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Substrate Specificity of α-L-Arabinofuranosidase from *Streptomyces diastatochromogenes* 065 toward Arabinose-Containing Oligosaccharides

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α-L-Arabinofuranosidase from *Streptomyces diastatochromogenes* 065 released only the terminal arabinose of arabinoxylo-oligosaccharides. The enzyme hydrolyzed methyl arabinofuranobiosides to arabinose and methyl arabinofuranoside in the order of (1→2)>(1→3)>(1→5)-linkages. The enzyme preferentially hydrolyzed the (1→3)-linkage over the (1→5)-linkage of methyl arabinofuranotrioside.

[Key words: α-L-arabinofuranosidase, *Streptomyces diastatochromogenes*, substrate specificity α-L-arabinofuranosidase, arabino-oligosaccharides, arabinoxylo-oligosaccharides]

It has been reported that L-arabinose selectively inhibits intestinal sucrase in an uncompetitive manner and reduces the glycaemic response in animals following sucrose ingestion (1). Based on this observation, arabinose can be used as a physiologically functional sugar possessing inhibitory activity against sucrose digestion. L-Arabinosyl residues are widely distributed in plant cell walls such as arabinan, arabinoxylan, arabinogalactan. α-L-Arabinofuranosidase [α-L-AFase (EC 3.2.1.55)] has proven to be an essential tool for the production of arabinose by degrading these polysaccharides. There have been many studies on α-L-AFase, but the substrate specificity of the enzyme has not frequently been reported because of the difficulty in preparing arabinofuranose-containing oligosaccharides. To clarify the specificity of α-L-AFase, we synthesized methyl 2-*O*-, 3-*O*- and 5-*O*-arabinofuranosyl-α-L-arabinofuranosides (arabinofuranobiosides) (2), and methyl 3,5-di-*O*-α-L-arabinofuranosyl-α-L-arabinofuranoside (arabinofuranotrioside) (3).

The purification of the enzyme from *Streptomyces diastatochromogenes* 065 and determination of the substrate specificity of the enzyme toward arabinofuranose-containing polysaccharides are undertaken (4–6), but the specificity of the enzyme towards arabinofuranose-containing oligosaccharides still remains to be studied. Therefore, we investigated the substrate specificity of α-L-AFase from *S. diastatochromogenes* 065 using several arabinoxylo- and arabino-oligosaccharides. The structures of the substrates used are shown in Fig. 1.

α-L-AFase from *S. diastatochromogenes* 065 was purified according to the method described previously (4).

The specificity of α-L-AFase from *S. diastatochromogenes* 065 toward arabinoxylo-oligosaccharides, namely *O*-α-L-arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (A_1X_2), *O*-β-D-xylopyranosyl-(1→4)-[*O*-α-L-arabinofuranosyl-(1→3)]-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (A_1X_3) and *O*-β-D-xylopyranosyl-(1→2)-*O*-α-L-arabinofuranosyl-(1→3)-*O*-β-D-xylopyranosyl-(1→4)-*O*-β-D-xylopyranosyl-(1→4)-D-xylopyranose (A_1X_4), was determined (Fig. 2). The sugars were prepared by the methods described previously (12, 13). The reaction mixture contained 0.5 ml (0.3 units) of α-L-AFase solution, 0.4 ml of McIlvaine buffer (pH 6.5) and 0.1 ml of 10% substrate (arabinoxylo-oligosaccharides). After 0-, 1-, 3-, 6-, 12- and 24-h incubations at 30°C, each of the reaction mixtures was heated in a boiling water-bath for 10 min to stop the reaction. Two μl of the mixture was subjected to thin-layer chromatography (TLC) analysis for the characterization of the hydrolysis products. TLC was developed by the ascending method using HPTLC-Alufolien Cellulose (Merck, Darmstadt, Germany) with a solvent system of 1-butanol-pyridine-water (6 : 4 : 3, v/v). Sugars on the plate were detected by heating at 140°C for about 5 min after spraying with a 1% methanol solution of *p*-anisidine hydrochloride. It was found that the enzyme only slightly hydrolyzed A_1X_2 (Fig. 2A), but did not hydrolyze A_1X_3 or A_1X_4 even after 24 h (Fig. 2B and C). The enzyme hydrolyzed A_1X_2 to a lower extent compared to other α-L-AFases from *Aspergillus niger* 5-16 (7) and *Bacillus subtilis* 3-6 (8).

The reaction mixture containing 0.1 ml of 1% arabinofuranobiosides (2) instead of arabinoxylo-oligosaccharides was treated in the same way. Twenty μl of the mixture was subjected to TLC analysis using HPTLC Kieselgel 60 (Merck) with a solvent system of chloroform-methanol-water (90 : 65 : 15, v/v). Sugars on the plate were detected by heating at 170°C for about 5 min after spraying with methanol-sulfuric acid (1 : 1, v/v). Figure 3 shows the time course of hydrolysis of arabinofuranobiosides by α-L-AFase from *S. diastatochromogenes* 065. The enzyme hydrolyzed the arabinofuranobiosides to arabinose and methyl α-L-arabinofuranoside in the order of (1→2)->(1→3)->(1→5)-linkages.

