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Research review paper

Sucrose synthase: A unique glycosyltransferase for biocatalytic glycosylation process development

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ABSTRACT

Sucrose synthase (SuSy, EC 2.4.1.13) is a glycosyltransferase (GT) long known from plants and more recently discovered in bacteria. The enzyme catalyzes the reversible transfer of a glucosyl moiety between fructose and a nucleoside diphosphate (NDP) (sucrose + NDP ↔ NDP-glucose + fructose). The equilibrium for sucrose conversion is pH dependent, and pH values between 5.5 and 7.5 promote NDP-glucose formation. The conversion of a bulk chemical to high-priced NDP-glucose in a one-step reaction provides the key aspect for industrial interest. NDP-sugars are important as such and as key intermediates for glycosylation reactions by highly selective Leloir GTs. SuSy has gained renewed interest as industrially attractive biocatalyst, due to substantial scientific progresses achieved in the last few years. These include biochemical characterization of bacterial SuSys, overproduction of recombinant SuSys, structural information useful for design of tailor-made catalysts, and development of one-pot SuSy-GT cascade reactions for production of several relevant glycosides. These advances could pave the way for the application of Leloir GTs to be used in cost-effective processes. This review provides a framework for application requirements, focusing on catalytic properties, heterologous enzyme production and reaction engineering. The potential of SuSy biocatalysis will be presented based on various biotechnological applications: NDP-sugar synthesis; sucrose analog synthesis; glycoside synthesis by SuSy-GT cascade reactions.

Contents

Abbreviations: GT, glycosyltransferase; NDP, nucleoside diphosphate; NMP, nucleoside monophosphate; NTP, nucleoside triphosphate; SuSy, sucrose synthase.

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1. Introduction

The synthesis of glycosides is of interest in the pharmaceutical, cosmetic, food and nutraceutical industry (De Bruyn et al., 2015b; Desmet et al., 2012; Gantt et al., 2011; Křen and Řezanka, 2008; Seibel et al., 2006; Weijers et al., 2008; Xiao et al., 2014). Selective installment of a glycosyl group on complex substrates is challenging for chemical synthesis and requires elaborate synthetic procedures (Desmet et al., 2012; Hsu et al., 2011). By contrast, the exceptional regio- and stereoselectivity of Leloir-type glycosyltransferases (GTs) allows single-step glycosylations at specific positions of complex substrates without the need for protecting group chemistry (Gantt et al., 2011). Furthermore, compared to other glycosidic-bond forming enzymes, Leloir GTs accept a broader substrate diversity. However, high costs and limited availability of nucleotide-activated sugar donors are currently the main limitations for their industrial application (Desmet et al., 2012; Gantt et al., 2011).

Several routes towards nucleoside diphosphate (NDP) sugar production, comprising chemical synthesis, or single- and multi-enzyme strategies, have been described in literature (Cai, 2012; De Bruyn et al., 2015b; Gantt et al., 2011). The single-enzyme strategies, exploiting reversibility of GT reactions, represent the simplest NDP-sugar syntheses (De Bruyn et al., 2015b; Gantt et al., 2011; Zhang et al., 2006). Among those, the synthase route using sucrose synthase (SuSy, EC 2.4.1.13) is attractive due to sucrose availability at low cost and in bulk quantities (Daude et al., 2012; De Bruyn et al., 2015b). The high glycosidic bond energy of sucrose (α-D-glucopyranosyl-(1 → 2)-β-D-fructofuranoside) is comparable to that of NDP-sugars (Neufeld and Hassid, 1963). Thus, SuSy is highly suited for economic production of costly NDP-glucose from NDP and sucrose (Scheme 1) (Elling, 1997). Moreover, the SuSy-catalyzed reaction in synthesis direction provides access to several sucrose analogs (Römer et al., 2001, 2003). Reports on NDP-sugar synthesis by SuSy (Baroja-Fernández et al., 2014; Elling, 1997; Elling et al., 2005) and *in situ* supply of the glycosyl donor substrate in SuSy-GT coupled enzymatic reactions (Bungaruang et al., 2013; Chen et al., 2001b; Luley-Goedl and Nidetzky, 2011; Rupprath et al., 2007) have outlined the enzyme's potential for biocatalysis.

