THE CELLULASE OF *TRICODERMA VIRIDE*

Purification and Properties of the β-D-glucosidase

By

Shogo SHIKATA*

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In the previous report (1969) four C_X-enzymes of *Tricoderma viride* were fractionated on the columns of ionexchangers. One of them was β-D-glucosidase, which hydrolyzed cellobiose and *p*-nitrophenyl-β-D-glucoside (PNG). Soluble carboxymethylcellulose (SCMC) was degraded step-wisely at cellobiose unit by the enzyme and followed by glucose formation.

The present paper is an investigation into the purification and properties of the β-glucosidase.

MATERIARS and METHODS

Preparation of enzyme. The separation of the C_X-components of *T. viride* has already been reported (Shikata, 1969) ; DEAE-IV component has been identified a β-D-glucosidase. The freeze-dried DEAE-IV protein was used here as the starting material for purifications.

Measurement of the β-glucosidase activity. The reaction mixture contained 0.1 ml of 0.0136 M PNG, 0.2 ml of 0.1 M acetate buffer, pH 4.5, and 0.1 ml of 0.05 % enzyme solution. After incubation at 30° C for 30 min, 10 ml of 0.1 M Na_2CO_3 were added to the mixture. The amount of *p*-nitrophenol liberated was then calculated from the extinction at 420 mμ,

Gel filtration. The DEAE-IV protein (16.5 mg) was applyed to a column of Sephadex G-50 (25×900mm) equilibrated with tris buffer (pH 8.6, $\mu = 0.05$). The elution was carried out with same buffer at a rate of 0.5 ml/min. Fractions (5 ml) were collected.

Disc electrophoresis. This was carried out as described by Ornstein (1964) and Davis (1964). A amount of sample protein loaded on a electrophoresis tube (6.5 × 75 mm) was 200 μg and the constant current was 5 mA/tube in a each experiment.

Ultracentrifuge. This was performed in a Hitachi analytical ultracentrifuge :

*Department of Chemistry, Faculty of Liberal Arts, Shinshu University, Matsumoto

1 % protein solution in 0.4 M acetate buffer, pH 4.5, was centrifuged at 60000 r. p. m. The temperature of the rotor was 12−12.5° C.

Effect of heat on the β-glucosidase activity. The mixtures of 0.1 ml of 0.05 % enzyme solution and 0.2 ml of 0.1 M acetate buffer, pH 4.5, were heated at temperature range 30−90° C for 10 min. After cooled with tap water 0.1 ml of 0.0136 M PNG was added to each mixture and the activity was measured.

Effect of pH. At the above mentioned assay condition, effects of pH were measured for two different buffers ; 0.1 M acetate buffer and 0.2 M Na_2HPO_4 - 0.1 M citric acid buffer were used and the pH ranges were 3.5−5.5 and 2.3−7.9 respectively.

RESULTS and DISCUSSION

The result of the ultracentrifuge was shown in Fig. 1. The peak in the figure had 4.6 of S value. The fraction was isolated with the aid of a seprating cell (at 60000 r. p. m. for 3 hr). A large portion of β-glucosidase activity contained in the original DEAE-IV protein was found in the fraction having S = 4.6.

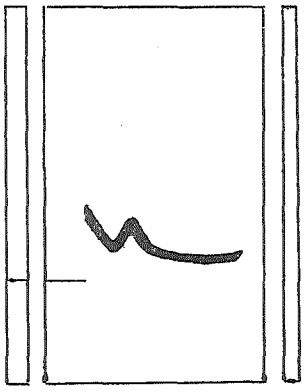

Fig. 1. Ultracentrifuge with DEAE-IV at 60000 r. p. m. for 50 min.

Two fractions were separated with a gel filtration on a column of Sephadex (Fig. 2). The bulk of the protein applyed to a column was eluted in a void volume (Peak I). The β-glucosidase activity remained fully in this portion but not the second peak (Peak II) (Table 1).

The results of the ultracentrifuge and gel filtration show that DEAE-IV fraction reported previously is a β-glucosidase containing a inactive protein (about 5 %, M. W. below 10000). It was confirmed furthermore by disc electrophoresis.

