Classification of Some \( \alpha \)-Glucosidases and \( \alpha \)-XYlosidases on the Basis of Substrate Specificity

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Three \( \alpha \)-glucosidases which passed under the names of transglucosidase (from Aspergillus niger), maltase (from Brewers yeast), and isomaltase (from Bakers yeast) for reasons of their substrate specificities and transfer actions, were purified to electrophoretically pure states.

These purified \( \alpha \)-glucosidases were made uniform in the hydrolyzing activities using \( p \)-nitrophenyl \( \alpha \)-glucopyranoside (\( \alpha \)-p-NPG) and were reacted with \( p \)-nitrophenyl \( \alpha \)-xylopyranoside (\( \alpha \)-p-NPX) or isopimere verso (xylopyranosyl-\( \alpha \)-1,6-glucopyranose), which are typical substrates of \( \alpha \)-xylosidase. Only \( A. \) niger \( \alpha \)-glucosidase among them hydrolyzed \( \alpha \)-p-NPX and isopimere verso. Further the substrate specificities of three \( \alpha \)-glucosidases and two \( \alpha \)-xylosidases (I and II from \( A. \) flavus MO-5) were investigated on maltose, isomaltose, \( \alpha \)-p-NPG, isopimere verso, and \( \alpha \)-p-NPX in detail, and kinetic parameters (\( K_m \), \( V_{max} \), and molecular activity (\( k_0 \)) were estimated and compared with each other.

In the comparison of kinetic parameters, \( A. \) niger \( \alpha \)-glucosidase showed a broad specificity, that is, containing isopimere verso in addition to maltose, isomaltose, and \( \alpha \)-p-NPG. Though this enzyme barely hydrolyzed \( \alpha \)-p-NPX too, the velocity was very slow. Though both yeast \( \alpha \)-glucosidases barely hydrolyzed \( \alpha \)-p-NPX or isopimere verso too, these substrates were not good for yeast enzymes. On the other hand, two \( \alpha \)-xylosidases showed narrow specificities, such that the substrates except for \( \alpha \)-p-NPX and isopimere verso were not hydrolyzed at all.

The action on isopimere verso by \( A. \) niger \( \alpha \)-glucosidase was completely the same as that on isomaltose at optimum pH, optimum temperature, inhibition pattern of hydrolyzing activity by 1-deoxynojirimycin, and transfer action pattern. Accordingly, we interpret these results as indicating that the hydrolyzations of isomaltose and isopimere verso by \( A. \) niger \( \alpha \)-glucosidase were catalyzed at the same active site. \( A. \) niger enzyme that has both \( \alpha \)-glucosidase activity and \( \alpha \)-xylosidase activity was shown to be classified in a middle position between \( \alpha \)-glucosidase and \( \alpha \)-xylosidase.

Many \( \alpha \)-glucosidases (EC 3.2.1.20) have been reported to exist in microorganisms, animal tissues, and plants, and extensive studies have been done. \( \alpha \)-Glucosidases are conventionally classified into three types (I, II, and III) on the basis of their substrate specificities. \( A. \) niger, \( A. \) flavus, \( S. \) cerevisiae, \( B. \) subtilis, \( A. \) aculeatus, and \( T. \) longibrachiatum \( \alpha \)-glucosidases in opposition to type I. The type III group, which comprises \( \alpha \)-glucosidases from pig serum, \( B. \) subtilis, \( A. \) aculeatus, and rice, and \( \alpha \)-glucan as well as malthooligosaccharides.

On the other hand, a few \( \alpha \)-xylosidases (EC 3.2.1.20) were reported to exist in microorganisms and plants too. Recently we purified two types of \( \alpha \)-xylosidases from \( A. \) flavus MO-5, and reported that all \( \alpha \)-xylosidases are classified into three types (A, B, and C) on the basis of their substrate specificities. \( A. \) niger, \( A. \) flavus, \( B. \) subtilis, \( A. \) aculeatus, and \( T. \) longibrachiatum \( \alpha \)-xylosidases in opposition to type I. The type III group, which comprises \( \alpha \)-glucosidases from pig serum, \( B. \) subtilis, \( A. \) aculeatus, and rice, and \( \alpha \)-glucan as well as malthooligosaccharides.

