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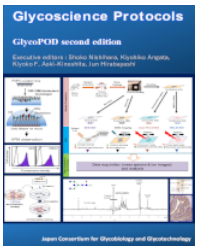

Smith degradation for glycan linkage analysis

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Introduction

Smith degradation, which comprises partial periodate oxidation and aldehyde reduction, was developed by Smith *et al.* for linkage analysis of carbohydrates (1,2). The periodate oxidation cleaves between hydroxyl carbons of a diol structure. The method was improved to high sensitivity for linkage analysis of oligosaccharides by Omichi and Hase (3–5).

Protocol

The following protocol is based on Reference 5 wherein linkage analysis of a reducing end saccharide in an oligosaccharide was performed. By analyzing the fluorogenic fragments generated using this method, the linkage position can be identified as shown in Figure 1.

Materials

1. Reagents were purchased from Fujifilm Wako Chemicals (Tokyo, Japan) of special grade or higher unless otherwise noted.

2. Wakosil-II 5C18HG column (6 × 150mm, #236-51151) was purchased from Fujifilm Wako Chemicals.

3. Fluorogenic standards for high-performance liquid chromatography (HPLC) analysis are prepared as described in Reference 3.

Instrument

1. HPLC apparatus.

Methods

1. Smith degradation
    a. Prepare a dried pyridylaminated (PA-) glycan sample (0.05–0.5 nmol).
    b. Dissolve in 20 μL of 0.1 M sodium acetate, pH 4.0 on ice.

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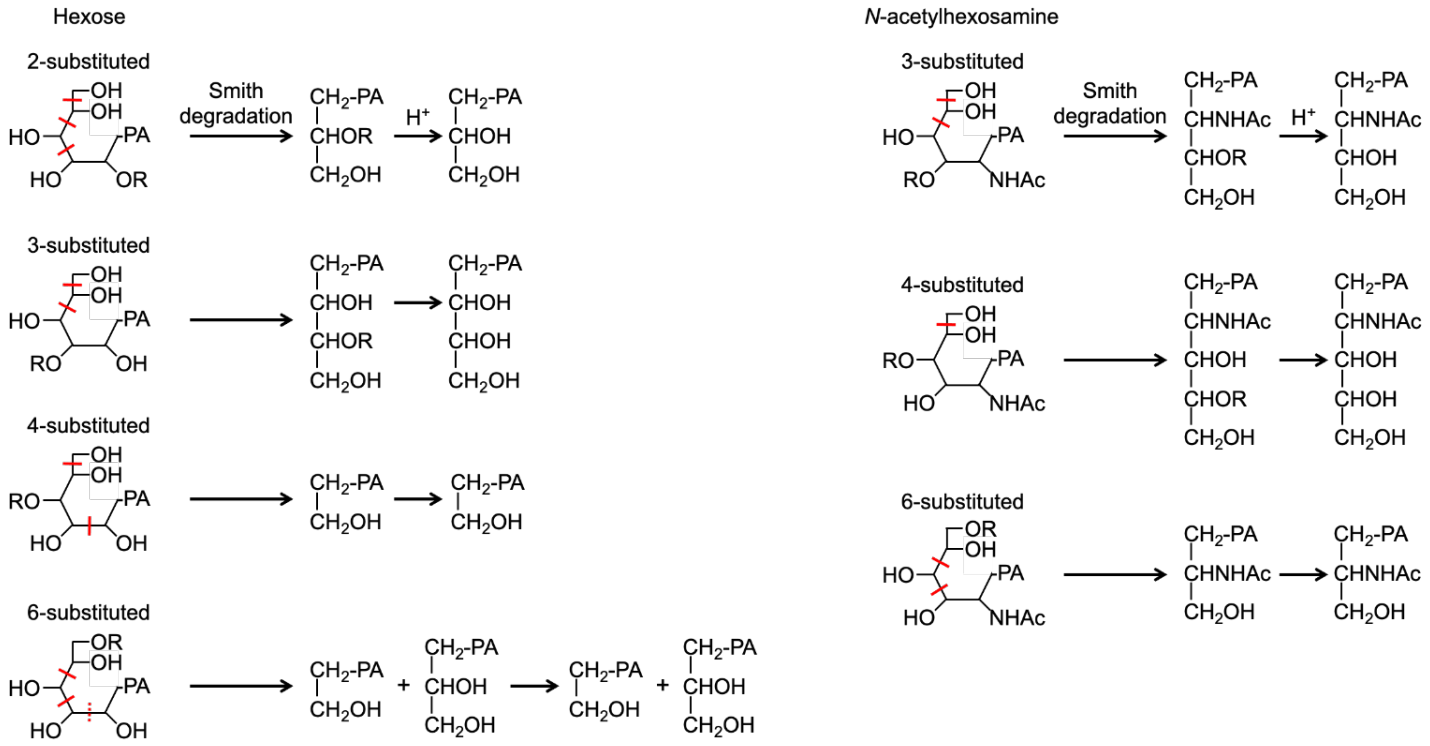