In Fig. 3 DEAE-IV shows two bands consisting of a thickly coloured band

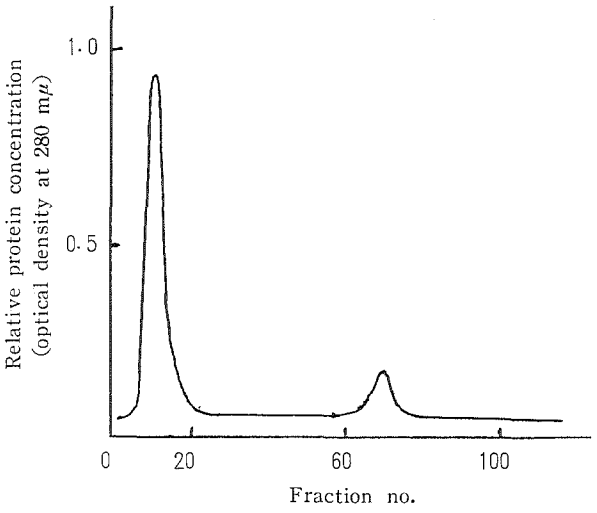

Fig. 2 Gel filtration on a column of Sephadex G-50.

	% of PNG hydrolyzed	% of yield of protein
Peak I	70	90
Peak II	0	5
DEAE-IV	68	—

Table 1. Activities and yields of proteins on Sephadex G-50. Assays were carried out as described in the text.

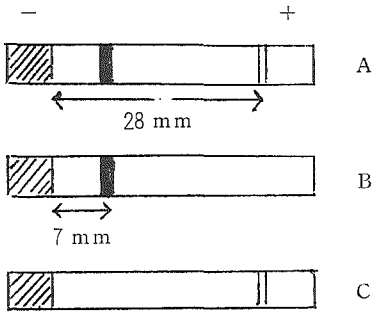

Fig. 3. Disc electrophoresis of DEAE-IV (A), Peak I (B) and Peak II (C). The left-hand oblique lines show gels for concentration. Electrophoresis was continued fof 30 min.

and a pale one. The migration of the former was 7 mm and that of the latter was 28 mm for 30 min. It coincides with the above conclusion that Peak I has only a thickly coloured band Peak II dose a pale one.

The results of heat and pH on the Peak I are shown in Fig. 4 and Fig. 5 respectively. The enzyme was thoroughly inactivated when heated at 80°C and pH 4.5 for 10 min. The pH - activity curves for two kinds of buffers mostly overlaped one another and the optimum was about pH 5. The results were just same with those on DEAE-IV.

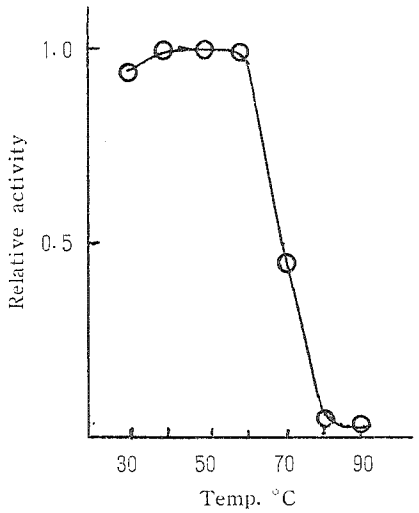

Fig. 4. Effect of heat on Peak I.

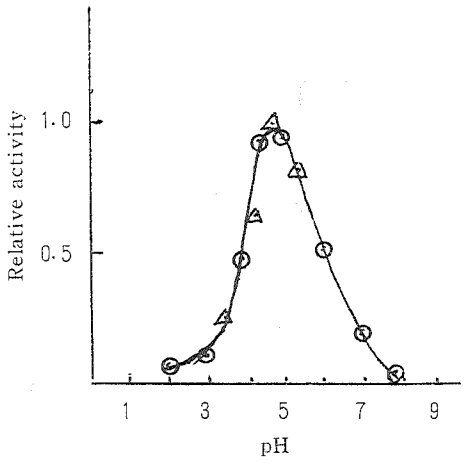

Fig. 5. Effect of pH on Peak I ; ⊙, 0.2 M Na_2HPO_4 - citric acid buffer ; △, 0.1 M acetate buffer.

REFERENCES

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SUMMARY

A β-D-glucosidase of *T. viride* was isolated as a pure protein by gel filtration. It was confirmd by ultracentrifuge and disc electrophoresis. The enzyme has a optimum pH 5 and was completely inactivated at 80 °C.