosidase from *Streptomyces* 9917S$_2$**

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_Received August 31, 1971_

The blood group B substance-degrading activity of *Streptomyces* 9917S$_2$ is induced by galactosides as α-galactosidase activity is. Purification of the α-galactosidase was attempted by chromatography on DEAE-Sephadex and Sephadex. The purified preparation was shown to be free from α- and β-glucosidases, β-galactosidase, α- and β-glucosaminidases, and α- and β-galactosaminidases activities. The blood group B substance-degrading activity was present only in this fraction. This enzyme preparation cleaves α-(1→3)- and α-(1→6)-galactosidic linkages. The activity is inhibited by D-galactose, melibiose, and raffinose and also by L-arabinose and D-xylose.

A number of highly specific protein-sugar binding reactions playing important biological functions has been known. Among them are agglutination reactions of red blood cells with their antigens. The reactions involve various problems of biological interest besides their originally ascribed significances. Human blood group A, B, and H substances are found not only in red blood cells but also in various mucous glands and the secreted mucus. Substances with similar serological activities, such as bacterial antigens, and human tumor glycolipids, are known in other organisms. Furthermore, hemagglutinins which are either specific or nonspecific for blood groups are present in various plant and animal tissues. Recently, some of these hemagglutinins are found to agglutinate certain tumor cells and to initiate mitosis in the culture of normal human leucocytes.

We have found that various kinds of hemagglutinins are produced in large quantities by bacteria and fungi, and that some of them strongly aggregate dissociated sea urchin and sponge cells. Studies on the structure of blood group substances capable of specifically binding hemagglutinins are required.

We have previously reported that culture fluids of certain species of *Streptomyces* decompose serological activities of blood group substances in human saliva and thus interrupt hemagglutination inhibitory action of saliva. In the present report, purification and some characteristics of blood group B substance-degrading enzyme produced by *Streptomyces* 9917S$_2$ are described.

**MATERIALS AND METHODS**

**Strain used and the culture condition.** *Streptomyces* 9917S$_2$ was used throughout the present work. A loopful of spores and mycelia was inoculated into 5 ml of 0.05 M phosphate buffer (pH 6.0) containing 2% lactose, 0.5% peptone (Kyokuto), 0.1% yeast extract (Difco), and 0.05% MgSO$_4$·7H$_2$O, and incubated for 48 hr at 25°C in a test tube with shaking. This was then added into 100 ml of the above medium in a 500-ml flask and culture continued for further 40 hr at 25°C with shaking.
Cells were then removed by filtration through a filter paper (Toyo Roshi, No. 2) and the filtrate was used to isolate α-galactosidase.

**Assay of glycosidases and blood group B substance-degrading activity.** Glycosidase activities were measured as described previously. B substance-degrading activity was quantitated by the hemagglutination inhibition test as described previously, though microtiter (Taiyo Bussan Co., Ltd., Tokyo) was used in some cases.

**Paper chromatography.** Galactose liberated from various substrates by the B substance-degrading enzyme was detected by paper chromatography. Samples were placed on a filter paper (Toyo Roshi No. 50). The chromatogram was developed with n-butanol: pyridine: water (6: 4: 3), and the location of sugars was detected by spraying an ammoniacal solution of silver nitrate.

**Chemicals.** DEAE-Sephadex A-50 and Sephadex G-100 were products of Pharmacia, Uppsala. Anti-B serum was obtained from Midori-Juji Co., Ltd., Osaka. Phenyl glycosides were purchased from Nakarai Chemicals, Ltd., Kyoto. Other chemicals were of the purest grades commercially available.

**RESULTS**

1. **Glycosidase activities in the culture filtrates**

   Effect of various sugars on the production of glycosidases was first examined. Table I shows that β-galactosidase and β-N-acetylglucosaminidase were formed in all cases. α-Galactosidase, on the other hand, was detected in lactose or D-galactose containing medium but not in D-glucose containing medium, suggesting that this enzyme is the blood group B substance decomposer.

2. **Purification of α-galactosidase**

   Preliminary experiment showed that the amount of enzymes in the culture filtrate was too small to apply the salting-out with ammonium sulfate. Thus the culture filtrate was directly applied to DEAE-Sephadex and the purification of α-galactosidase was made as shown in Fig. 1.

