Food Chemistry 227 (2017) 202–210

Contents lists available at ScienceDirect

Food Chemistry

journal homepage: www.elsevier.com/locate/foodchem

A novel two-step enzymatic synthesis of blastose, a β-D-fructofuranosyl-(2↔6)-D-glucopyranose sucrose analogue

Alfonso Miranda-Molina [1], Edmundo Castillo [1], Agustin Lopez Munguia *

Departamento Ingeniería Celular y Biocatálisis, Instituto de Biotecnología, Universidad Nacional Autónoma de México, Av. Universidad 2001, Col. Chamilpa, C.P. 62209 Cuernavaca-Mor., Mexico

ARTICLE INFO

Article history:
Received 15 September 2016
Received in revised form 12 January 2017
Accepted 17 January 2017
Available online 19 January 2017

Chemical compounds studied in this article:
Trehalose (PubChem CID: 7427)
Sucrose (PubChem CID: 5988)
Levan (PubChem CID: 440946)
D-Glucose (PubChem CID:5793)
D-Fructose (PubChem CID: 5984)
Erlose (PubChem CID: 193471)
Neokestose (PubChem CID: 107452)
D-Galactose (PubChem CID: 6036)
DL-Xylose (PubChem CID: 644160)
Lactosucrose (PubChem CID: 174626)
Levanobiose (PubChem CID: 439555)

Keywords:
Blastose
Trehalose
Levansucrase
Trehalase
Regioselectivity
Prebiotics

ABSTRACT

Blastose, a natural disaccharide found in honey, is usually found as a byproduct of fructo-oligosaccharide synthesis from sucrose with fructosyltransferases. In this study, we describe a novel two-step biosynthetic route to obtain blastose, designed from a detailed observation of *B. subtilis* levansucrase (SacB) acceptor structural requirements for fructosylation. The strategy consisted first in the synthesis of the trisaccharide *O*-β-D-Fru*f*-(2↔6)-*O*-α-D-Glc*p*-(1↔1)-α-D-Glc*p*, through a regioselective β-D-transfructosylation of trehalose (Tre) which acts as acceptor in a reaction catalyzed by SacB using sucrose or levan as fructosyl donor. In this reaction, levansucrase (LS) transfers regioselectively a fructosyl residue to either C_6-OH group of the glucose residues in Tre. The resulting trisaccharide obtained in 23% molar yield based on trehalose, was purified and fully characterized by extensive NMR studies. In the second step, the trisaccharide is specifically hydrolyzed by trehalase, to obtain blastose in 43.2% molar yield based on the trisaccharide. This is the first report describing the formation of blastose through a sequential transfuctosylation-hydrolysis reaction.

1. Introduction

Besides the carbohydrate diversity offered by nature, glycodiversification may be generated through chemical and/or biochemical transformations, with biocatalysis as the preferred strategy for glycosylation and glycoside synthesis (Flieger et al., 2005; Gimeno-Pérez, Linde, Fernández-Arrojo, Plou, & Fernández-Lobato, 2015). In this context, successful processes have been developed for the synthesis of sucrose analogs and sucrose derivatives, of major interest as alternative chiral syntons for the production of a wide variety of glycoderivatives, such as tailor-made glycoconjugates and prebiotic oligosaccharides such as inulo-levan- and neofructooligosaccharides, among other derivatives (Daudé, Remaud-Siméon, & André, 2012; Zambelli et al., 2014).

Blastose (*O*-β-D-Fru*f*-(2↔6)-D-Glc*p*), is a reducing sucrose isomer, and the basis of the neofructooligosaccharide (neoFOS) series also known as neosugars. There is an increasing interest in neoFOSs synthesis due to their reported superior bifido-stimulating effect, as well as their chemical and thermal stability. In particular, neokestose (*O*-β-D-Fru*f*-(2↔6)-*O*-α-D-Glc*p*-(1↔2)-β-D-Fru*f*), the first fructosylation product of blastose, has been shown to improve the population of *Bifidobacteria* and *Lactobacilli* to a greater extent than commercial FOS, to inhibit *Clostridia* and the development of cancer cells (Ozimek, Kralj, van der Maarel, & Dijkhuizen, 2006).

