Regioselective Synthesis of $\alpha$-L-Fucosyl-containing Benzyl Disaccharides by use of $\alpha$-L-Fucosidases of Aspergillus niger

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INTRODUCTION

The $\alpha$-L-fucosyl group is found in glycoprotein or glycolipids (Spiro, 1970), (1-2)-linked or (1-3), (1-4) or (1-6) linked to D-glycosyl residue.

Recently, Svenson and Katasumi Ajisaka reported an enzymic synthesis of methyl $\alpha$-L-fucopyranosyl-$\beta$-D-galactopyranosides by a transglycosylation of the L-fucosyl residue of para nitrophenyl $\alpha$-L-fucopyranoside to methyl $\beta$-D-galactopyranoside and N-acetylglucosamine with the aid of an $\alpha$-L-fucosidase from porcine liver and microorganism. Moreover, an enzyme obtained from microorganism is desirable for a preparative scale synthesis of oligosaccharides, as a large amount of the enzyme can be obtained easily. We report herein the regioselective synthesis of disaccharides containing a (1-3) linked $\alpha$-L-fucosyl group to N-acetylbenzyl glucosamine by using of $\alpha$-L-fucosidase. These $\alpha$-L-fucosyl-containing disaccharides are important not only starting materials for the synthesis of high molecular weight oligosaccharides as components of glycoproteins or glycolipids, but also can use for developing clinical test.

MATERIALS AND METHODS

Material

Q-Sepharose and Bio-gel P2 were purchased from Pharmacia-LKB and Bio-Rad respectively. As a source of aspergillus niger culture broth, "Rhozyme" was purchased from Gencor.

Semipurification of $\alpha$-L-fucosidase

Rhozyme (2.8 g) was dissolved in 20 mM potassium phosphate buffer (280 ml, pH 7.0) and dialyzed extensively against the same buffer (pH 7.0) during 48hrs. The dialyzed Rhozyme was counted the protein amounts and fucosidase activity, and then the Rhozyme solution was applied onto a Q-sepharose column (2.6 x 30 cm) which eluted with a salt gradient (Grove & Serif, 1981) from 0 to 0.5 M NaCl in 20 mM potassium phosphate buffer (pH 7.2, 2L in total), flow rate (5 ml/15 min., 1 drop/3 sec).

Fractions containing $\alpha$-L-fucosidase activity were pooled from fraction No.168-222, and concentrated 360 ml to 5 ml by a membrane filtration with Amicon PM30.

$\alpha$-L-fucosidase activity measured with PNP-fucoside as a substrate was 0.115 unit/ml.

Fucosidase activity;

The activity was determined with PNP-fucoside as modified A'Daniello method (Skood, 1975; D'Aniello, 1982; Weston et al., 1992). For enzyme assay, each volumes of semipurified fucosidase fraction from Rhozyme are diluted to 100 $\mu$l, of 5 mM/ml PNP-fucoside dissolved in 0.3 M sodium acetate buffer pH 5.0. The same substrate mixture was incubated in a shaking water bath at 37°C for 1 hr. The enzyme reaction was terminated by adding 1 ml of 0.2 M Na2CO3. Absorbance of para-nitrophenol (PNP) was read on a Beckman spectrophotometer at a wave length of 400
nm. Galactosidase activity; substrate- PNP-β-D-galactoside, N-acetylglucosaminidase activity; substrate PNP-β-D-N-acetylglucosamine, mannosidase activity; substrate PNP-β-D-mannoside, the manual is same above.

Protein Assay

It's determined by Bio Rad protein assay reagent at A 595 nm.

Synthesis of α-L-fucosyl 1-3-N-acetylglucosamine;

N-acetyl glucosamine 125 mg and PNP-α-L-fucopyranoside 25 mg were dissolved in 0.1 M acetate buffer (pH 5.0, 2.1 ml) and N,N-dimethyl formamide (250 µl). The semipurified α-L-fucosidase solution (600 µl, 0.07 unit) was added to the substrate solution, and the mixture was incubated for 13 hrs. at 37°C (Svensson & Thiem, 1990; Yazawa et al., 1986). The reaction mixture was heated in a boiling water bath for 10 min. (15,000 rpm, 30 min.), the denatured enzyme was centrifuged off, and the supernatant was applied onto Biogel P2 (200-400 mesh; 1.2x80 cm), and the reaction mixture was eluted with 0.1 M pyridine acetate pH 5.5 (BeMiller, 1980). The eluant was collected from fraction No.40-60 (flow rate 1.13/15 min.) (Fig. 4).

α-L-fucopyranoside 25 mg were dissolved in 0.1 M acetate buffer (pH 5.0, 2.1 ml) and N,N-dimethyl formamide (250 µl). The semipurified α-L-fucosidase solution (600 µl, 0.07 unit) was added to the substrate solution, and then treated the same manual above. (Svensson & Thiem, 1990; Yazawa et al., 1986).

The eluant was collected from fraction No.40-60 (flow rate 1.13/15 min.) (Fig. 4).

Assay of carbohydrate contents and purification;

The carbohydrate contents of each fraction was measured (Dubois et al., 1956; Clamp et al., 1967) with thioglycolic acid-H2SO4 reagent and UV-detection (248 nm). Fractions containing the benzyl-disaccharide were applied on C18 Sep-Pack (elute with methanol), and silica gel (chloroform-methyl alcohol-water, 13:6:1, RF; 0.49) after concentration.