Note

Synthesis of Laminarioligosaccharides Using Crude Extract of Euglena gracilis z Cells

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Laminarioligosaccharides (LOS) are important compounds for studying the specificity of β-glucan degrading enzymes, especially for β-1,3-glucans. They are now prepared by partial acid hydrolysis of β-1,3-glucans such as laminaran or pachyrnan.† In this study, the authors have developed an enzymatic method to prepare a series of LOS from glucose and α-glucose-1-phosphate (G-1-P) by the combined action of laminariobiose phosphorylase (EC 2.4.1.31)1 (LBPase) and β-1,3-oligoglucan phosphorylase (EC 2.4.1.30)2 (LOPase) as follows:

\[ \text{Glc + G-1-P} \rightarrow \text{LG}_1 + \text{Pi} \]

\[ \text{LG}_1 + \text{G-1-P} \rightarrow \text{LG}_2 + \text{Pi} \]

\[ \vdots \]

\[ \text{LG}_n + \text{G-1-P} \rightarrow \text{LG}_{n+1} + \text{Pi} \]

These enzymes are known to present in Euglena gracilis cells and have been partially purified.2-3 Furthermore, no other phosphorylases that react on α-G-1-P have been known in Euglena. For the above reasons, Euglena was used as the source of the enzyme in the following experiment.

* Euglena gracilis z (IAM E-6) was obtained from the Institute of Applied Microorganisms, The University of Tokyo. It was grown in a standard heterotrophic medium4 for 5 days at 30°C in the dark. The harvested cells (1 g) were suspended in 4 ml of 50 mM Tris-HCl buffer (pH 7.0), Buffer A, and disrupted by sonication. The suspension was centrifuged and the supernatant was used as the enzyme solution. It contained 1.11 U/ml of LBPase and 1.66 U/ml of LOPase activities. These activities were measured by the amount of Pi5 produced from 10 mM G-1-P and glucose or laminariobiose, respectively. One unit of either activity was defined as the amount of the enzyme that produces 1 μmol Pi per minute at 37°C in Buffer A. Neither G-1-P phosphatase nor phosphoglucomutase activity was de-

Fig. 1. Changes in the Concentration of G-1-P.
Initial concentration of glucose was: ○, 100; □, 50; △, 25; ●, 10; and ■, 5 mM.

Fig. 2. Concentration of Laminarioligosaccharides after 48 hr Incubation.
The initial concentrations of glucose are: A, 100 mM (1.8%); B, 50 mM (0.9%); C, 25 mM (0.45%); D, 10 mM (0.18%); and E, 5 mM (0.09%). Oligosaccharides were identified by comparing their retention times with those of standard laminarioligosaccharides. The average D.P. of the saccharides are calculated as A, 1.8; B, 2.6; C, 4.1; D, 6.6; and E, 8.4.

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Symbols: LG₁, LG₂, LG₃, ..., LGₙ stand for laminariobiose, laminaritriose, laminaritetraose, ..., n-mer of laminarioligosaccharide, respectively.
tected in the solution.

LOS was synthesized in the reaction mixtures (5 ml) consisting of Buffer A containing 400 μl of the enzyme solution, 100 mM G-1-P, 5 mM MgCl₂, 0.02% NaN₃, and varying concentrations (5 to 100 mM) of glucose. The reactions were done at 37°C for up to 48 hr and were followed by measuring the concentration of remaining G-1-P using phosphoglucomutase-glucose-6-phosphate dehydrogenase system. Figure 1 shows the course of the five reactions with various initial glucose concentrations. As is expected, the reaction proceeds more rapidly with a higher initial glucose concentration, and less G-1-P remained with a higher initial concentration of glucose after 48 hr of incubation.

The composition of LOS in the reaction mixtures obtained after the incubation for 48 hr was analyzed by HPLC equipped with an RI detector using the Hibercolumn LiChrosorb NH₂ (Cica-Merck, Japan) with 65% acetonitrile as the solvent. The samples were put on the column after deionization by Amberlite IR-120B and Amberlite IRA-93. The results are shown in Fig. 2. The retention times of the resulting oligosaccharides corresponded to those of the standard laminarioligosaccharides (D.P. 2–7, Yaizu Suisan Chemicals, Japan). It is clear that the average D.P. of saccharides in the reaction mixtures increased in proportion to the decrease in the initial concentrations of glucose. For instance, the value obtained with 100 mM glucose was 1.8, but 8.4 was obtained with 5 mM glucose. Figure 1 also indicates that the amount of G-1-P consumed varied rather slightly compared with the initial concentra-

tion of glucose. When the latter changed by 20 times (from 5 mM to 100 mM), the former did only less than twice (from 49% to 81%). This suggests the possibility that the equilibrium point of this multi-step reaction is set mainly by the ratio of P₄ to G-1-P. These results indicate that LOS of various average D.P. can be obtained by choosing an appropriate ratio of the concentration of glucose to that of G-1-P. G-1-P is an expensive reagent, but it can be easily prepared from starch using potato juice (containing phosphorylase). The extract of Euglena cells can also be easily prepared, so the method mentioned above can be an alternative for the practical preparation of LOS.

References