Blastose consists of a glucose and a fructose molecule linked by a glycosidic bond between the C6-OH of the glucose residue and

* Corresponding author.
E-mail address: agustin@ibt.unam.mx (A. Lopez Munguia).
[1] Both authors made equal contribution to this work.

http://dx.doi.org/10.1016/j.foodchem.2017.01.094
0308-8146/

the C2-OH reducing carbon of the β-D-fructose residue (Gotor, 2002; Mower & Hancock, 1975; Seibel, Moraru, & Gotze, 2005; Tanaka, Yamamoto, Oi, & Yamamoto, 1981). Up to now, the chemical synthesis of blastose has not been described, while in enzymatic reactions, blastose has only been obtained in combination with various fructo-oligosaccharides as a secondary product of fructosyltransferase reactions with sucrose as substrate (Seibel et al., 2006). Blastose was initially described in submerged cultures of members of the fungal genus *Claviceps,* such as *C. africana* and *C. sorghi* where it was produced in concentrations up to 22 g/L from 100 g/L of sucrose (Flieger et al., 2005). It has also been obtained in reactions catalyzed by levansucrase from *Cladosporium cladosporioides* mycelium (34 g/L from 600 g/L of sucrose) (Tanaka et al., 1981) and in the reaction of sucrose with levansucrase from *Zymomonas mobilis* (Santos-Moriano et al., 2015). In the latter case, the authors demonstrated that blastose is the hydrolysis product of the synthesized neokestose, although it was not possible to rule out that blastose could also be synthesized by fructosylation of glucose as acceptor. Blastose (1.3–23.8 g/L) was also obtained from 171 g/L sucrose reactions with *Bacillus megaterium* levansucrase (Homann, Biedendieck, Götze, Jahn, & Seibel, 2007). Prebiotic FOS containing blastose (8–22 g/L) include those produced by the yeast *Xanthophyllomyces dendrorhous* β-fructofuranosidase (Gimeno-Pérez et al., 2015). In fact, the major interest in FOS research relies in their prebiotic properties, and blastose itself has been shown to support the growth of probiotic bacteria such as *Bifidobacteria* and *Lactobacillus* in the human gut. With its low caloric intake (2 kcal/g) and low glycemic index, its consumption has been associated to lower colon cancer risks, the prevention of urogenital infections, the improved absorption of Ca^{2+} and Mg^{2+} and the reduction of blood lipid levels (Delzenne, 2003; Sabater-Molina, Larqué, Torrella, & Zamora, 2009). As blastose purification from FOS mixtures is an inefficient and cumbersome process, its direct enzymatic synthesis may be an alternative production system (Flieger et al., 2005).

Bacillus subtilis levansucrasee (SacB), E.C. 2.4.1.10, is an enzyme capable to transfer the fructosyl residue from sucrose to a wide variety of acceptor substrates including water (hydrolysis reaction). In general, SacB is capable to catalyse the synthesis of levan, a D-fructose β-2,6-linked polymer; polymerization starts with a first fructose transfer to sucrose, which is followed by multiple transferences in a number that depends on several factors (Ortiz-Soto, Rivera, Rudiño-Piñera, Olvera, & López-Munguía, 2008). Glucose and fructose –byproducts of the reaction- can also act as acceptors. Eventually, and depending on their structure, other sugars added to the reaction may also act as fructosyl acceptors, resulting in either fructosides or heterooligosaccharides, such as galacto-fructosides, *xylo*-fructosides or lactosucrose (Mena-Arizmendi et al., 2011; Muramatsu & Nakakuki, 1995; Ning et al., 2010). In the synthesis of fructosides through LS acceptor reactions from sucrose, a major drawback is the presence of the residual sucrose, as well as free glucose and fructose, byproducts of the transferase and hydrolysis reactions. We have recently demonstrated the importance of SacB *exo*-levanase activity, which allows the use of levan as substrate for fructosylation of acceptor molecules (Mendez-Lorenzo et al., 2015). It is possible to take advantage of this SacB property, to fructosylate acceptor molecules without contamination with glucose and sucrose. The *exo*-type hydrolysis of levan releases fructose (monosaccharide) which can be fructosylated to levanbiose (disaccharide). Residual levan may be eliminated by filtration or precipitation.

