Acceptor reaction of a novel transfructosylating enzyme from *Bacillus* sp.

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Abstract

Many different oligosaccharides were produced by transferring the fructose residue of sucrose to maltose, cellobiose, lactose and sucrose (self–transfer), where their yields of fructosylated acceptor products accounted for 26~30% (w/w). The maximum conversion yield (30%) was obtained in fructosyl cellobioside formation with 500 g sucrose/l (substrate) and 200 g cellobiose/l (acceptor). These four acceptors gave various products having DP (degree of polymerization) 2~7 by successive transfer reactions.

Introduction

The enzymatic synthesis of a wide variety of oligosaccharides has been achieved by transfer reactions between a donor and various kinds of acceptors[1-3]. Transfer reaction usually takes place from a specific donor to a different acceptors, forming structurally different acceptor products[1-3]. Typical transfer reactions are usually found in dextransucrase (EC 2.4.1.5), inulosucrase (EC 2.4.1.9), levansucrase (EC 2.4.1.10), etc[3-5]. In this study, a series of acceptor reactions was carried out using the enzyme with various sucrose as a fructosyl donor and a wide variety of sugars as acceptors.

Materials and methods

Chemicals

The sucrose used as a substrate for fermentaion medium as carbon source and acceptor reaction was food–grade commercial product, while other chemicals were of reagent grade.

Enzyme preparation

Bacillus sp., was cultivated at 37℃ for 14 h in a 250 ml flask containing 50 ml of medium as described previously[6]. After removal of cells by centrifugation at 10,000 × g, the supernatant was concentrated by dialysis and membrane filtration (MWCO 30,000) and then centrifuged at 10,000 × g, for 10 min. Crude enzyme solution was further concentrated by ammonium sulfate precipitation (20–60% saturation) followed by dialysis at 4℃ overnight against 50 mM sodium phosphate buffer (pH 6.0). The resulting enzyme solution was directly used without further purification.

Enzyme reaction

Enzyme reactions were carried out in 10 ml test tubes containing 5 ml reaction mixture (3 ml sucrose in 50 mM sodium phosphate buffer (pH 6.0), various acceptors 1 ml, enzyme solution 1 ml) for 120 h at 50℃ in a water bath.

Analytical methods

Enzyme activity was determined by measuring the amount of product released under the following conditions: reaction mixture consisted of 500 g sucrose/l in 50 mM sodium phosphate buffer (pH 6.0) 5 ml, enzyme solution 5 ml at 50℃. One enzyme unit was defined as the amount of enzyme required to produce 1 μmole of 1-kestose (GF_2: G and F mean glucosyl and fructosyl moiety in sucrose molecule, respectively) per min. The reaction mixtures were analyzed by HPLC using an Aminex HPX-42C column (0.78 × 30 cm, Bio-Rad) and a refractive index detector (Shimadzu Co., Kyoto, Japan). The column temperature was maintained at 85℃ and water was used as a mobile phase at 0.6 ml/min.

Results

Acceptor specificity

Table. 1. Acceptor specificity of the transfructosylating enzyme from *Bacillus* sp[a].

Acceptor (g/l)[b]	Conversion yield (%)
D-Arabinose	ND[c]
D-Ribose	2.0
D-Xylose	20.7
D-Fructose	ND
D-Galactose	12.2
D-Glucose	16.1
D-Mannose	ND
L-Sorbose	11.2
D-Cellobiose	24.6
D-Lactose	21.5
D-Maltose	20.9
D-Sucrose	20.2
D-Melezitose	ND
D-Raffinose	ND
Stachyose	ND
Glycerol	ND
D-Inositol	ND
D-Mannitol	ND
D-Sorbitol	2.3
Xylitol	ND

[a] All reactions were carried out at 50 °C and pH 6.0 with 140 U/g substrate of enzyme for 90 h.

[b] The acceptor concentrations were used 250 g/l. [c] ND means not detected.