The reaction mixture containing 0.1 ml of 10% arabinofuranotrioside (3) instead of arabinoxylo-oligosac-

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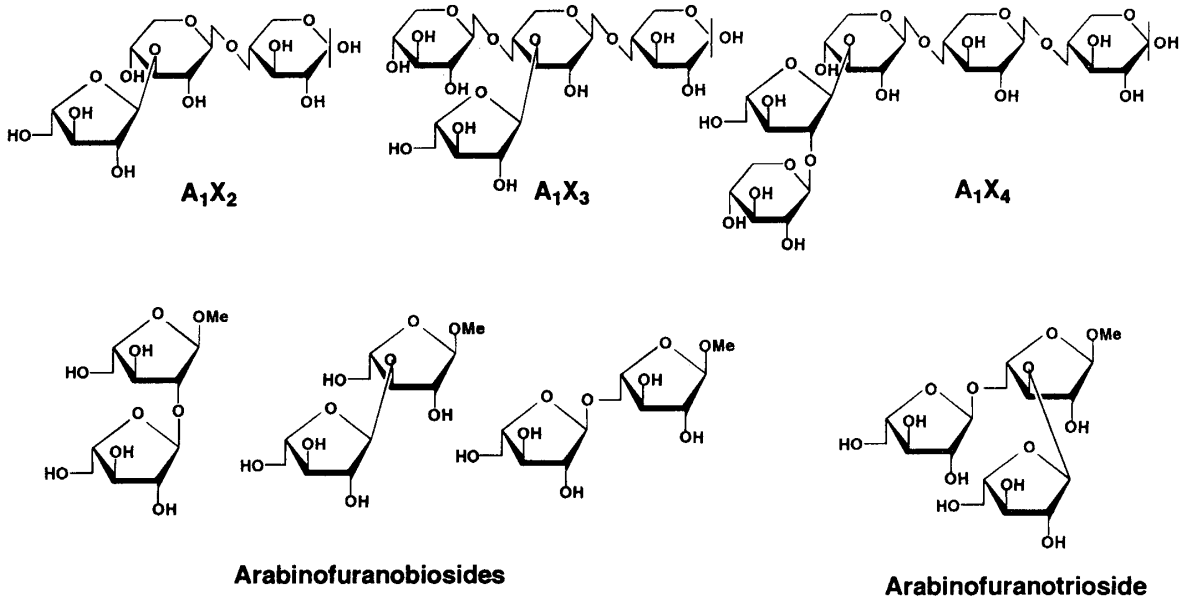

FIG. 1. The structures of arabinoxylo-oligosaccharides and arabino-oligosaccharides.

charides was incubated at 30°C for 3 h. Then, the reaction mixture was applied to a C_{18} column (LiChrospher 100 RP-18, Cica-Merck) equilibrated with 1.7% CH_3CN at a flow rate of 0.5 ml/min. Sugars eluted from the column were detected using a RI detector. The enzyme preferentially hydrolyzed the (1→3)-linkage rather than the (1→5)-linkage of arabinofuranotrioside as shown in Fig. 4. This specificity of the enzyme toward arabino-oligosaccharides was almost the same as that of the α-L-AFase from *B. subtilis* 3-6 (9).

The substrate specificity of α-L-AFase from *S. diastatochromogenes* 065 towards arabinofuranose-containing polysaccharides has been reported, and it was found that the enzyme released arabinose from arabinan (hydrolysis rate of approximately 30%), but did not or only slightly released arabinose from arabinoxylan and gum arabic (4, 6). Arabinans analyzed to date have been found to have (1→5)-linkages in the main-chain and to be highly branched at the *O*-2 and/or *O*-3 positions with single or multiple arabinofuranose residues as side chains (10). Tanaka *et al.* (11) proposed that one of the reasons for the incomplete degradation of arabinan by the enzyme is the presence of (1→2)-linkages and galactose. However, our results indicate that methyl 2-*O*-α-L-arabinofuranosyl-α-L-arabinofuranoside was the best substrate among the arabinofuranobiosides for the α-L-AFase from *S. diastatochromogenes* 065 (Fig. 3).