The present study describes the formation of blastose through sequential two-step transfuctosylation-hydrolysis reactions using two different enzymes in separate steps. A fructosyl unit is first transferred from sucrose or levan to trehalose by SacB. In the second step the trisaccharide *O*-β-D-Fru*f*-(2↔6)-*O*-α-D-Glc*p*-(1↔1)-α-D-Glc*p* **4** is selectively hydrolysed by a commercial trehalase to form blastose **5** (Scheme 1).

2. Experimental section

2.1. Materials

Trehalose, sucrose, D-fructose and D-glucose were supplied by Sigma–Aldrich Inc. (MO, USA). Acetonitrile was of HPLC grade and were supplied by Burdick & Jackson (MI, USA). Blastose and low molecular weight levan were prepared in our laboratory (Porras-Dominguez, Avila-Fernandez, Miranda-Molina, Rodriguez-Alegria, & Lopez-Munguia, 2015). All other reagents were of the highest available purity.

2.2. Enzymes

Levansucrase (E.C. 2.4.1.10) from *B. subtillis* (SacB) was produced and purified in our laboratory according to the method reported previously (Mena-Arizmendi et al., 2011; Porras-Dominguez et al., 2015). Trehalase (prokaryote), a liquid enzyme preparation was obtained from Megazyme. α-amylase from *Aspergillus niger* and α-glucosidase from rice were supplied by Sigma–Aldrich Inc. (MO, USA). CGTase from *Thermoanaerobacter* sp. (Toruzyme 3.0 L, a liquid enzyme preparation) was obtained from Novozyme, *Thermotoga maritima* β-glucosidase was prepared in our laboratory (Miranda-Molina et al., 2010).

2.3. Enzyme assays

2.3.1. Transfructosilation reactions with levansucrase

Bacillus subtilis levansucrase (SacB) was selected for fructosylation. SacB activity was determined from the initial rate of reducing sugar release from a 10% w/v sucrose solution at 37 °C in 50 mM acetate buffer (pH 6.0) using the dinitrosalicylic acid (DNS) method with glucose as a standard. One activity unit (U) was defined as the amount of enzyme releasing the equivalent of 1 μmol of reducing sugar from sucrose per minute (mol min^{-1}).

Purified SacB was added to a reaction solution (1.5 ml) containing sucrose (200, 400, 600 and 800 mM), and trehalose (600 mM), or sucrose (800 mM) and trehalose (200, 400, 600 and 800 mM), or 600 mM of trehalose and 130 g/L of low molecular weight levan (800 mM of fructose). The final activity in the mixture was adjusted to 1 U/mL in reaction with sucrose as a fructosyl donor and 7.5, 10 and 25 U/mL in reaction with levan. Reaction mixtures were incubated at 37 °C at 650 rpm in an Eppendorf Thermomixer comfort (Hamburg, Germany). At different reaction times (0–48 h), 150 μL aliquots were withdrawn, boiled for 15 min to inactivate the enzyme, and conveniently diluted with water (1:10 or 1:20) for analysis HPLC. In all cases, reactions without trehalose were carried out as controls.

All fructosylation reactions were analyzed by HPLC analyses, performed in a Waters HPLC system (Waters Corp., MA, USA) equipped with a 600E system controller, using a Prevail Carbohydrate ES 5 μm column (4.6 × 250 mm, Grace Davison, Deerfield, IL, USA). Detection was performed using a refractive index detector (model 2410, Waters) equilibrated at 30 °C. Acetonitrile/water 75:25 (v/v), degassed with helium, was used as mobile phase at 1.0 mL/min. Fructoside yields were calculated from trehalose conversion.

2.3.2. Hydrolysis reactions with trehalase

The trehalase activity was determined from the initial rate of reducing sugar release from a 10 g/L trehalose solution at 37 °C in 50 mM acetate buffer (pH 5.0); using the dinitrosalicylic acid (DNS) method with glucose as a standard. One activity unit (U)