In this paper, we describe the action on \( \alpha \)-p-NPX and isopimere verso by three purified commercial \( \alpha \)-glucosidases [Transglucosidase L “Amano” from \( A. \) niger, maltase

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from Sacch. sp. (Brewers yeast), and isomalto from Bakers yeast\), and the substrate specificities of three \(x\)-glucosidases and two \(x\)-xylosidas\(
\) and Asp. niger \(x\)-xylosidase too.

Materials and Methods

Materials. Q-Sepharose and Superdex 200 were purchased from Pharmacia LKB Biotechnical. PL-SAX and TSK-gel G3000SWx1. They were purchased from Polymer Laboratories and Tosoh. Transglucosidase L \("Amano\) from Asp. niger, maltase from Sacch. sp. (Brewers yeast), and isomalto from Bakers yeast were \(\) obtained from Amano Pharmaceutica, Toyobo, and Sigma Chemicals, respectively. \(x\)-P-NP and \(x\)-X-NPX were purchased from Merek and Nacalai Teque. Isoprimeverose was prepared in our laboratory as described previously.\textsuperscript{15} Under materials used were analytical or commercial grade.

Enzyme preparations. Transglucosidase \("Amano\) was dialyzed against 20 mm phosphate buffer (pH 6.8), and purified by Q-Sepharose, Superdex 200, PL-SAX, and TSK-gel G3000SWx1. The final active fractions were combined and dialyzed against 20 mm acetate buffer (pH 5.0), and used as the purified enzyme preparation. Maltase and isomalto were dissolved in 20 mm phosphate buffer (pH 6.8) and purified by PL-SAX and TSK-gel G3000SWx1. The final active fractions of each enzyme were combined and dialyzed against 20 mm phosphate buffer (pH 6.8), and used as the purified enzyme preparations.

Purified \(x\)-maltosidase I and II were obtained by the cell-free extract of cultured mycella of Asp. flavus MO-5 as described in our previous paper.\textsuperscript{15,13}

The purity of each enzyme preparation was confirmed by gel filtration chromatography using TSK-gel G3000SWx1 and SDS-polyacrylamide gel electrophoresis (PAGE). The protein concentrations of purified enzyme preparations used in these studies were 5267 \(\mu\)g/ml (Asp. niger \(x\)-glucosidase), 2867 \(\mu\)g/ml (Bakers yeast \(x\)-glucosidase), 34 \(\mu\)g/ml (Bakers yeast \(x\)-xylosidase), 42 \(\mu\)g/ml (Asp. flavus \(x\)-xylosidase I), and 47 \(\mu\)g/ml (Asp. flavus \(x\)-xylosidase II).

Enzyme assay. One unit of \(x\)-glucosidase activity was defined as the amount of enzyme which released 1 \(\mu\)mol of \(p\)-nitrophenol from \(x\)-P-NP per min at pH 5.0 (Asp. niger \(x\)-glucosidase) and 6.8 (yeast \(x\)-glucosidas\() with the temperature at 37°C.

One unit of \(x\)-xylosidase activity was defined as the amount of enzyme which released 1 \(\mu\)mol of \(p\)-nitrophenol from \(x\)-X-NPX per min at pH 4.5 and temperature 45°C (\(x\)-xylosidase I) and pH 6.0 and 40°C (\(x\)-xylosidase II).

Actions on \(x\)-P-NP and isoprimeverose by three purified \(x\)-glucosidases. The reaction mixture, containing 50 \(\mu\)l of 20 mm \(x\)-P-NP or 1% (w/v) isoprimeverose solution in 50 \(\mu\)l of enzyme solution (2 or 4 \(\mu\)l) was incubated for 20h at 37°C. After incubation, the products were analyzed by thin-layer chromatography (TLC).