**TABLE I. Effect of Sugar on the Production of Glycosidase Activities in Streptomyces 9917S2**

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Activity in the medium containing*&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Lac</td>
</tr>
<tr>
<td>Phenyl-α-D-glucoside&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>β-&lt;sup&gt;b&lt;/sup&gt;</td>
<td>170</td>
</tr>
<tr>
<td>α-D-galactoside&lt;sup&gt;b&lt;/sup&gt;</td>
<td>200</td>
</tr>
<tr>
<td>β-&lt;sup&gt;b&lt;/sup&gt;</td>
<td>300</td>
</tr>
<tr>
<td>α-D-glucosaminide&lt;sup&gt;b&lt;/sup&gt;</td>
<td>8</td>
</tr>
</tbody>
</table>

<sup>a</sup> Absorbance at 660 nm. One tenth ml of culture filtrate was mixed with 0.1 ml of 20 mM phenylglycoside solution in 0.1 M citrate buffer (pH 6.0) and incubated for 10 min at 35°C. At the end of incubation, 0.1 ml of 0.4 M trichloroacetic acid solution, 1.0 ml of 0.4 M Na₂CO₃ solution, and 0.1 ml of Folin reagent were added in order. After 30 min, 1.5 ml of H₂O was added to the mixture and the amount of the phenol liberated was measured colorimetrically at 660 nm.

<sup>b</sup> Minimal amount of B substance for complete hemagglutination inhibition (1, when heated culture filtrate was used). One twentieth ml of culture filtrate was mixed with 0.05 ml of B substance solution and kept at 35°C. After 6 hr, the mixture was heated for 10 min at 100°C, and hemagglutination inhibition test was made.
Culture fluid
filtration through Toyo Roshi No. 2 filter paper

Filtrate, 1.5 liter
adsorption on DEAE-Sephadex A-50 (wet 240 ml) equilibrated with 0.05 M phosphate buffer (pH 6.0) elution with 0.05 M phosphate buffer (pH 6.0) containing 0.6 M NaCl, 400 ml

Eluate
concentration with “Dia Filter” G-20T, 2 kg/cm², dialysis against 0.05 M phosphate buffer (pH 6.0) at 4°C, overnight

Dialyzed fraction
DEAE-Sephadex A-50 column chromatography (1.6 x 25 cm, equilibrated with 0.05 M phosphate buffer, pH 6.0) elution with a linear gradient of NaCl formed by 200 ml of 0.05 M phosphate buffer (pH 6.0) and 200 ml of the same buffer containing 0.6 M NaCl

Eluate
concentration and desalting with a collodion bag

Filtrated fraction
Sephadex G-100 column chromatography (2 x 48 cm, equilibrated with 0.05 M phosphate buffer, pH 6.0) re-chromatography on Sephadex G-100 (2 x 48 cm)

Purified preparation

Fig. 1. Diagram of Purification Procedures of the β-Galactosidase from Streptomyces 9917S2.

β-Galactosidase was almost completely separated from other enzyme activities (pirot experiment, Fig. 2) in the first step (DEAE-Sephadex adsorption) of the purification under the conditions employed.

In practice, 1.5 liter of the culture filtrate was added into 120 ml (wet volume) of DEAE-Sephadex A-50 equilibrated with 0.05 M phosphate buffer (pH 6.0), the mixture was stirred for 1 hr, and filtered under the reduced pressure. The filtrate was added into another 120 ml of DEAE-Sephadex A-50 and treated as above. Two batches of the DEAE-Sephadex A-50 which adsorbed enzymes were then combined and eluted with 400 ml of 0.05 M phosphate buffer (pH 6.0) containing 0.6 M NaCl.

The eluate was concentrated to about 20 ml through a “Dia Filter” G-20T (Nippon Shinku Gijutsu Co., Ltd., Chigasaki) with pressure (2 kg/cm² with argon gas) and then the concentrate was dialyzed against 0.05 M phosphate buffer (pH 6.0). The dialyzed sample was then adsorbed to DEAE-Sephadex A-50 equilibrated with 0.05 M phosphate buffer (pH 6.0) in a column (1.6 x 25 cm). The column was eluted with a linear gradient of sodium chloride (0~0.6 M) in the above phosphate buffer. Fractions (5 ml each) were collected. All the operations were carried out at 4°C.

