Reaction mechanism of translated xylanase from *Thermatoga maritima* MSB 8 and preparation of propyl-glycosides

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Abstract

A thermostable xylanase from *Thermotoga maritima* (Xyn B) cleaves several pNP-glycosides of monosaccharides. We found that the initial product of the cleavage of pNP-xyloside (pNP-Xyl) was a disaccharide, not xylose, indicating that xylosyl unit of pNP-Xyl was transglycosylated to another pNP-Xyl. We determined that the disaccharide was xylobiose which has the linkage of the β 1-4, and described the reaction mechanism of the Xyn B. Also, we produced the several pNP-glycosides and propyl-disaccharides from the transglycosylation of Xyn B with varial glycosides and/or 1-propanol. All reaction products were purified by column chromatography (Toyo-pearl HW-40C, 45 cm×2.5 cm or 45 cm × 2.5 cm× 2). The isolated products were analyzed by means of 1D and 2D NMR.

Introduction

Over the past few decades, xylanases have received a great deal of attention, mainly due to their many and varied industrial applications such as in the pulp and paper industry, and in the food and feed industries. In addition, there has also been an interest in xylanases for use in the production of xylose, xylobiose and xylo-oligomers.[1,2] *Thermotoga maritima* MSB 8 possesses two xylanase genes, *xyn* A and *xyn* B, In the previous report, The *xyn* B gene was isolated from the genomic DNA of *T. maritima*, cloned, and expressed in *Escherichia coli*. Xyn B was purified to homogeneity by heat treatment, affinity chromato -graphy and ion-exchange column chromatography. The purified enzyme, Xyn B, cleaves several pNP-glycosides of monosaccharides. We found that the initial product of the cleavage of pNP-xyloside (pNP-Xyl) was a disaccharide, not xylose, indicating that xylosyl unit of pNP-Xyl was transglycosylated to another

pNP-Xyl. We need to determine the linkage of the disaccharide whether it is β 1-4 or other ones. We suggested the reaction procedure based on the several results in the previous report. However, the detail reaction mechanism of the XynB was not clear. In the present study, we described the reaction mechanism of the XynB and isolated two pNP-disaccharides produced from the reaction of pNP-glycosides, and propyl-glycosides from the reaction with pNP-glycoside and 1-propanol using the Xyn B.

Material and Method

Production and purification Xyn B

The Xyn B was produced and purified according to the previous report.[1] The purified Xyn B was used with proper dilution.

Preparation of xylobiose, pNP-fucobioside, pNP-arabinobioside by transglycosylation reaction

A mixture of 0.5% of pNP-Xyl, pNP-Fuc, or pNP-Ara, 0.1% of NaN_3, and properly diluted Xyn B in 50 mM MES buffer (pH 6.1) was incubated for 24 hours at 30°C. Then, Amberlite MB3 was added to stop the reaction and to remove the p-nitrophenol formed. The products were filtrated and concentrated. The concentrated transglycosylation products were purified by column chromatography (Toyo-pearl HW-40C, 45 cm× 2.5 cm or 45 cm× 2.5 cm× 2). The isolated products were analyzed by means of 1D and 2D NMR.

Preparation of propyl-xyloside, propyl-fucoside, propyl-arabinoside by trans-glycosylation reaction

A mixture of 0.5% of pNP-Xyl, pNP-Fuc, or pNP-Ara, 0.1% of NaN_3, 4% of 1-propyl alcohol and properly diluted Xyn B in 50 mM MES buffer (pH 6.1) was reacted 16 hours at 60°C. Then, Amberlite MB3 was added to stop the reaction and to remove the p-nitrophenol formed. The products were filtrated and concentrated. The concentrated transglycosylation products were purified by column chromatography (Toyo-pearl HW-40C, 45 cm× 2.5 cm or 45 cm ×2.5 cm ×2). The isolated products were analyzed by means of ^{1}H, ^{13}C decoupled, DEPT135, COSY, HSQC, HMBC.