Substrate specificity. In the case of maltose, isomalto, and isoprimeverose, the reaction mixture contained 200 \(\mu\)l of substrate at various concentrations, 200 \(\mu\)l of 0.1 M acetate buffer (pH 4.5, 5.0, or 6.0) or phosphate buffer (pH 6.8), and 100 \(\mu\)l of each purified enzyme solution. The mixture was incubated for 15 min at 37°C, 40°C, or 45°C. The glucose released was measured by the glucose oxidase-peroxidase method\textsuperscript{17,18} with some modifications, using Glucose-AR II reagent (Wako Pure Chemical Industries). The xylene released from isoprimeverose was ignored in a measurement of the initial velocity, because the sensitivity to xylene by this glucose oxidase-peroxidase method was about 1/150 that of glucose.

In the case of \(x\)-P-NPG and \(x\)-X-NPX, the reaction mixture contained 100 \(\mu\)l of substrate at various concentrations, 200 \(\mu\)l of 0.1 M acetate buffer (pH 4.5, 5.0, or 6.0) or phosphate buffer (pH 6.8), and 100 \(\mu\)l of each purified enzyme solution. The reaction solution was stopped by the addition of 400 \(\mu\)l of 0.2 M Na\(\text{CO}_3\) and the \(p\)-nitrophenol released was measured by the absorbance at 400 nm.\textsuperscript{19}

Transfer actions on \(\text{[U-}^{14}\text{C]}-\text{glucose from isomalto or isoprimeverose by Asp. niger }x\)-glucosidase. The reaction mixture, containing 100 \(\mu\)l of 10% (w/v) isomalto, or isoprimeverose, 10 \(\mu\)l of 10% (w/v) glucose, 30 \(\mu\)l of distilled water, 10 \(\mu\)l of [U-\(^{14}\text{C}\)]-glucose (74 Kdpm, 1.25 \(\mu\)g), and 50 \(\mu\)l of Asp. niger \(x\)-glucosidase (isomaltose, 0.05 u/m; isoprimeverose, 4.0 u/m) was incubated at 40°C for 0.5, 1, 2, 4, 8, and 24 h. The experiment without [U-\(^{14}\text{C}\)]-glucose was done simultaneously. After heating, the reaction mixture was spotted on TLC plate and chromatographed. Finally, the autoradiogram was prepared by placing the TLC plate in contact with RX X-ray film (Fuji) for 4 days. The identification of products was done by both R\_ values of TLC and retention times of high pressure liquid chromatography (HPLC) in comparison with standard saccharides. HPLC was done under the following conditions: column, Hiber LiChrosorb NH\(\text{\textsubscript{2}}\) (Merek); mobile phase, acetonylter-water (75:25, v/v), flow rate, 1.0 ml/min; oven temperature, 40°C.

Protein assay. Protein was measured with a BCA (bicinchoninic acid) Protein Assay kit (Pierce) by the method of Smith et al.\textsuperscript{20} and bovine serum albumin was used as a standard.

Electrophoresis. SDS-PAGE by the Laemmli system\textsuperscript{21} was used for confirmation of the purification. Each enzyme preparation was put on a precast SDS 8-16% gradient gel (TEF Corporation), and run at 20 mA for 2 h. After electrophoresis, proteins were stained with Coomassie Brilliant Blue G-250. A molecular weight marker kit (Bio-Rad Laboratories) was used for standard proteins.

Gel filtration chromatography and estimation of molecular weight. TSK-gel G3000SWx1 was used for final purification of each enzyme and for measurement of the molecular weight. The analysis was done by HPLC using an LC-6A pump, SPD-6A UV-VIS spectrophotometric detector, and C-R4A Chromato Pac (all from Shimadzu Corporation) under the following conditions: mobile phase, 50 mm phosphate buffer (pH 6.8) containing 0.3 M NaCl; flow rate, 1.0 ml/min; oven temperature, room temperature; UV, 280 nm. Molecular weight marker kit (Daichi Pure Chemicals) was used as standard proteins.