Figure 1: Overview of linkage analysis by Smith degradation. The sites that are cleaved by Smith degradation are indicated by red bars. A partially cleaved site is indicated by a dotted bar. CH_2OH-CHOH-CH_2-PA is also produced from 6-substituted hexoses because the CHO-CHOH bond in CHO-CHOH-CH_2-PA is rather stable against periodate oxidation compared with the CHOH-CHOH bond.

c. Add 20 µL of ice-cold 50 mM sodium metaperiodate.
d. Incubate at 0°C for 15 min in the dark.
e. Add 40 µL of 0.26 M sodium borohydride.
f. Incubate at 25°C for 1 h.
g. Add 10 µL of acetic acid and mix well to decompose residual borohydride.
h. Add 30 µL of 2 M sodium hydroxide to adjust the pH to 4.5.

2. Purification of the fluorogenic fragment derived from the reducing end

a. Apply the reaction mixture into a reversed-phase column (Wakosil-II 5C18HG, 6 × 150 mm) equilibrated with 50 mM ammonium acetate, pH 4.5, containing 0.025% 1-butanol. The condition is as provided below: Flow rate: 2.0 mL/min.

Detection: excitation 315 nm, emission 400 nm.

b. Elute by increasing the concentration of 1-butanol as shown below. If the sample is a disaccharide, elute at 0.025% 1-butanol: 0–11 min: 0.025%. 11–35 min: 0.025%–1%, linearly.

c. Collect the major fluorogenic product and lyophilize.

3. Acid hydrolysis and HPLC analysis (**Note 1**)

a. Dissolve the purified product in 25 µL of 4 M trifluoroacetic acid.

b. Heat at 100°C for 3 h.

c. Dry up under reduced pressure.

If the reducing end is *N*-acetylhexosamine, perform Steps d–g provided below to re-*N*-acetylation; otherwise, proceed to Step h.

d. Dissolve the dried product in 100 μL of the methanol, pyridine, and water mixture (6:3:2, v/v/v).

e. Add 6 μL of acetic anhydride and mix well.

f. Incubate at 25°C for 30 min.

g. Dry up under reduced pressure.

h. Dissolve in 50 μL of water.

i. Analyze by reversed-phase HPLC. The analytical condition is as below: Column: Wakosil-II 5C18HG, 6 × 150mm. Solvent: 50 mM ammonium acetate, pH 5.6, containing 0.025% 1-butanol. Isocratic elution. Flow rate: 2.0 mL/min. Detection: excitation 315 nm, emission 400 nm.

Note

1. In the case of oligosaccharides, HPLC analysis was performed after trimming off the nonreducing end part of the product using acid hydrolysis to facilitate the analysis. In the case of disaccharides, Steps a–g can be omitted.

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