Note

Synthesis of Laminarioligosaccharides Using Crude Extract of *Euglena gracilis* z Cells

Motomitsu KITAOKA, Takashi SASAKI*
and Hajime TANIGUCHI*[†]

Nippon Petrochemicals Co. Ltd.,
3-1 Uchisaiwai-cho 1-chome,
Chiyoda-ku, Tokyo 100, Japan

* National Food Research Institute,
1-2 Kannondai 2-chome, Tsukuba,
Ibaraki 305, Japan

Received November 27, 1990

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

⋮

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 $\text{Pi}^{5)}$ produced from 10 mM G-1-P and glucose or laminaribiose, 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 phosphoglucumutase activity was de-

Fig. 1. Changes in the Concentration of G-1-P.

Initial concentration of glucose was: $\circ$, 100; $\square$, 50; $\triangle$, 25; $\bullet$, 10; and $\blacksquare$, 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.

[†] Corresponding author.

Symbols: LG_2 , LG_3 , LG_4 , ---, LG_n stand for laminaribiose, 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 μ l of the enzyme solution, 100 mM G-1-P, 5 mM $MgCl_2$, 0.02% NaN_3 , 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.⁶⁾ 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 a RI detector using the Hibercolumn LiChrosorb NH_2 (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 Pi 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

- 1) W. J. Whelan, in "Methods in Carbohydrate Chemistry," Vol. 1, ed. by R. L. Whisler and M. L. Wolfrom, Academic Press, New York, 1962, pp. 330-333.
- 2) S. H. Goldemberg, L. R. Marechal and B. C. De Souza, *J. Biol. Chem.*, **241**, 45 (1966).
- 3) L. R. Marechal, *Biochim. Biophys. Acta*, **146**, 417 (1967).
- 4) L. R. Marechal and S. H. Goldemberg, *J. Biol. Chem.*, **239**, 3163 (1964).
- 5) O. H. Lowry and J. N. Lopez, *J. Biol. Chem.*, **162**, 421 (1946).
- 6) G. Michael, in "Methods of Enzymatic Analysis," 2nd English Ed., Vol. 4, ed. by H. U. Bergmeyer, Academic Press, New York, 1974, pp. 185-191.