Results

Purifications of three \(x\)-glucosidas\()

Three commercial \(x\)-glucosidas\() were purified to electro-photically pure states by these chromatographies (Fig. 1). Each yeast \(x\)-glucosidase showed a single band. Asp. niger \(x\)-glucosidase had two components as reported already by Kita et al.\textsuperscript{51} Native molecular weights of Asp. niger, Brewers yeast, and Bakers yeast \(x\)-glucosidas\() were estimated to be 125,000, 52,000, and 45,000 by gel filtration, respectively.

Actions on \(x\)-P-NP and isoprimeverose by three purified \(x\)-glucosidas\()

The hydrolyzing activities of three \(x\)-glucosidas\() were made uniform using \(x\)-P-NPG, which is a common substrate,
Fig. 2. Analyses of Products from Isoprimeverose and α-p-NPX by α-Glucosidases on TLC.
The reaction mixture, containing 50 μl of substrate (1% iso primeverose or 20 mM α-p-NPX) and 50 μl of enzyme solution (2 or 4 u/ml) was incubated for 20 h at 37°C. TLC was done on a Merck TLC plate of silica gel 60. The sugars were detected by heating at 130°C for 10 min after spraying with 50% sulfuric acid in methanol. (a), iso primeverose. A solvent system of TLC was acetonitrile-water (80:20, v/v), and time of development was twice. (b), α-p-NPX. A solvent system of TLC was acetonitrile-water (88:12, v/v), and time of development was once. M1, xylose; M2, glucose; B, no treatment; 1 and 2, Asp. niger α-glucosidase (2 and 4 u/ml); 3 and 4, Brewers yeast α-glucosidase (2 and 4 u/ml); 5 and 6, Bakers yeast α-glucosidase (2 and 4 u/ml).

Fig. 3. Lineweaver-Burk Plots for Hydrolysis of Isoprimeverose and α-p-NPX by α-Glucosidases and α-Xylosidases.
(a), iso primeverose. The reaction mixture, containing 200 μl of substrate at various concentrations, 200 μl of 0.1 M acetate buffer (pH 4.5, 5.0, and 6.0) or phosphate buffer (pH 6.8), and 100 μl of enzyme solution was incubated for 15 min at 37°C, 40°C, or 45°C. (b), α-p-NPX. The reaction mixture, containing 100 μl of substrate at various concentrations, 200 μl of 0.1 M acetate buffer (pH 4.5, 5.0, and 6.0) or phosphate buffer (pH 6.8), and 100 μl of enzyme solution was incubated for 15 min at 37°C, 40°C, or 45°C. v, initial velocity for hydrolysis of substrate (mM substrate per mg of protein per min); s, substrate concentration (mM). ○, Asp. niger α-glucosidase; △, Brewers yeast α-glucosidase; ●, Bakers yeast α-glucosidase; □, Asp. flavus MO-5 α-xylosidase I; ▲, Asp. flavus MO-5 α-xylosidase II.

and were reacted with α-p-NPX and iso primeverose, which are typical substrates of α-xylosidase. The reaction mixtures were analyzed by TLC (Fig. 2). Only Asp. niger α-glucosidase and iso primeverose, because xylose was detected in the reaction mixtures of both substrates. But the two α-glucosidases from yeast did not apparently hydrolyze either.

Though there were difference of actions on α-p-NPX or iso primeverose between Asp. niger α-glucosidase and yeast α-glucosidases, there was a problem with the protein concentration of enzymes used in this test, because the concentration of Asp. niger α-glucosidase was higher than that of yeast α-glucosidases due to the difference of substrate specificity for aryl glucoside (α-p-NPG).