Effect of substrate/acceptor ratio on the acceptor reaction

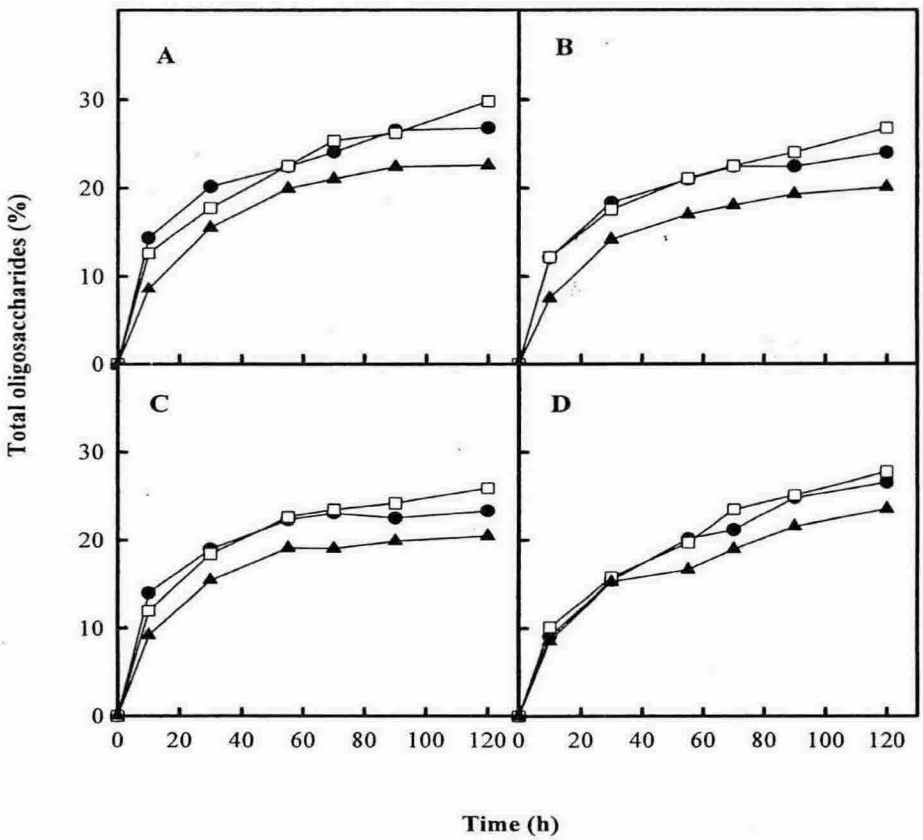

Figure. 1. Typical time course of various sugars on the acceptor reaction of transfructosylating enzyme from *Bacillus* sp. (A) D–cellobiose, (B) D–lactose, (C) D–maltose, (D) sucrose. Symbols: (●) 500 g sucrose/l (substrate) – 400 g acceptor/l, (□) 500 g sucrose/l (substrate) – 200 g acceptor/l, (▲) 500 g sucrose/l (substrate) – 50 g acceptor/l.

References

1. Muramatsu M and Nakakuki T (1995) Enzymatic synthesis of novel fructosyl and oligofructosyl trehaloses by *Aspergillus sydowi* β–fructofuranosidase.*Biosci.Biotech.Biochem.* **59**:208–212.

2. Duan KJ, Sheu DC, Lin CT (1995) Transglycosylation of a fungal alpha–glucosidase. The enzyme properties and correlation of isomalto–oligosaccharide production. *Ann. NY. Acad.Sci.* **750**: 325–328.

3. Heincke K, Demuth B, Jordening HJ, Buchholz K (1999) Kinetics of the dextransucrase acceptor reacton with maltose– experimental results and modeling. *Enzyme Microb. Technol.*

24: 523–534.

4. Jung KH, Yun JW, Kang KR, Lim JY and Lee JH (1989) Mathematical model for enzymatic production of fructo–oligosaccharides from sucrose. *Enzyme Microb. Technol.***11**: 491–494.

5. kitaoka M, Robyt JF (1999) Mechanism of the action of *Leuconostoc mesenteroides* B-512 FMC dextransucrase: kinetics of the transfer of D–glucose to maltose and the effects of enzyme and substrate concentrations. *Carbohyd. Res.* **320**: 183–191.

6. Park JP, Bae JT, Yun JW (1999) Critical effect of ammonium ions on the enzymatic reaction of a novel transfructosylating enzyme for fructo–oligosaccharide production from sucrose. *Biotechnol. Lett.* **21:** 491–494.