The maximum percentage hydrolysis of arabinan by α-L-AFase from *S. diastatochromogenes* 065 has been found to be 30% at most despite of additional treatment of the same enzyme (6). The methylation analysis of digested arabinan by this enzyme indicated that the enzyme released arabinose exowisely from the non-reduc-

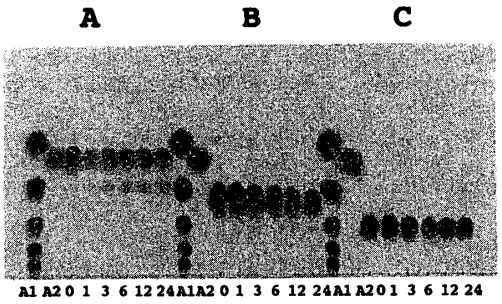

FIG. 2. TLC analysis of arabinoxylo-oligosaccharide digestion by α-L-arabinofuranosidase from *S. diastatochromogenes* 065. Hydrolysates: (A) from A_1X_2; (B) from A_1X_3; (C) from A_1X_4. A1, Authentic xylose to xylopentaose from top to bottom; A2, authentic arabinose. The authentic markers, xylose to xylopentaose were prepared by the method described in the previous paper (14).

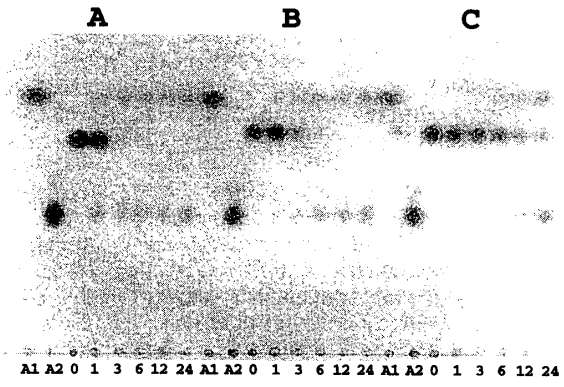

FIG. 3. TLC analysis of methyl α-L-arabinofuranobioside digestion by α-L-arabinofuranosidase from *S. diastatochromogenes* 065. (A) Products from methyl 2-*O*-α-L-arabinofuranosyl-α-L-arabinofuranoside; (B) products from methyl 3-*O*-α-L-arabinofuranosyl-α-L-arabinofuranoside; (C) products from methyl 5-*O*-α-L-arabinofuranosyl-α-L-arabinofuranoside. Markers used: A1, methyl α-L-arabinofuranoside; A2, arabinose.

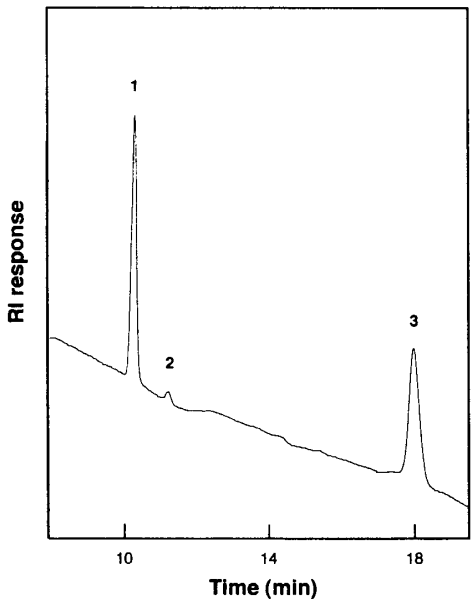

FIG. 4. HPLC of hydrolysate of methyl α-L-arabinofuranotrioside treated with α-L-arabinofuranosidase from *S. diastatochromogenes* 065. Peak 1, Methyl 5-*O*-α-L-arabinofuranosyl-α-L-arabinofuranoside; peak 2, methyl 3-*O*-α-L-arabinofuranosyl-α-L-arabinofuranoside; peak 3, methyl 3,5-di-*O*-α-L-arabinofuranosyl-α-L-arabinofuranoside.

ing end of arabinan remaining the proportion of the main-chain and side chain as previously reported (6). Thus, the mode of action of α-L-AFase from *S. diastatochromogenes* 065 on sugar beet-arabinan was speculated to be as follows. Firstly, the enzyme hydrolyzes single or multiple α-L-arabinofuranosyl side chains attached at the *O*-3 or *O*-2 positions of the main chains consisting of contiguous (1→5)-linked α-L-arabinofuranosyl residues. Secondly, the enzyme stepwise hydrolyzes the (1→5)-linkages of the arabinosyl residues in the main chains starting from the non-reducing terminals.

In the previous paper (4), we concluded that the α-L-AFase from *S. diastatochromogenes* 065 is not good at hydrolyze low molecular weight substrates. However, in this study we found that the enzyme hydrolyzes various low molecular weight substrates such as arabinofuranobiosides (Fig. 3) and arabinofuranotrioside (Fig. 4). Therefore, it may be speculated that this enzyme hydrolyzes the (1→5)-linkages of arabinan to a lesser extent and further, that the presence of trace amounts of galactose in arabinan renders the hydrolysis difficult. However, a detailed structural analysis of arabinan in relation to its hydrolysis would be necessary to develop an economically feasible method for arabinose production.

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