Substrate specificities of α-glucosidases and α-xylosidases
To confirm the actions on α-p-NPX and iso primeverose by α-glucosidases, the substrate specificities of three α-glucosidases were examined on maltose, isomaltose, α-p-NPG, iso primeverose, and α-p-NPX. Substrate specificities of two α-xylosidases (I and II) from Asp. flavus MO-5 were also examined in the same way.

Kinetic parameters for hydrolysis of these substrates by each enzyme were obtained from Lineweaver-Burk plots. Figure 3 shows the double reciprocal plots, the relationship between the initial velocity (v) and the substrate concentration (s), for the hydrolysis of α-p-NPX and iso primeverose (data for maltose, isomaltose, and α-p-NPG not shown).
### Table 1. Michaelis Constant (\(K_m\)), Maximum Velocity (\(V_{max}\)), and Molecular Activity (\(k_o\)) for Hydrolysis of Substrates by \(\alpha\)-Glucosidases and \(\alpha\)-Xylosidases

<table>
<thead>
<tr>
<th>Type (Enzyme)</th>
<th>Origin (Enzyme)</th>
<th>MW*</th>
<th>Maltose</th>
<th>Isomaltose</th>
<th>Asp. niger (Transglucosidase)</th>
<th>brewers yeast (Maltase)</th>
<th>Bakers yeast (Isomaltase)</th>
<th>MW*</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>52,000</td>
<td>45,000</td>
<td>125,000</td>
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<tr>
<td>Substrate</td>
<td>(K_m) (mm)</td>
<td>(V_{max})</td>
<td>(k_o) (sec^{-1})</td>
<td>(K_m)</td>
<td>(V_{max})</td>
<td>(k_o)</td>
<td>(K_m)</td>
<td>(V_{max})</td>
</tr>
<tr>
<td>Maltose</td>
<td>15.38</td>
<td>77.54</td>
<td>67.20</td>
<td>—</td>
<td>6.94</td>
<td>44.44</td>
<td>33.33</td>
<td>6.29</td>
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<tr>
<td>Isomaltose</td>
<td>34.48</td>
<td>5.95</td>
<td>5.16</td>
<td>1.30</td>
<td>171.35</td>
<td>128.52</td>
<td>138.52</td>
<td>0.52</td>
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<tr>
<td>(\alpha-p)-NPG</td>
<td>0.22</td>
<td>156.25</td>
<td>135.42</td>
<td>30.30</td>
<td>60.61</td>
<td>126.27</td>
<td>3.96</td>
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<td>Isopimeroverose</td>
<td>71.43</td>
<td>5.95</td>
<td>5.16</td>
<td>4.17</td>
<td>0.03</td>
<td>0.02</td>
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<td>(\alpha-p)-NPX</td>
<td>0.27</td>
<td>0.02</td>
<td>0.02</td>
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<td>0.02</td>
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### (\(\alpha\)-Xylosidase)

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<th>C</th>
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<tr>
<td></td>
<td></td>
<td>400,000</td>
<td>Asp. flavus MO-5 ((\alpha)-Xylosidase I)</td>
<td>Asp. flavus MO-5 ((\alpha)-Xylosidase II)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>180,000</td>
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<tr>
<td>Substrate</td>
<td>(K_m)</td>
<td>(V_{max})</td>
<td>(k_o)</td>
<td>(K_m)</td>
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<td>Maltose</td>
<td>—</td>
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<td>—</td>
<td>—</td>
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<tr>
<td>Isomaltose</td>
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<td>—</td>
<td>—</td>
</tr>
<tr>
<td>(\alpha-p)-NPG</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
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<td>Isopimeroverose</td>
<td>4.00</td>
<td>58.82</td>
<td>392.13</td>
<td>47.62</td>
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<tr>
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<td>1.32</td>
<td>4.44</td>
<td>29.60</td>
<td>0.97</td>
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</table>

* MW, molecular weight.

**Fig. 4.** Effects of pH and Temperature on the Activity of Asp. niger \(\alpha\)-Glucosidase for Hydrolysis of Isomaltose and Isopimeroverose.

