Note

Isomelezitose formation by glucansucrases

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ABSTRACT

Several glucansucrases were surveyed for their ability to produce isomelezitose, a trisaccharide with the structure α-D-glucopyranosyl (1 → 6) β-D-fructofuranosyl (2 ↔ 1) α-D-glucopyranoside. Nearly all strains tested, with one exception, produced at least trace levels of isomelezitose. Yields were low but significant, ranging from less than 1% to approximately 5% based on sucrose. This trisaccharide may arise in either of two ways: glucopyranosyl transfer to the 6^{Fru}-OH position of sucrose, or to the anomeric −OH position of isomaltulose. This study indicates that isomelezitose formation may be a general phenomenon of many glucansucrase reactions.

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1. Introduction

Glucansucrases are members of the GH family 70 of glycosyl hydrolase/transferase enzymes [1]. They utilize sucrose as the high-energy α-D-glucopyranosyl donor, producing polymers of α-D-glucopyranosyl units linked in a variety of ways. D-Fructose is released, and may accumulate in the reaction medium if not removed via further metabolism. It has long been known that subsequent glucosyl transfer to fructose can result in leucrose (α-D-glucopyranosyl (1 → 5) β-D-fructopyranose [2] and isomaltulose (α-D-glucopyranosyl (1 → 6) D-fructose) [3]. Côte et al. [4] showed that in the presence of fructose, Leuconostoc citreum NRRL B-21297 alternansucrase (EC 2.4.1.140) also produces various trisaccharides, including 6^{Glc}-O-glucosylated leucrose and 2^{Fru}-O-glucosylated isomaltulose (isomelezitose). More recently, Shi et al. [5] found isomelezitose in acceptor reaction mixtures from Weissella confusa dextransucrase, suggesting that perhaps isomelezitose formation was more widespread among glucansucrases than previously suspected.

Therefore, we have undertaken a brief survey of several glucansucrases available in our laboratory to determine which glucansucrases produce isomelezitose, and in what amounts.

2. Experimental

2.1. Enzyme preparations

The following bacterial strains were obtained from the USDA-ARS Culture Collection (Peoria, IL, USA): Leuconostoc mesenteroides NRRL B-512F, B-523, B-1118, B-1121, B-1297, and B-1438; Leuconostoc citreum NRRL B-742, B-21297, and B-23183; Lactobacillus satsumensis NRRL B-1254 and B-59839. Bacteria were grown in a modified MRS medium containing sucrose, as previously described [6]. After cell removal by centrifugation (10,000 × g, 20 min), the culture fluids were concentrated by tangential-flow ultrafiltration using a 100,000 MW cutoff membrane, and diafiltered against 20 mM pH 5.5 sodium acetate buffer containing 2 mM calcium chloride and 1.5 mM sodium azide. The concentrated and dialyzed culture fluids were used without further purification. Activity ranged from 0.5 to 7.0 Units glucansucrase/mL.

Insoluble-glucan producing glucansucrase B-1118 DsrI and soluble-glucan producing B-1118 DsrS were prepared by cloning and expression in E. coli as previously described [7] and were electrophoretically homogeneous.

2.2. Reactions

All enzyme reactions were carried out at room temperature in 20 mM pH 5.5 sodium acetate buffer containing 2 mM calcium chloride and 1.5 mM sodium azide as a preservative. To compare

the products from various enzymes, 1 mL of an enzyme preparation was mixed with 6 mL of 1M sucrose (2 g total sucrose). When all sucrose had been consumed, as determined by thin-layer chromatography, any water-insoluble glucan was removed by centrifugation. The resulting aqueous samples were chromatographed over a 5 cm × 150 cm column of Bio-Gel P-2 (fine mesh), eluted with water. Fractions containing isomelezitose, as determined by TLC, were combined and freeze-dried, then dried in vacuo at 50 °C overnight. Isomelezitose was positively identified by chromatographic mobility, MALDI-TOFS, and ^{1}H and ^{13}C-NMR as previously described [4].

2.3. Analytical methods

Glucansucrase activity was measured by monitoring the incorporation of ^{14}C-glucose into methanol-insoluble glucan [7] using a modification of the technique first described by Germaine et al. [8].

Reactions were also monitored chromatographically. Thin-layer chromatography was carried out using silica gel 60 plates with three solvent ascents of acetonitrile-water 4:1 (v/v). Sugars were made visible using N-(1-naphthyl) ethylenediamine dihydrochloride in 3% (v/v) sulfuric acid in methanol [9]. The amount of isomelezitose in each reaction was determined by scanning the TLC plate on a desktop scanner (Epson Perfection V200 Photo) in black-and-white photographic reflectance mode. The image was saved as a 300dpi jpeg file, which was subsequently analyzed densitometrically, using Un-Scan-It software version 6.1 (Silk Scientific, Orem, Utah), and comparing spot densities and sizes to those of a standard isomelezitose solution spotted on the same plate.