Figure 3 shows the elution pattern of the dialyzed enzyme fraction through the DEAE-Sephadex A-50 column. β-Galactosidase activity was further removed, but α-galactosidase and β-N-acetyl-glucosaminidase were not separated from each other. Thus fractions 21~35 were combined, concentrated in a collodion bag to less than 1 ml. The concentrate was applied to a Sephadex G-100 column (2 x 48 cm) equilibrated with 0.05 M phosphate buffer. The column was eluted and fractions were

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**TABLE II. SUMMARY OF THE PURIFICATION OF α-GALACTOSIDASE FROM Streptomyces 9917S2**

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Protein (mg)</th>
<th>α-Galactosidase activity (U/mg)</th>
<th>Yield</th>
<th>Purification ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Culture filtrate</td>
<td>2210</td>
<td>51840</td>
<td>22.4</td>
<td>100</td>
</tr>
<tr>
<td>DEAE-Sephadex A-50 (1st)</td>
<td>738</td>
<td>24720</td>
<td>33.5</td>
<td>47.7</td>
</tr>
<tr>
<td>DEAE-Sephadex A-50 (2nd)</td>
<td>270</td>
<td>14040</td>
<td>52.0</td>
<td>27.1</td>
</tr>
<tr>
<td>Sephadex G-100 (1st)</td>
<td>43.2</td>
<td>7200</td>
<td>166.7</td>
<td>13.9</td>
</tr>
<tr>
<td>Sephadex G-100 (2nd)</td>
<td>15.6</td>
<td>5040</td>
<td>323.1</td>
<td>9.7</td>
</tr>
</tbody>
</table>

*α) 1 μmole phenol/10 min.

*β) Determined by the method of Lowry and Folin.
FIG. 2. DEAE-Sephadex Chromatography of the Culture Filtrate of *Streptomyces* 9917S-2.

One liter of culture filtrate was concentrated to about 50 ml with "Dia-Filter" G-20T and the concentrate was applied to a 2 × 25 cm-column of DEAE-Sephadex A-50.

- - - Absorbance at 280 mµ.
△ - △ β-Galactosidase activity.
○ - ○ α-Galactosidase activity.
× - × β-N-Acetyl-glucosaminidase activity.

FIG. 3. The Second DEAE-Sephadex Chromatography.

- - - Absorbance at 280 mµ.
△ - △ β-Galactosidase activity.
○ - ○ α-Galactosidase activity.
× - × β-N-Acetyl glucosaminidase activity.
collected as above. As shown in Fig. 4, α-galactosidase and β-N-acetyl-glucosaminidase activities were completely separated by this procedure, and the blood group B substance-degrading activity was observed only in the α-galactosidase fraction. Re-chromatography of the α-galactosidase fraction (fractions 12–17) on the same Sephadex G-100 column resulted in a single symmetrical peak of α-galactosidase activity (Fig. 5).

Table II summarizes the results. Specific activity of the α-galactosidase was increased 15-fold over the culture filtrate.

3. Properties of the purified α-galactosidase

Using the α-galactosidase preparation purified as above, properties of the enzyme were
Blood Group Substance-degrading Enzymes Obtained from Streptomyces sp. Part II

investigated, especially with regard to the blood group B substance-degrading activity. Assay procedures for glycosidase and B substance-degrading activities were as previously described except that 0.05 ml of the purified enzyme preparation (0.2 µg protein), instead of 0.2 ml of the culture filtrate, was used in the present study. Under these conditions, the rate of hydrolysis was proportional to the amount of protein up to 0.4 µg.

As shown in Fig. 6, the optimal pH was found to be 4.5-6.0 for both α-galactosidase and B substance-degrading activities. The Km

![Graph](image)

**Fig. 7.** Lineweaver-Burk Plot of the Purified Enzyme for Phenyl-α-galactoside. (pH 6.0, 35°C)

![Graph](image)

**Fig. 8.** Thermal Stability of the Purified Enzyme. Enzyme preparations were heated for 10 min, cooled, and the activities remained were measured.

- ○○ B substance degradation (Heated enzyme, n=0).
- ●● Phenyl-α-D-galactoside hydrolysis.

![Graph](image)

**Fig. 9.** Temperature-Activity Curves of the Purified Enzyme.

- ○○ B substance degradation, (Heated enzyme, n=0).
- ●● Phenyl-α-D-galactoside hydrolysis.