(a) pH activity curve. The reaction mixture, containing 200 \(\mu\)l of 0.5% (w/v) isomaltose or isopimeroverose, 200 \(\mu\)l of McIlvaine buffer (pH 2.5–6.0) or 0.5 M phosphate buffer (pH 6.0–7.5), and 100 \(\mu\)l of enzyme solution (isomaltose: 0.002 U/ml; isopimeroverose: 0.030 U/ml) was incubated for 15 min at 37°C. (b) Temperature activity curve. The reaction mixture, containing 200 \(\mu\)l of 0.5% (w/v) isomaltose or isopimeroverose, 200 \(\mu\)l of 0.1 M acetate buffer (pH 5.5), and 100 \(\mu\)l of enzyme solution was incubated for 15 min at various temperatures (30–70°C). ○, isomaltose; ●, isopimeroverose.

In Table 1, the Michaelis constant (\(K_m\)), the maximum velocity (\(V_{max}\)) and the molecular activity (\(k_o\)) are listed. \(k_o\) was calculated from the \(V_{max}\) and the molar concentration of the enzyme (\(e_o\)) using the formula \(k_o = \frac{V_{max}}{e_o}\).

With respect to maltose, isomaltose, and \(\alpha-p\)-NPG having \(\alpha\)-glucosidic linkages, three \(\alpha\)-glucosidases showed the actions that are the same as the results reported already. These \(\alpha\)-glucosidases barely hydrolyzed \(\alpha-p\)-NPX, and the \(V_{max}\) and \(k_o\) were very small. With respect to isopimeroverose, Bakers yeast \(\alpha\)-glucosidase did not hydrolyze this substrate,
and Brewers yeast enzyme barely hydrolyzed it. On the other hand, *Asp. niger* α-glucosidase hydrolyzed isopimverose better than yeast enzymes. Though the affinity of this α-glucosidase for isopimverose was low, the $V_{\text{max}}$ and $k_0$ were about one-third to one-sixth of those of substrates having α-glucosidic linkage.

On the other hand, the two α-xylosidases did not hydrolyze the substrates having α-glucosidic linkages at all, but hydrolyzed only α-β-NPX and isopimverose, which have α-xylosidic linkages.

**Comparison of actions on isomaltose and isopimverose by *Asp. niger* α-glucosidase**

The substrate specificities supported the idea that *Asp. niger* α-glucosidase has an α-xylosidase activity. To discover whether this enzyme catalyzes hydrolysis of both isomaltose and isopimverose at the same active site, some actions on hydrolysis of both substrates were examined and compared to each other.

First, optimum pH and optimum temperature of *Asp. niger* α-glucosidase on both substrates were examined (Fig. 4). Optimum pH and optimum temperature were pH 5.5 and 60°C, respectively, and this was the same without distinction of the substrate.

Second, inhibition patterns by 1-deoxyxojirimycin on hydrolysis of both substrates were investigated (Fig. 5). The hydrolysis of isomaltose and isopimverose by *Asp. niger* α-glucosidase was inhibited noncompetitively by 1-deoxyxojirimycin, which is an inhibitor for α-glucosidase. The inhibitor constant ($K_i$) of 1-deoxyxojirimycin for this enzyme was calculated from linear plots of Fig. 5. The $K_i$ values of inhibition on isomaltose and isopimverose were $1.32 \times 10^{-4}$ mm and $1.07 \times 10^{-4}$ mm, nearly equal.