3. Results and discussion

Isomelezitose was formed in reactions with sucrose by all glucansucrases tested except for one, although the relative amounts varied. By thin-layer chromatography, it was not possible to discern if Leuconostoc citreum NRRL B-742 was producing trace amounts, but upon column chromatography of the mixture and closer examination, isomelezitose could neither be detected nor isolated. All other strains tested yielded enough isomelezitose to be positively identified by ^{1}H-NMR spectroscopy.

It was obvious from thin-layer chromatography that Leuconostoc mesenteroides strain NRRL B-1297 produced the highest amounts, and this strain was used for subsequent production, isolation, and characterization of the trisaccharide on a useful scale and subsequent characterization by ^{1}H and ^{13}C NMR spectrometry. The ^{13}C NMR spectrum shown in Fig. 1 is identical to previously published

spectra [4,5,10]. Initially, we calculated approximate yields by isolation of isomelezitose using preparative chromatography on a column of Bio Gel P-2, but this technique proved tedious, time-consuming, and did not completely separate isomelezitose from other products in the lower-yielding strains. We subsequently developed a densitometric method based on thin-layer chromatography, which proved faster and more reproducible. Fig. 2 shows a comparison of isomelezitose yields from nine wild-type strains of glucansucrase available in our laboratory, as well as two cloned glucansucrases from the type strain of Leuconostoc mesenteroides, NRRL B-1118 [7].

Fig. 3 shows that isomelezitose is formed by strain NRRL B-1297 at a wide range of sucrose concentrations, with relative yields increasing somewhat at higher levels of sucrose. Although lower sucrose concentrations, typically on the order of 100 mM, are often used in published studies on glucansucrases, higher concentrations may more closely represent situations found in natural fermentations, such as sugar syrups or decaying plant matter. Thus, it is likely that isomelezitose would be present at measurable levels in a number of naturally fermented foods, as well as contaminated sugar cane or beet syrups.

As discussed in our previous work [4], there are two conceivable pathways to isomelezitose. One is via the direct glucosylation of sucrose, and the other involves the initial formation of isomaltulose via glucosylation of fructose [2], followed by subsequent glucosyl transfer to the anomeric carbon of isomaltulose to form a sucrose-type linkage. At present, we have no good data to support either pathway over the other. Although it could be argued that the formation of a sucrose-type linkage is less likely due to the unfavorable thermodynamics, such a transfer has been described in the formation of lactulosucrose via L. mesenteroides NRRL B-1299 glucansucrase [11]. In that case, the reaction mechanism was elucidated using ^{14}C-labeled sucrose. However, L. mesenteroides NRRL B-512F dextransucrase was investigated more recently for its ability to transfer glucosyl units from sucrose to isomaltulose, and it was found that the products were glucosylated at the 6-OH position of the glucosyl moiety, forming a series of 6^{Glc}-O-isomaltodextrinyl isomaltulose oligosaccharides [12], suggesting that at least in that case, isomelezitose was not formed. Shi [13] has proposed that the question could be answered by judicious use of labeled substrates and mass spectrometry; such information may yield useful insights regarding the mechanism of glucansucrases.

Although several of these strains have been isolated and described since Jeanes et al. [14] published their comprehensive survey in 1954, we wish to point out that there may be some correlation between isomelezitose yields and glucan yields. The strains
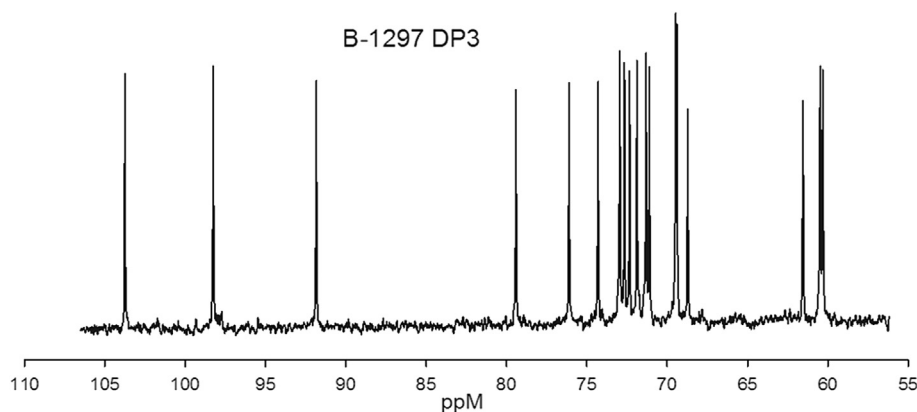

Fig. 1. Proton-decoupled ^{13}C-NMR spectrum of isomelezitose produced by L. mesenteroides NRRL B-1297 glucansucrase and isolated by Bio-Gel P-2 chromatography. Spectrum is identical to published spectra [4,5,10].