Glycogen Synthetase from Pullularia pullulans

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In the previous papers1~3 the authors have reported on the structure and synthesis of polysaccharides produced by Pullularia pullulans. In the course of studies on the biosynthetic mechanism of pullulan, we have found glycogen synthetase in the organism. The enzyme catalyzes the transfer of glucose into glycogen from UDP-glucose.

Pullularia pullulans S-1 supplied by professor S. Ueda, Kyushu University, was used throughout this study. Preparation of enzyme was carried out as described below. The organism was cultured for 24~28 hr as described in the previous paper.1) Cells harvested (wet weight 18~20 g) by centrifugation were washed with water, frozen, ground with 2 times their weight of alumina, extracted with 70 ml of water and centrifuged at 600~g for 20 min. The supernatant solution was recentrifuged at 30,000~g for 1 hr and the precipitate was suspended in 4 ml of water. The suspension obtained was used as enzyme solution. Enzyme preparation was analyzed for protein content (70~80 mg/ml), and diluted so as to give the protein content 60 mg/ml. Standard reaction mixture contained 1.2 μmole of UDP(14C)-glucose (8.3×10^4 cpm/μmole), 1.8 μmole of MgCl₂, 3.0 mmoles of glucose-6-phosphate, 15.0 mmoles of Tris-HCl buffer (pH 7.5) and 6.0 mg of enzyme protein in a total volume 300 μl. The mixture was incubated at 30°C for 30 min and boiled for 3 min to stop the reaction. An aliquot of the reaction mixture was spotted on Toyo filter paper No. 51A and chromatographed with descending method using the solvent ethanol-ammonium acetate (pH 7.5) (7:3). The origin of chromatography was cut out and the radioactivity of the origin was counted in toluene scintillation fluid. The radioactivity in the origin was calculated as glucose incorporated into glycogen from UDP(14C)-glucose.

In the standard reaction mixture, 3.9 μmoles of 14C-glucose was transferred from UDP(14C)-glucose into glycogen per 30 min. The time course curve in this experiment was almost linear. The relationship between the concentration of glucose-6-phosphate and enzyme activity is shown in Fig. 1.

FIG. 1. Effect of the Glucose-6-phosphate Concentration on Enzyme Activity.

The optimum pH of the reaction was 7.5 in Tris-HCl buffer. Effect of glycogen concentration on the reaction rates was shown in Fig. 2. Oyster glycogen was used. The reaction rate in the presence of glycogen, 27 mg/ml, was about 24 times that in the absence of glycogen.

Product resulting from the standard reaction mixture was characterized by acid hydrolysis and treatment with glycogen-hydrolyzing enzymes. Product was obtained from standard reaction mixture incubated for 2 hr at 30°C by precipitation with 50% aqueous ethanol. The precipitation was repeated thrice. The product was hydrolyzed with 1 N HCl (100°C, 3 hr), glucoamylase, β-amylase, pullulanase and β-amylase plus pullulanase. The reaction mixture, with standards, was chromatographed on Toyo filter paper No. 50 in n-butanol-pyridine-water (6:4:3). The radioactive spots were cut out and counted in toluene scintillation fluid. These results are shown in Table I. Treatment of the product formed in the standard reaction mixture

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1 Polysaccharide Production by Pullularia pullulans. Part IV. See Reference 3) for the Part III.
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TABLE I. ANALYSIS OF PRODUCT FORMED IN THE STANDARD INCUBATION MIXTURE

<table>
<thead>
<tr>
<th>Product treatment</th>
<th>% of total ¹⁴C incorporated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Glucose</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>HCl</td>
<td>80</td>
</tr>
<tr>
<td>Glucoamylase</td>
<td>93</td>
</tr>
<tr>
<td>β-Amylase</td>
<td>0</td>
</tr>
<tr>
<td>Pullulanase</td>
<td>0</td>
</tr>
<tr>
<td>Pullulanase plus β-amy</td>
<td>0</td>
</tr>
</tbody>
</table>

a) 1 N HCl, 100°C, 3 hr.
b) Maltotriose-Maltodecaose

Fig. 2. Effect of Glycogen Concentration on the Reaction Rates.

with these hydrolyzing enzymes suggested that the product formed was an α-1, 4-glucan with some α-1, 6-linkages. The glycogen synthetase was detected in the cells of *Pullularia pullulans* cultured for 24~28 hr. From 48 hr-culture, however, glycogen synthetase could not be obtained, though the cells cultured for 48 hr synthesized pullulan at maximum rate.

Both glycogen and pullulan are α-glucans consisting of same type of linkage. In order to study on synthetic mechanism of pullulan, it may be required that the metabolic relationship between pullulan and glycogen is elucidated.

REFERENCES