Finally, transfer action patterns on high concentrations of both substrates were observed by autoradiograms using [U-14C]-glucose (Figs. 6 and 7). In the case of isomaltose, *Asp. niger* α-glucosidase transferred the glucosyl residue of a non-reducing end side of isomaltose to [U-14C]-glucose, and produced 14C-labelled isomaltose, maltose, isomaltotriose, and panose by α-1,4 and α-1,6 glucosyl transfer actions as shown in Fig. 6(a). In the case of isopimverose, TLC of the reaction mixture without [U-14C]-glucose did not have any difference between 0 h and 24 h, and looked like there was no reaction (Fig. 7(b)), but a spot of 14C-labelled isopimverose appeared in the autoradiogram as shown in Fig. 7(a). Further, a spot of 14C-labelled xylobiose (xylopyranosyl-α-1,4-xylopyranose) appeared in the autoradiogram using [U-14C]-xylose as shown in Fig. 7(c). These transfer products were identified on the basis of the $R_f$ values of TLC and retention times of HPLC. Accordingly, this enzyme transferred the xylosyl residue of a non-reducing end side of isopimverose to glucose or xylose released, and produced isopimverose and xylobiose by α-1,6 and α-1,4 xylosyl transfer actions. *Asp. niger* α-glucosidase showed the same transfer action pattern.
without distinction of the structure between xylose and glucose that are on the non-reducing end side of substrate.

Discussion

In this paper we described detailed substrate specificities of five enzymes (three α-glucosidases and two α-xylosidases) on maltose, isomaltose, α-p-NPG, isoprimerose, and α-p-NPX. The substrate specificities of five enzymes were compared on the basis of $K_m$ and $k_0$ in Table I.

The values of kinetic parameters for maltose, isomaltose, and α-p-NPG by three α-glucosidases were similar to the results reported already, and showed special features of each enzyme that are fundamental for classification of α-glucosidases by Chiba and Shimomura.21 With respect to α-p-NPX and isoprimerose, the features of α-glucosidases were found to be as follows on the basis of their $K_m$ and $k_0$. In the case of Brewers yeast α-glucosidase, the affinity for α-p-NPX was very high, as good as α-p-NPG. But the velocity was very slow, this enzyme could scarcely hydrolyze α-p-NPX. The affinity and velocity on isoprimerose were very low and slow. In the case of Bakers yeast α-glucosidase, the specificity on α-p-NPX was the same as that of Brewers yeast α-glucosidase. This enzyme could scarcely hydrolyze isoprimerose. In the case of Asp. niger α-glucosidase, the specificity on α-p-NPX was the same as that of yeast α-glucosidases. Though the affinity for isoprimerose was low, the velocity was very high. The $k_0/K_m$ value, which is a factor for synthetic judgment of substrate specificity, was 4.17 on isoprimerose. Though this $k_0/K_m$ value was about one-thirteenth of isomaltose (56.14), it was almost equal to the value (4.37) on maltose of Brewers yeast α-glucosidase (maltase) and the value (4.80) on isomaltose of Bakers yeast α-glucosidase (isomaltase). We interpret this results as indicating that isoprimerose for Asp. niger α-glucosidase is a substrate as good as maltose for maltase and isomaltose for isomaltase. In the view of these facts, it seems tested α-glucosidases have loose specificities that either glucose and xylose is the non-reducing end side of substrates. Asp. niger α-glucosidase especially showed a broad specificity that was clearly different from yeast α-glucosidases. With respect to whether this enzyme catalyzes hydrolysis of both isomaltose and isoprimerose at the same active site, some examinations using both substrates were done as mentioned above. We interpret these results as indicating that a single active site is concerned in an appearance of both α-glucosidase activity and α-xylosidase activity. In short, Asp. niger α-glucosidase has an α-xylosidase activity.

On the other hand, α-xylosidases I and II did not hydrolyze the substrates except for α-p-NPX and isoprimerose, and did not show α-glucosidase activity at all. Accordingly, it is clear these α-xylosidases have narrow specificities in that the non-reducing end side of the substrate is always xylose.