Results

Time coursed reaction with pNP-glycosides and analysis of the structure of the product by NMR

The time courses of the reaction with 0.5 % pNP-Xyl, pNP-Fuc, or pNP-Ara were tracked by using TLC. As shown in the time course TLC, the pNP-Xyl reaction produced the mainly disaccharide. The linkage and structure of the disaccharide was confirmed by the NMR analysis. Based on the NMR analysis, this disaccharide was confirmed as β-1,4- xylobiose. In the reaction with other glycosides, intermediates were found on TLC at the position of pNP-disaccharides. The intermediates produced from pNP-Fuc and pNP-Ara reactions were confirmed to be pNP-fucobioside which had the linkage of β-1,3 and the pNP-arabinobioside which had the linkage of α-1,3 by NMR analysis.

Transglycosylation of pNP-glycosides and 1-propanol by Xyn B

The reaction with the pNP-glycosides and 1-propanol using Xyn B produced the correspondent propyl glycosides. The structures of these products were analyzed by 1D and 2D NMR. The structures of these products were propyl-monosaccharides, not disaccharides.

Proposed reaction mechanism of Xyn B and pNP-xyloside

The cleavage of pNP-Xyl by Xyn B of *T. maritima* was not caused by simple hydrolysis and a transglycosylation step must occur in the reaction. The result that the main product of the reaction was xylobiose and the intermediate of the reaction with another glycosides was pNP-glycobiose suggests that the reaction proceeded in one of the following two schemes.

Scheme 1

pNP-Xyloside + pNP-Xyloside -> pNP-xylobiose + pNP

pNP-xylobiose -> pNP + Xylobiose

Scheme 2

pNP-Xyloside -> pNP + Xylose

pNP-xyloside + xylose -> pNP + xylobiose

The scheme 1 means that the xylosyl unit of pNP-Xyl is transferred to another pNP-Xyl molecule to form pNP-xylobioside followed by the hydrolysis into p-nitrophenol and xylobiose. The scheme 2 means that a pNP-Xyl is initially hydrolyzed into p-nitrophenol and xylose and a transglycosylation takes place with the resultant xylose and another pNP-Xyl to form xylobiose.

Though we could not find the pNP-xylobiose in the time coursed reaction with the pNP-Xyl, scheme 1 was still possible because Xyn B showed much higher affinity with pNP-Xylobiose than pNP-xyloside. Thus, the reaction mechanism of Xyn B was determined with the result of the alcohol reaction with pNP-Xyl.

The difference in the schemes can be determined by detecting the alkyl-xylobioside as explained below.

2 pNP-Xyl -> pNP-xylobiose + ROH -> pNP + Xyl_2-OR

(followed the Scheme 1)

2 pNP-Xyl -> pNP-Xyl + xyl + pNP + ROH -> 2 pNP + xyl + xyl-OR

(followed the Scheme 2)

The major intermediate of the reaction with pNP-Xyl and 1-propanol was propyl-xyloside, not propyl-xylobioside. Also, the reaction with pNP-Xyl and methyl-xyloside did not give methyl-xylobioside.

Based on these results, it is indicated that the transglycosylation reaction mechanism of Xyn B follows the Scheme 2.

Reference

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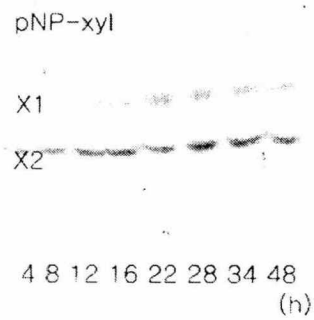

Fig 1. Thin layer chromatography of the hydrolysis products formed from pNP X2

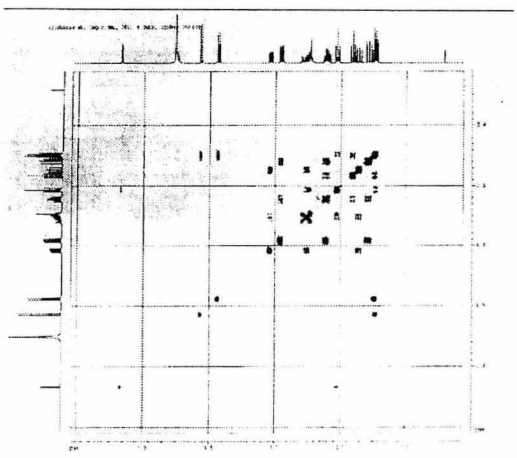

Fig 2. COSY analysis for the hydrolysis product considered disaccharide.