β-Galactosidases from Jack Bean and *Streptococcus* Have Different Cleaving Abilities towards Fucose-Containing Sugars

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We examined the sugar-cleaving abilities of β-galactosidases from jack bean and *Streptococcus* towards sugars containing fucose residues, and found that jack bean β-galactosidase has an ability to cleave the β1-3 linkage between galactose (Gal) and fucose (Fuc) residues, but not β1-4 linkage. On the other hand, streptococcal β-galactosidase was found to cleave the linkage in both Galβ1-4Fuc and Galβ1-3Fuc disaccharide units. Such a difference in sugar-cleaving abilities between these 2 β-galactosidases will be useful for structural analysis of glycans, especially those from species belonging to Protostomia, such as *Caenorhabditis elegans*.

Key words β-galactosidase; galactose β1-4 fucose; Protostomia; *Caenorhabditis elegans*

Glycosylation is one of the important post-translational protein modifications that affect various biological phenomena, such as development and immunity.[1)] Diversity in the glycans attached to proteins greatly increases the functional diversity of the proteins. Therefore, to understand the functions of glycoproteins, it is essential to elucidate the structures of their constituent sugar chains. One of the effective approaches for structural analysis of glycans is the use of exoglycosidases having strict specificity, followed by the analysis of the products by mass spectrometry (MS) and/or HPLC.[2)] The use of glycosidases with unique cleaving abilities provides essential information about the sequences and types of linkages of glycans, which is difficult to obtain only by MS or HPLC. Since this approach depends on the exact cleaving abilities of glycosidases, it is important to gather data about the specificity of these enzymes.

During structural analyses of *N*-glycans in the glycoproteins of *Caenorhabditis elegans*,[3,4)] we found that some galactose (Gal) residues were not removed by jack bean β-galactosidase,[5,6)] an agent usually used to liberate Gal residues from glycans. On the other hand, the Gal residues were removed by streptococcal β-galactosidase.[7)] These findings indicated that these 2β-galactosidases differ in their sugar-cleaving abilities. Based on the structures of *C. elegans* *N*-glycans,[8)] which had been reported to have a certain extent of difference from those of vertebrates, the Gal residues which jack bean β-galactosidase failed to remove is presumed to attach to a fucose (Fuc) residue *via* β1-4 linkage. In the present study, we compared the sugar-cleaving abilities of these 2 enzymes, and found that only streptococcal β-galactosidase is able to remove the Gal residue from Galβ1-4Fuc disaccharide, a unit structure found in glycoconjugates of some species belonging to Protostomia.[9—13)]

MATERIALS AND METHODS

Materials The substrates used in the present study were sugars labeled with a fluorophore (2-aminopyridine; abbreviated as PA) *via* a linker derived from mannitol. Galβ1-4Fuc-PA and Galβ1-3Fuc-PA (Figs. 1A, B) were chemically synthesized.[14,15)] D3-PA, D4-PA, and E3-PA are PA derivative of natural *N*-glycans which contain the Galβ1-4Fuc unit isolated from *C. elegans* (Figs. 1C—E), and they were prepared as reported previously.[16)]

Glycosidase Treatment In order to study the ability of galactosidases to eliminate Gal residue, PA-sugar (10 pmol) was treated with 5 mU of jack bean β-galactosidase[17)] (Seika-

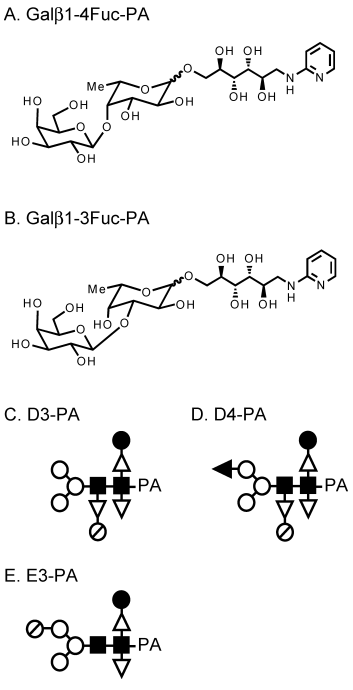

Fig. 1. Structures of Sugars Labeled with 2-Aminopyridine (PA) (PA-Sugars)

Structures of Galβ1-4Fuc-PA (A) and Galβ1-3Fuc-PA (B) and the expected structures of D3-PA (C), D4-PA (D) and E3-PA (E). Open circle, mannose; open circle with a diagonal line, hexose; filled circle, galactose (Gal); filled square, *N*-acetylglucosamine (GlcNAc); open triangle, fucose (Fuc); filled triangle, methylated fucose.

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gaku Kogyo, Tokyo, Japan) in 25 μl of 40 mM sodium citrate, pH 3.5, containing 0.02% bovine serum albumin, or with 1 mU of streptococcal β-galactosidase[7] (Seikagaku Kogyo) in 25 μl of 40 mM ammonium acetate, pH 5.5, containing 0.02% bovine serum albumin, for 24 h at 37 °C, according to the recommendation of the manufacturer.

