Note

Syntheses of Alkyl β-D-Mannopyranosides and β-1,4-Linked Oligosaccharides Using β-Mannosidase from Rhizopus niveus

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β-1,4-Linked manno-oligosaccharides were obtained regioselectively when mannose was incubated with β-mannosidase from Rhizopus niveus. By use of the obtained β-1,4-linked manno-oligosaccharides as a donor, the transglycosylation reaction was conducted with the same enzyme, and β-linked alkyl glycosides were obtained from various alcohols. In the reaction using the β-1,4-linked manno-oligosaccharides as a donor and acceptor, β-1,4-linked manno-oligosaccharides were obtained by a transglycosylation mechanism.

Key words: β-mannosidase; Rhizopus niveus; β-D-mannosyl oligosaccharide; reverse hydrolysis; transglycosylation

β-1,4-Linked manno-oligosaccharides such as Manβ1→4GlcNac, Manβ1→4Glc, and Manβ1→4Man are components of sugar chains in glycoproteins and glycolipids. In addition, β-D-mannosyl oligosaccharides such as Manβ1→4Man or Manβ1→4Manβ1→4Man are also used predominantly by human intestinal bifidobacteria. Therefore, an efficient method for the synthesis of β-linked manno-oligosaccharides has been needed. However, the chemical synthesis of β-mannosyl linkages is one of the most difficult problems in carbohydrate chemistry. Enzymatic synthesis of Manβ1→4GlcNac has been reported by Usui et al. using a transglycosylation reaction with β-mannanase (EC 3.2.1.78). They used Manβ1→4Manβ1→4Man as a donor in the transglycosylation reaction, and they obtained this trisaccharide from the hydrolyzate of mannan. However, natural mannan consists of a β-1,4-linked manno-backbone to which another carbohydrate group is attached, so it is difficult to isolate pure manno-oligosaccharides. The isolation procedure is inefficient and cumbersome.

In this study, we developed a convenient method for the synthesis of Manβ1→4Man or Manβ1→4Manβ1→4Man enzymatically using the β-mannosidase (EC 3.2.1.25) from R. niveus. Moreover, we synthesized β-1,4-linked mannotetraose and mannopentaose as well as various β-linked alkyl mannosides by the transglycosylation reaction using Manβ1→4Man as a donor.

According to the procedure of Hashimoto et al., the β-mannosidase was purified from the culture broth of R. niveus (commercial name “Sumizyme MC”), which was purchased from Shin-nihon Chemicals Co., Ltd. (Osaka, Japan). Powdered culture broth of R. niveus (100 g) was dissolved in 300 mL of water. The precipitate was removed by centrifugation at 10,000 x g at 4°C for 30 min. The supernatant was put on a DEAE Sepharose FF column (2.6 x 30cm) and the column run using a NaCl linear gradient (360 mL of 25mM potassium phosphate buffer (pH 7.0) and 360 mL of the same buffer made to 0.5M NaCl). The flow rate was maintained at 4 mL per min. Figure 1 shows the chromatogram using a DEAE Sepharose FF column for the purification of β-mannosidase. The fractions containing β-mannosidase activity (fractions No. 32-40) were collected and concentrated. We used this partially purified enzyme preparation for the following synthetic reaction without further purification. One unit of β-mannosidase was defined as the enzyme activity that liberated 1 μmol of p-nitrophenol from pNp-β-Man per min.

A solution consisting of 35 g of mannose and R. niveus β-mannosidase (2.5 units) in 50 mL of sodium acetate buffer (pH 5.0, 0.1 M) was incubated at 37°C. After 24 h, the enzyme was inactivated by heating the solution in boiling water for 5 min. The reaction mixture was put on an activated carbon column. At first, mannose was washed out with water, then oligosaccharides were eluted by a stepwise increase of acetonitrile concentration from 2% (v/v) to 6% (v/v) as shown in Fig. 2. The amounts of reducing sugar in each fraction were measured by a phenol-sulfuric acid method. The fractions corresponding to two major peaks, A and B, were collected and concentrated separately to give syrups.

