THE CELLULASE OF TRICODERMA VIRIDE

Purification and Properties of the β-D-glucosidase

By

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In the previous report (1969) four C₆-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.

MATERIALS and METHODS

Preparation of enzyme. The separation of the C₆-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 Na₂CO₃ were added to the mixture. The amount of p-nitrophenol liberated was then calculated from the extinction at 420 μm.

Gel filtration. The DEAE-IV protein (16.5 mg) was applied to a column of Sephadex G-50 (25 × 900mm) equilibrated with tris buffer (pH 8.6, μ = 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:

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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 Na₂HPO₄ - 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 separating 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.](image)

Two fractions were separated with a gel filtration on a column of Sephadex (Fig. 2). The bulk of the protein applied 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.

<table>
<thead>
<tr>
<th></th>
<th>% of PNG hydrolyzed</th>
<th>% of yield of protein</th>
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<tbody>
<tr>
<td>Peak I</td>
<td>70</td>
<td>90</td>
</tr>
<tr>
<td>Peak II</td>
<td>0</td>
<td>5</td>
</tr>
<tr>
<td>DEAE-IV</td>
<td>68</td>
<td></td>
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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 for 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 overlapped 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 Na₂HPO₄ - citric acid buffer; △, 0.1 M acetate buffer.
REFERENCES


SUMMARY

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