**Table III. Effect of Inhibitors on the Enzyme Activities**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Concentration (×10⁻³ M)</th>
<th>B Substance degradation</th>
<th>Phenyl-α-D-galactoside hydrolysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>—</td>
<td>512</td>
<td>185</td>
</tr>
<tr>
<td>EDTA</td>
<td>10</td>
<td>512</td>
<td>190</td>
</tr>
<tr>
<td>Iodoacetate</td>
<td>1</td>
<td>256</td>
<td>110</td>
</tr>
<tr>
<td>p-CMB</td>
<td>0.1</td>
<td>2</td>
<td>60</td>
</tr>
<tr>
<td>NaCN</td>
<td>1</td>
<td>256</td>
<td>205</td>
</tr>
<tr>
<td>Na₃D</td>
<td>1</td>
<td>256</td>
<td>190</td>
</tr>
<tr>
<td>NaF</td>
<td>3</td>
<td>512</td>
<td>195</td>
</tr>
<tr>
<td>α, α'-Dipyridyl</td>
<td>1</td>
<td>256</td>
<td>185</td>
</tr>
<tr>
<td>N-Ethylmaleimide</td>
<td>1</td>
<td>512</td>
<td>190</td>
</tr>
</tbody>
</table>

*Minimal amount of B substance for complete hemagglutination inhibition (1, when heated enzyme was used).

Absorbance at 660 nm. See the footnote in Table I.
value of the enzyme for phenyl-α-galactoside was $1 \times 10^{-3}$ M (Fig. 7).

The enzyme was unstable at temperatures higher than 45°C, and heating at 55°C for 10 min resulted in the complete loss of the activities (Fig. 8). In the presence of the substrate, however, the maximum α-galactosidase activity was found at 50–55°C, while the highest B substance degrading activity was at 40–50°C (Fig. 9). The difference is probably due to the different incubation period in assay procedures (10 min vs. 8 hr., see Methods).

The activities of α-galactosidase and B substance degradation were inhibited with p-chloromercuribenzoate (p-CMB). Moniodoacetate at the concentration of $1 \times 10^{-3}$ M also inhibited the enzyme activities. (Table III). Among the metallic ions examined, Ag⁺ and Hg²⁺ at the concentration $5 \times 10^{-4}$ M inhibited the enzyme activities (Table IV). These results suggest that the activities are mediated by SH-enzyme.

**TABLE IV. EFFECT OF METALLIC IONS ON THE ENZYME ACTIVITIES**

<table>
<thead>
<tr>
<th>Metal</th>
<th>Concentration ($\times 10^{-4}$ M)</th>
<th>Activity B Substance degradation (±)</th>
<th>Phenyl-α-D-galactoside hydrolysis (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>--</td>
<td>512</td>
<td>185</td>
</tr>
<tr>
<td>Ag⁺</td>
<td>5</td>
<td>8</td>
<td>145</td>
</tr>
<tr>
<td>Ba²⁺</td>
<td>5</td>
<td>512</td>
<td>190</td>
</tr>
<tr>
<td>Ca²⁺</td>
<td>5</td>
<td>512</td>
<td>190</td>
</tr>
<tr>
<td>Co²⁺</td>
<td>5</td>
<td>236</td>
<td>190</td>
</tr>
<tr>
<td>Cu²⁺</td>
<td>5</td>
<td>512</td>
<td>200</td>
</tr>
<tr>
<td>Fe³⁺</td>
<td>5</td>
<td>512</td>
<td>185</td>
</tr>
<tr>
<td>Hg²⁺</td>
<td>5</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>Mg²⁺</td>
<td>5</td>
<td>512</td>
<td>195</td>
</tr>
<tr>
<td>Mn²⁺</td>
<td>5</td>
<td>312</td>
<td>240</td>
</tr>
<tr>
<td>Ni²⁺</td>
<td>5</td>
<td>512</td>
<td>190</td>
</tr>
<tr>
<td>Pb²⁺</td>
<td>5</td>
<td>512</td>
<td>200</td>
</tr>
<tr>
<td>Sn²⁺</td>
<td>5</td>
<td>512</td>
<td>190</td>
</tr>
<tr>
<td>Zn²⁺</td>
<td>5</td>
<td>512</td>
<td>190</td>
</tr>
</tbody>
</table>

a) Minimal amount of B substance for complete hemagglutination inhibition (1, when heated enzyme was used).
b) Absorbance at 660 mµ.