Abbreviation: pNp-β-Man, p-nitrophenyl β-D-mannopyranoside.
of 162 mg and 65 mg, respectively. Although these yields were not high, mannose is cheap and can be easily recovered from the reaction mixture. The peaks A and B were identified as Manβ1→4Man and Manβ1→4Manβ1→4Man, respectively, by comparing the 13C-NMR spectra with the literature.7) Manβ1→4Man is a disaccharide found in α2-acid glycoprotein, and this method for the preparation of this disaccharide would be helpful for the study of function and structure relationship of sugar chains in this glycoprotein. Besides, the mannobiose or mannnotriose obtained in this reaction can be used as a donor in the transglycosylation reaction for the synthesis of Manβ1→4GlcNAc using β-mannanase.41

It is generally recognized that the reverse hydrolysis reaction is not regioselective and products with various linkages are formed simultaneously.8-11) It should be noted that a β-1,4-linked product was predominantly formed by the R. niveus β-mannosidase.

To examine the transglycosylation activity of the β-mannosidase from R. niveus, the reaction was done using Manβ1→4Man as a mannosyl donor and various alcohols as acceptors. A solution consisting of 5% (w/v) of Manβ1→4Man and 25% (v/v) alcohol of various kinds was incubated with β-mannosidase (5 milliunits/ml) in 100 mM sodium acetate buffer (pH 5.0) at 37°C for 10 h. The results are summarized in Table. Various primary and secondary alcohols acted as good acceptors.

A solution containing 5% (w/v) of Manβ1→4Man as a donor

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<tr>
<th>Table</th>
<th>Acceptor Specificity of β-Mannosidase</th>
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<tr>
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<td>Acceptors</td>
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<td></td>
<td>Methanol</td>
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<td>Ethylene glycol</td>
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<td>Benzyl alcohol</td>
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Transfer yield (%) = Transfer product + Mannose

n.d., not detected.

Fig. 3. HPLC Analysis of the Transglycosylation Reaction Using Mannobiose as Donor and Acceptor.

As well as an acceptor and β-mannosidase (5 milliunits/ml) in 100 mM sodium acetate buffer (pH 5.0) was incubated at 37°C. Figure 3 shows the HPLC chromatogram of the reaction mixture containing Manβ1→4Man. Other than the peaks A and B, which were confirmed as mannose and Manβ1→4Man, respectively, three new peaks were observed. The peaks C, D, and E in Fig. 3 were identified by mass spectra as β-1,4-linked mannnotriose, mannnotetraose, and mannopentaose, respectively. It is assumed that mannnotriose was formed by the transfer of the mannosyl residue to Manβ1→4Man, then mannnotetraose and mannopentaose were formed by further transfer of a mannosyl residue to mannnotriose and mannnotetraose.

The factors controlling the regioselectivity in the transglycosylation reaction have been reported to have a strong correlation with the substrate specificity of the enzyme in the hydrolysis reaction.11,12) These results on the transglycosylation reaction using β-mannosidase from R. niveus agree with this rule. In contrast, the reverse hydrolysis reaction is known to show low regioselectivity, because the oligosaccharides of various linkages are formed in equilibrium. Consequently, the reverse hydrolysis reaction usually gives a mixture of oligosaccharides of various linkages including the oligosaccharides which are difficult to obtain by the transglycosylation reaction. In this study, however, the reverse hydrolysis reaction afforded Manβ1→4Man predominantly. The specificity of β-mannosidase from R. niveus to hydrolyze the β-1,4-linkage may be extremely high in comparison with the other linkages. It is assumed that Manβ1→4Man reached the equilibrium rapidly, in contrast, the rate for the other oligosaccharides to reach equilibrium would be extremely slow. Therefore, the amounts of the products other than β-1,4-linked oligosaccharides could be negligible at the time when Manβ1→4Man reached equilibrium.

In conclusion, R. niveus β-mannosidase was effective for the selective synthesis of not only β-linked alkyl mannosides but also β-1,4-linked mannoooligosaccharides. These results suggest a wide applicability for the synthesis of β-mannosyl linkages considering that the chemical approach is very difficult.

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References

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