Note

Characterization of α -D-xylosidase II from *Aspergillus niger*Junichi MATSUSHITA,* Yoji KATO
and Kazuo MATSUDADepartment of Agricultural Chemistry, Faculty of
Agriculture, Tohoku University, Sendai
980, Japan

Received February 6, 1987

In our previous study,^{1,2)} we reported that two components of α -D-xylosidase [α -D-xylosidase I: α -D-xylosidase II = 70:30 (activity ratio)] were detected in the culture filtrate of *Aspergillus niger* strain AMS 4111 grown in a medium containing Sanzyme 1000-digested Glyloid 2A. The major component, α -D-xylosidase I of AMS 4111, was purified and characterized in detail.²⁾ This paper describes the purification and characterization of the minor component, α -D-xylosidase II of AMS 4111. Unless otherwise stated, all materials and experimental procedures used for this study were the same as those described in previous papers.^{1,2)}

A concentrate (about 100 ml) of the 10-day-old culture

filtrate (about 2 l) of strain AMS 4111 was chromatographed on DEAE-Sephadex A-25: the activity of α -D-xylosidase was detected in the breakthrough fractions. When the fractions containing α -D-xylosidase were then chromatographed on CM-Sephadex in 20 mM Na-acetate buffer (pH 5.5), the enzyme activity was separated into two components: one in the breakthrough fractions (α -D-xylosidase II) and the other in fractions eluted from the CM-Sephadex column at 0.1 M NaCl with a linear gradient (α -D-xylosidase I).²⁾ The breakthrough fractions were concentrated and put on a column (1.0 $\times$ 7.0 cm) of CM-Sephadex C-50 pre-equilibrated with 20 mM Na-acetate buffer (pH 5.0). After washing of the column with the same buffer (90 ml), the adsorbed materials were eluted with a linear salt gradient produced by mixing 120 ml each of the same buffer and 0.2 M NaCl in the same buffer. α -D-xylosidase activity was detected in the adsorbed fractions (Fig. 1a). The active fractions were concentrated with a collodion bag (Sartorius membrane filter, Collodion-bag 12) and put on a column (2.2 $\times$ 110 cm) of Sephadex G-200 pre-equilibrated with 20 mM Na-acetate buffer (pH 5.0) and the column was eluted with the same buffer. Two activity peaks were detected (Fig. 1b). The minor activity peak having a molecular weight of about 500,000 is probably α -D-xylosidase I, because the molecular weight of α -D-xylosidase I had been estimated to be 490,000.²⁾ Therefore, the major activity peak detected at the void fraction of the Sephadex column was named α -D-xylosidase II preparation and characterized. Thus, α -

Fig. 1a. Chromatography on a CM-Sephadex Column of α -D-Xylosidase II Fraction from the First CM-Sephadex Chromatography.

○, α -D-xylosidase activity, which was assayed as follows: a mixture of 50 μ l of the enzyme solution, 50 μ l of 8 mM *p*-nitrophenyl α -D-xylopyranoside, and 100 μ l of 0.2 M NaOAc-HCl buffer (pH 3.5) was incubated at 45°C for 10 min. The reaction was stopped by the addition of 2.8 ml of 0.1 M Na₂CO₃, and *p*-nitrophenol released was measured by the absorbance at 420 nm. ●, protein. ---, concentration of NaCl in Na-acetate buffer (pH 5.0). Fractions 20 to 32 were combined.

Fig. 1b. Chromatography on a Sephadex G-200 Column of α -D-Xylosidase II Fraction from the CM-Sephadex Column.

○, α -D-xylosidase activity; ●, protein. Fractions 68 to 84 were combined.

* Present address: Chugai Pharmaceutical Co., Ltd., Tokyo.

TABLE I. SUMMARY OF THE PURIFICATION OF α -D-XYLOSIDASE II FROM *A. niger*

Step	Total protein (mg)	Total activity (U)	Specific activity (U/mg protein)	Purification (fold)
1. Culture filtrate	2,000	21.76 ^a	1.1×10^{-2}	1
2. DEAE-Sephadex A-50	21.0	17.76 ^a	84.6×10^{-2}	77
3. CM-Sephadex C-50	7.2	5.62	78.0×10^{-2}	71
4. 2nd CM-Sephadex C-50	1.2	5.42	452.0×10^{-2}	411
5. Sephadex G-200	0.7	2.82	403.0×10^{-2}	366

^a α -D-Xylosidase I and α -D-xylosidase II.

xylosidase in the culture filtrate (step 1) was purified about 366-fold, with about 13% recovery of the activity (Table I).

To check the purity of the α -D-xylosidase II preparation, polyacrylamide gel electrophoresis in 7.5% gel, gel electrophoresis in SDS-polyacrylamide, and isoelectric focusing using an Ampholine gradient with pH 5.0 to pH 7.0 were done. However, the purity of the preparation was not checked in each case because the enzyme protein did not move from the origin. Gel filtration on Sephadex G-200 showed that the enzyme has a high molecular weight, over 800,000.

Partially purified α -D-xylosidase II was most active at pH 2.5~3.0 and was stable in the range from pH 3.0 to pH 7.0 at 45°C for 10 min. The temperature optimum for the enzyme activity was 40~45°C and the enzyme was stable up to 60°C. These results agree well with those for α -D-xylosidase I which were reported previously.²⁾ The Michaelis constant for *p*-nitrophenyl α -D-xylopyranoside of α -D-xylosidase II was calculated from Lineweaver-Burk plots. The apparent *K_m* and *V_{max}* values were 10.5 mM and 22.6 μ mol/min/mg protein.

Upon incubation with partially purified α -D-xylosidase II, isoprimeverose (6-*O*- α -D-xylopyranosyl-D-glucose) yielded glucose and xylose. The partially purified α -D-xylosidase II could also split off the α -D-xylopyranosyl residue on the non-reducing terminal of the backbone of oligoxyloglucans such as α -D-xylopyranosyl-(1 $\rightarrow$ 6)- β -D-glucopyranosyl-(1 $\rightarrow$ 4)-{[α -D-xylopyranosyl-(1

$\rightarrow$ 6)-] β -D-glucopyranosyl-(1 $\rightarrow$ 4)-}₂-D-glucose. Oligoxyloglucans used for this study were tri- (Xyl:Glc = 1:2), tetra- (Xyl:Glc = 2:2), penta- (Xyl:Glc = 2:3), and hepta-saccharide (Xyl:Glc = 3:4). This indicates that the action of α -D-xylosidase II on isoprimeverose and oligoxyloglucans is the same as that of α -D-xylosidase I.

We previously reported that until the growth of *A. niger* mycelia reached a maximum (2nd day of cultivation), no significant amount of α -D-xylosidase was produced in the culture broth, and thereafter the level of α -D-xylosidase increased with the time of cultivation and the decrease of mycelial weight (after the 3rd day of cultivation).¹⁾ Furthermore, about 60% of the total activity was extracted from the 2-day-old mycelia and the extract from the mycelia gave a single α -D-xylosidase activity peak having a molecular weight of 490,000.¹⁾ These previous findings and the current results suggest that α -D-xylosidase II, which has the same general properties and action mechanism on the tested oligoxyloglucans as those of α -D-xylosidase I, is a component strongly bound to mycelial wall and secreted into the culture broth with the autolysis of the mycelia.

REFERENCES

- 1) Y. Kato, J. Matsushita and K. Matsuda, *J. Ferment. Technol.*, **63**, 389 (1985).
- 2) J. Matsushita, Y. Kato and K. Matsuda, *J. Biochem.*, **98**, 825 (1985).