Eleven sugars and 2 aminosugars were tested for their effects on the enzyme reactions. Galactose and galactose-containing sugars such as melibiose, raffinose, and D-galactosamine were used for this purpose. The effects of sugars on the enzyme activities are shown in Table V.

**TABLE V. EFFECT OF SUGARS ON THE ENZYME ACTIVITIES**

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Concentration ($\times 10^{-3}$ M)</th>
<th>Activity B Substance degradation (±)</th>
<th>Phenyl-α-D-galactoside hydrolysis (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>--</td>
<td>512</td>
<td>150</td>
</tr>
<tr>
<td>L-Ara</td>
<td>33</td>
<td>4</td>
<td>70</td>
</tr>
<tr>
<td>D-Fru</td>
<td>33</td>
<td>512</td>
<td>150</td>
</tr>
<tr>
<td>D-Gal</td>
<td>33</td>
<td>4</td>
<td>10</td>
</tr>
<tr>
<td>D-Glu</td>
<td>33</td>
<td>512</td>
<td>150</td>
</tr>
<tr>
<td>D-Man</td>
<td>33</td>
<td>512</td>
<td>150</td>
</tr>
<tr>
<td>Mel</td>
<td>33</td>
<td>4</td>
<td>35</td>
</tr>
<tr>
<td>Raf</td>
<td>33</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>L-Rha</td>
<td>33</td>
<td>512</td>
<td>150</td>
</tr>
<tr>
<td>D-Rib</td>
<td>33</td>
<td>512</td>
<td>150</td>
</tr>
<tr>
<td>L-Sor</td>
<td>33</td>
<td>256</td>
<td>150</td>
</tr>
<tr>
<td>D-Xyl</td>
<td>33</td>
<td>4</td>
<td>30</td>
</tr>
<tr>
<td>D-GalN</td>
<td>33</td>
<td>16</td>
<td>125</td>
</tr>
<tr>
<td>D-GluN</td>
<td>33</td>
<td>512</td>
<td>200</td>
</tr>
</tbody>
</table>

a) Minimal amount of B substance for complete hemagglutination inhibition (1, when heated enzyme was used).
b) Absorbance at 660 mµ.

c) At the end of incubation, 0.01 ml of the reaction mixture was applied to paper chromatography, and the galactose liberated from substrates was determined qualitatively.

**TABLE VI. ANOMERIC SPECIFICITY OF THE α-GALACTOSIDASE OF Streptomyces 9917S2**

Incubation was made with 2 µg enzyme protein, at pH 6.0 and at 35°C.

<table>
<thead>
<tr>
<th>Substrate</th>
<th>Degradation (±)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phenyl-α-D-galactoside</td>
<td>+</td>
</tr>
<tr>
<td>Phenyl-β-D-galactoside</td>
<td>-</td>
</tr>
<tr>
<td>Phenyl-N-acetyl-α-D-galactosaminide</td>
<td>-</td>
</tr>
<tr>
<td>Phenyl-N-acetyl-β-D-galactosaminide</td>
<td>-</td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
</tr>
<tr>
<td>Melibiose</td>
<td>+</td>
</tr>
<tr>
<td>Raffinose</td>
<td>+</td>
</tr>
<tr>
<td>B substance</td>
<td>+</td>
</tr>
</tbody>
</table>
mine were all inhibitory. L-Arabinose and D-xylose were also inhibitory, while other sugars tested were not (Table V).

Table VI shows the substrate specificity of the purified enzyme. Melibiose and raffinose were hydrolyzed besides phenyl-α-galactoside and the B substance, indicating that the enzyme cleaves α-(1→6)-galactosidic linkage as well as α-(1→3)-galactosidic linkage.

**DISCUSSION**

Relatively little is known on α-galactosidase, compared to β-galactosidase. In bacteria, the presence of α-galactosidase activity has been reported only with *Aerobacter aerogenes*, *Diplococcus pneumoniae*, *Streptococcus bovis*, *Streptomyces olivaceus*, *Str. fraidae*, *Str. roseospinus* nov. sp., *Escherichia coli*, and some soil bacteria. Main concern in these studies was to elucidate the bacterial enzymes which hydrolyze raffinose and melibiose. The α-galactosidases from these bacteria effectively cleave α-(1→6)-galactosidic linkages of the above sugars, but whether the enzymes are also able to cleave other α-galactosidic linkages has been left unclear.