It is generally considered that α-glucosidases and α-xylosidases are different enzymes with respect to the substrate specificity, and both enzymes are classified into the three types within each category as mentioned above. But it was seen by our results that the substrate specificities of the enzymes partially overlapped. This means that some α-glucosidases hydrolyzed substrates for α-xylosidase. Accordingly it is difficult to distinguish α-glucosidase from α-xylosidase accurately, and it is indicated that a major reclassification of the α-glucosidase family including both enzymes on the basis of substrate specificity is possible. Although discussion of substrate specificity is very complicated, the classification on the basis of the comparison of $k_0/K_m$ values of tested enzymes in this study was done. These enzymes were divided into the following three groups as shown in Table II. Group 1 (yeast α-glucosidases) comprises α-glucosidases that have little α-xylosidase activity. Group 2 (Asp. niger α-glucosidase) comprises α-glucosidase that has α-xylosidase activity too. And group 3 (Asp. flavus α-xylosidases) comprises α-xylosidase without α-glucosidase activity at all. We think Asp. niger enzyme is in a middle position between α-glucosidase and α-xylosidase. This classification is a trial on the basis of both α-glucosidase activity and α-xylosidase activity, because substrate specificities of only five enzymes of the many α-glucosidases and α-xylosidases were studied. Furthermore the investigation of Sacch. logos α-glucosidase (type II), plant
Table II. Classification of α-Glucosidases and α-Xylosidases on the Basis of $k_d/K_m$ Values

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Substrate</th>
<th>Maltose</th>
<th>Isomaltose</th>
<th>α-p-NPG</th>
<th>Isoprimerove</th>
<th>α-p-NPX</th>
<th>Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brewers yeast α-glucosidase</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>1</td>
</tr>
<tr>
<td>Bakers yeast α-glucosidase</td>
<td>NR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>NR</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Aasp. niger α-glucosidase</td>
<td>+ +</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>2</td>
</tr>
<tr>
<td>Aasp. flavus MO-5 α-xylosidase I</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>3</td>
</tr>
<tr>
<td>Aasp. flavus MO-5 α-xylosidase II</td>
<td>NR</td>
<td>NR</td>
<td>NR</td>
<td>–</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

The judgment of substrate specificity of each enzyme was done as follows on the basis of $k_d/K_m$ (mm$^{-1}$·sec$^{-1}$) value: +++, 100 ≤ $k_d/K_m$ < 100; +, 10 ≤ $k_d/K_m$ < 10; –, 0 ≤ $k_d/K_m$ < 1; NR, not reacted.

α-glucosidases (type III), animal α-glucosidases (type III), Bacillus sp. α-xylosidase (type A), and plant α-xylosidases (type B), for example, will be necessary. Accordingly it is still unknown whether our theory of a new classification holds good on the basis of each classification of both enzymes described in the introduction or not.

Kimura et al. reported a comparison of amino acid sequence among some α-glucosidases, including Aasp. niger α-glucosidase.33 In the presentation, they reported that the amino acid sequence of Aasp. niger α-glucosidase has a high homology to that of α-glucosidases from animals, but don’t have a homology to that of α-glucosidases from Sacch. cerevisiae and Bacillus cereus. In view of this results, they proposed that α-glucosidases are generally divided two categories at least. In our study too, Aasp. niger α-glucosidase and yeast α-glucosidase were distinguished at the point of α-xylosidase activity as mentioned above.

By the way, it is known that α-1,3-xylosidic linkages are contained in oligosaccharides of human urine33 and in sugar chains of bovine blood clotting factors.35 Though their biological roles are unknown, some importance are anticipated. Up to the present, there has been no report of an α-xylosidase from animals. Human α-glucosidases exist in extensive tissues universally, but their biological roles are unknown although some have been studied.26 If the substrate specificities of animal α-glucosidases are similar to that of Aasp. niger α-glucosidase and have α-xylosidase activity, we hope animal α-glucosidases take part in decomposition of sugar chains containing α-1,3-xylosidic linkages and function as a switch for some signal in vivo. This may be one part of the biological role of animal α-glucosidases. To confirm the validity of this suggestion, we will investigate the substrate specificities of animal α-glucosidases on substrates having α-xylosidic linkages.

References