Reversed-Phase HPLC Analysis Reversed-phase HPLC was performed on a Palpak type R column (0.46×25 cm; Takara Biomedicals, Shiga, Japan) equilibrated with 0.1 M ammonium acetate, pH 4.0, at a flow rate of 1.0 ml/min. Fluorescence signals of the eluted PA-sugar was monitored using a fluorescence spectrophotometer at an excitation wavelength of 310 nm and an emission wavelength of 380 nm.

RESULTS AND DISCUSSION

Jack bean β-galactosidase cleaves the β1-4 linkage between Gal and *N*-acetylglucosamine (GlcNAc) residues more efficiently than the β1-3 linkage,[5,6] indicating that this enzyme preferentially removes the Gal residue linked to the penultimate sugar unit *via* a β1-4 linkage rather than a β1-3 linkage. However, we found that jack bean β-galactosidase could not remove the Gal residue from *C. elegans N*-glycans, though the Gal residues were presumed to be attached to Fuc residue *via* a β1-4 linkage based on the reported structures of *C. elegans N*-glycans.[8,12]

To clarify this discrepancy, we examined the ability of jack bean β-galactosidase by using PA-sugars containing Gal-Fuc linkages, namely, Galβ1-4Fuc-PA, Galβ1-3Fuc-PA, D3-PA, D4-PA, and E3-PA (Fig. 1) as substrates. After treatment of these PA-sugars with jack bean β-galactosidase, the products were analyzed by reversed-phase HPLC (Fig. 2A). This enzyme was found to cleave only Galβ1-3Fuc-PA; it failed to remove Gal residue from Galβ1-4Fuc-PA, D3-PA, D4-PA and E3-PA. These findings clearly showed that jack bean β-galactosidase preferentially hydrolyzes the linkage in Galβ1-3Fuc over that in Galβ1-4Fuc. Such observations have not been reported in case of Gal-GlcNAc disaccharide.

Hydrolytic rate of jack bean β-galactosidase was reported to be affected by the type and linkage of the penultimate sugar unit, and application of such a property for structural analysis of sugar chain has been proposed.[6] The present result showed that Galβ1-3Fuc and Galβ1-4Fuc can be distinguished from each other, indicating that jack bean β-galactosidase has useful properties.

Next, we examined the sugar-cleaving ability of streptococcal β-galactosidase toward PA-sugars (Fig. 2B). As opposed to jack bean β-galactosidase, this enzyme was able to cleave all substrates. The present findings indicate that jack bean β-galactosidase and streptococcal β-galactosidase have different sugar-cleaving abilities, *i.e.*, the former can only cleave Galβ1-3Fuc but not Galβ1-4Fuc, whereas the latter can cleave both. Moreover, in order to rule out the influence of varying experimental conditions, we also examined sugar-cleaving ability of jack bean β-galactosidase under the identical conditions used for streptococcal β-galactosidase, and obtained essentially the same results (data not shown).

The Galβ1-4Fuc disaccharide unit has been reported in nematodes, octopus, squid, and keyhole limpet,[9—13] all of these species belong to the Protostomia. A family of galactosyltransferases (GT92) responsible for the generation of the Galβ1-4Fuc disaccharide unit has also been found in various eukaryotic species except mammals,[18] suggesting the presence of the Galβ1-4Fuc unit in these species. Although the presence of the Galβ1-3Fuc disaccharide unit has not been reported thus far, its existence continues to remain a possibility, especially some of GT92 galactosyltransferases might be able to generate this linkage.

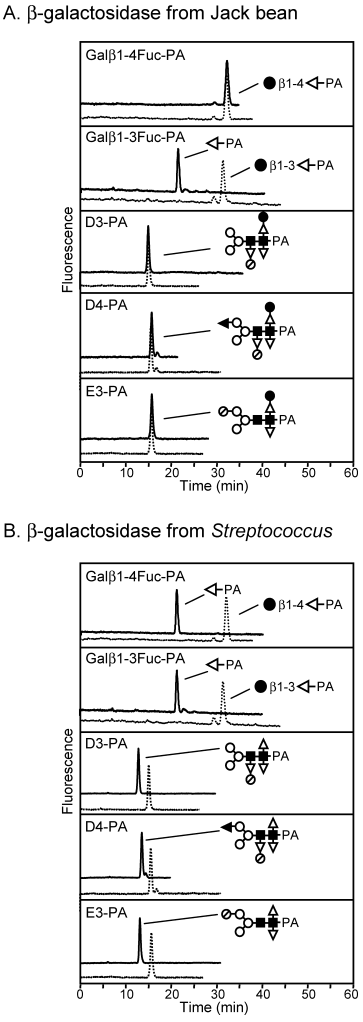

Fig. 2. Reversed-Phase HPLC Analysis of PA-Sugars Treated with β-Galactosidases

(A) Reversed-phase HPLC chromatograms of PA-sugars before (broken line; control) and after (solid line) treatment with jack bean β-galactosidase. The expected structures of the sugars are shown. Symbols are identical to those in Fig. 1. (B) Elution profiles of PA-sugars before (broken line; control) and after (solid line) treatment with streptococcal β-galactosidase.

The differences found in the present study between the sugar-cleaving abilities of β-galactosidases from jack bean and *Streptococcus* towards fucose-containing sugars should be useful, especially for the analysis of glycans from protostomes, because they enable distinction between Galβ1-4Fuc and Galβ1-3Fuc disaccharide unit, and thereby contribute to research on glycoconjugates.

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