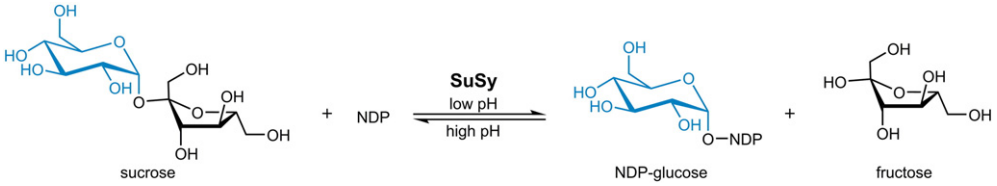
The main route of sucrose synthesis in plants and bacteria is based on the sequential action of sucrose phosphate synthase and sucrose phosphate phosphatase. Sucrose phosphate synthase generates sucrose-6-phosphate starting from fructose-6-phosphate and an activated sugar donor such as UDP-glucose, and sucrose phosphate phosphatase cleaves off the phosphate group releasing sucrose (Cumino et al., 2010; Porchia and Salerno, 1996; Salerno and Curatti, 2003). Further metabolization of sucrose by SuSy leads to fructose and NDP-glucose. The latter is used in the biosynthesis of starch or cellulose in plants or glycogen and other (structural) polysaccharides in cyanobacteria (Baroja-Fernández et al., 2003; Curatti et al., 2008; Haigler et al., 2001; Koch, 2004). SuSy was discovered in plants already in 1955 (Cardini et al., 1955). For about 50 years SuSy research was almost entirely focused on plant enzymes. Recent reports on SuSys from bacteria revealed a difference in nucleotide substrate preference for bacterial and plant enzymes (Diricks et al., 2015; Figueroa et al., 2013). Furthermore, the higher thermostability of bacterial SuSys might be useful for future industrial applications. However, the enzymes' synthetic use has not been fully explored yet. In view of the potential of SuSy to aid glycoside synthesis by GTs, a survey of biocatalytic process development of SuSy is presented. Summarized are catalytic properties, heterologous enzyme production and biotechnological applications of SuSy, including possible future perspectives.

2. Characteristics of sucrose synthases

2.1. Classification, structure and molecular mechanism of sucrose synthases

SuSys belong according to the systematic sequence-based classification of GT enzymes to family GT-4 (Coutinho et al., 2003). This is the second largest GT family with approximately 48,300 members in the Carbohydrate Active Enzymes (CAZy) database to date (Lombard et al., 2014). SuSy enzymes are retaining GTs and adopt a GT-B fold (Lairson et al., 2008; Zheng et al., 2011). Bacterial and plant SuSys are typically homotetramers composed of subunits with a molecular mass of around 90 kDa (Barratt et al., 2001; Bungaruang et al., 2013; Delmer, 1972; Elling and Kula, 1993a; Figueroa et al., 2013; Klotz et al., 2003; Morell and Copeland, 1985; Nakai et al., 1997a; Porchia et al., 1999; Römer et al., 2004; Sauerzapfe et al., 2008; Schäfer et al., 2004; Tanase and Yamaki, 2000; Wu et al., 2015). The suggested tetrameric form was confirmed by the only available X-ray crystal structures of SuSys from *Arabidopsis thaliana* (AtSus1, Fig. 1A) and *Nitrosomonas europaea* (Wu et al., 2015; Zheng et al., 2011). The plant and the bacterial SuSy showed a sequence identity of 50.3% and high structural conservation. Each SuSy monomer consists of four distinct domains. Here, only the plant domains are described to understand the effect of enzyme phosphorylation at the major phosphorylation site, which contributes to the fine-tuning of their activity (Fig. 2; Fig. S1). Two serine residues were identified as phosphoacceptors within the N-terminal regulatory domains (Hardin et al., 2003; Huber et al., 1996; Tsai and Wang, 2003). The *A. thaliana* AtSus1 polypeptide chain comprises a cellular targeting domain (CTD, residues 11–127), an early nodulin 40 (ENOD40) peptide binding domain (EPBD, residues 157–276), a typical N- ($GT\text{-}B_N$) and C-terminal ($GT\text{-}B_C$) domain of GT-B GTs, and a C-terminal extension (Zheng et al., 2011). The CTD contains the major phosphorylation site at Ser^{13} and the EPBD the second phosphorylation site at Ser^{167}. A unique feature of SuSys within GT-B enzymes is the ~10 Å helix Nα1 in the $GT\text{-}B_N$ domain (Fig. 1). The 3D-structure of AtSus1 reveals that conformational changes in the CTD of one subunit could be transmitted to the active site of the adjacent subunit via EPBD and helix Nα1, which in turn interacts with pyrophosphate of UDP and fructose, as illustrated in Fig. 1B. Thus, distortion of helix Nα1 upon Ser-phosphorylation in the CTD could explain modulation of biochemical properties of plant SuSys (for detailed information see Section 3 *Heterologous production of sucrose synthases* and Section 4.1 *Disaccharide synthesis*). Another conformational rearrangement proposed for plant and bacterial SuSys is induced by substrate binding (Wu et al., 2015). Based on comparative structural analysis of AtSus1 in closed conformation and *N. europaea* SuSy in open conformation an open/close induced fit mechanism was suggested. This is in good agreement with similar large conformational changes in other retaining GT-B GTs (Buschiazzo et al., 2004; Sheng et al., 2009).

Family GT-4 comprises a wide variety of GTs, including SuSy, sucrose phosphate synthase, trehalose synthase, trehalose phosphorylase, and many others, which are characterized by broad acceptor and donor



Scheme 1. Reaction scheme of sucrose synthase (SuSy). NDP is a nucleoside diphosphate.