Serological activity of blood group B substances derives from α-(1→3)-galactosidic linkage at the nonreducing terminal of blood group mucopolysaccharides, and the B substance-degrading enzymes are α-galactosidases which cleave this linkage.

The α-galactosidase from coffee beans hydrolyzes both raffinose (α-(1→6)-galactosidic linkage) and the B substance (α-(1→3)-linkage). On the other hand, α-galactosidases from yeast, sweet almonds, *Platella vulgata*, *Mortierella vinacea*, *Clostridium maebashi*, and some soil bacteria have not been examined for their ability to hydrolyze α-(1→6)-linkage.

In the present study, we have attempted to characterize the α-galactosidase of *Streptomyces 9917S2*, especially to elucidate whether the enzyme cleaves α-(1→3)-galactosidic linkage. First, it was found that the B substance-degrading activity is inducible by lactose (a β-galactoside) as α-galactosidase is. Generally, bacterial α-galactosidases are inducible enzymes except in the case of *S. bovis*. However, it has not been studied whether bacterial B substance-degrading enzymes are inducible. α-Galactosidases are induced both by α- and β-galactosides in *E. coli B*, *Str. olivaceus*, and bacterium M 12, but only by α-galactosides in *A. aerogenes* and *E. coli IAM 1277*.

Purification of the α-galactosidase from *Streptomyces 9917S2* has also resulted in the purification of the blood group B substance-degrading activity, both activities falling into the same fraction throughout the purification processes. The final preparation, which was 15-fold enriched in the specific activity of α-galactosidase, was free from α- and β-glucosidases, β-galactosidase, α- and β-glucosaminidases, and α- and β-galactosaminidases.

Several bacterial α-galactosidases have so far been purified, but purification of bacterial B substance-degrading enzymes has not been made except for partial purification of the enzyme from *Clostridium maebashi*. Some bacterial α-galactosidases have been reported to be unstable in various purification procedures. The α-galactosidase of *Streptomyces 9917S2* was, however, quite stable and isolated in a highly purified state.

Properties of the purified α-galactosidase preparation examined in the present study indicate that the activities of α-galactosidase and B substance degradation is attributed to the same enzyme protein. The enzyme has a broad optimal pH range, 4.5~6.0, for both reactions. This value corresponds to that reported for α-galactosidases from many other organisms, though some of α-galactosidases have been reported to possess their optimal pH values ranging from 6.5 to 8.2.

The activities of α-galactosidase and the B substance degradation from *Streptomyces 9917S2* were inhibited by d-galactose, melibiose, and...
raffinose, and also by L-arabinose and D-xylose. The first three sugars are probably acting as competitive inhibitors. These sugars are also reported to be inhibitory on the B substance-degrading enzyme of Cl. maebashi. How-\textsuperscript{44}ever, further investigation will need for the elucidation for inhibition by L-arabinose and D-xylose. ƒ¿-Galactosidases from plants, yeasts, and molds have been known to have β-L-arabinosidase activity.\textsuperscript{22} The crystalline α-galactosidase of M. vinacea hydrolyzes methyl-β-L-arabinoside.\textsuperscript{35} This may be the case with the enzyme from Streptomyces 9917S.\textsuperscript{2} Inhibition by D-xylose may have more difficulty to explain, although there is a report that the α-galactosidase activity of M. vinacea is inhibited by D-xylose.\textsuperscript{35}

Present study has shown that α-galactosidase from Streptomyces 9917S\textsubscript{2} cleaves α-(1→6)- and α-(1→3)-galactosidic linkages. It is now under investigation with a further purified enzyme preparation whether this enzyme can cleave other α-galactosidic linkages.

Addendum: Taxonomical characteristics of Streptomyces 9917S\textsubscript{2} will be reported by Dr. A. Shimazu of the Institute of Applied Microbiology, University of Tokyo, in the near future.

Acknowledgement. The authors would like to express their thanks to Dr. T. Tomiyama of the Faculty of Medicine, University of Tokyo, for his helpful advice in serological techniques.

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