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Research review paper

Amylosucrase as a transglucosylation tool: From molecular features to bioengineering applications

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ABSTRACT

Amylosucrase (EC 2.4.1.4, ASase), an outstanding sucrose-utilizing transglucosylase in the glycoside hydrolase family 13, can produce glucans with only α-1,4 linkages. Generally, on account of a double-displacement mechanism, ASase can catalyze polymerization, isomerization, and hydrolysis reactions with sucrose as the sole substrate, and has transglycosylation capacity to attach glucose molecules from sucrose to extra glycosyl acceptors. Based on extensive enzymology research, this review presents the characteristics of various ASases, including their microbial metabolism, preparation, and enzymatic properties, and exhibits structure-based strategies in the improvement of activity, specificity, and thermostability. As a vital transglucosylation tool of producing sugars, carbohydrate-based bioactive compounds, and materials, the bioengineering applications of ASases are also systematically summarized.

1. Introduction

The carbohydrate-active enzymes (CAZymes) refer to a series of enzymes that can build and breakdown complex carbohydrates and glycoconjugates (Cantarel et al., 2009). Recently, CAZymes have attracted a lot of attention owing to their ability of catalyzing the synthesis of complex oligosaccharides or polysaccharides from monosaccharides, as well as the glycosylation of nucleic acids, proteins, and polyphenols (André et al., 2014). In the CAZymes database, a diverse number of sucrose-utilizing enzymes have been proved to be useful tools for the diversification of carbohydrate-based molecules (André et al., 2010; Meng et al., 2016). Among them, amylosucrase (ASase, EC 2.4.1.4) is a sucrose-utilizing transglucosylase that can catalyze the synthesis of an amylose-like polymer named α-1,4-glucans, and the transglucosylation of a variety of exogenous acceptors.

ASase is an atypical member of the glycoside hydrolase family 13 possessing polymerization activity (Moulis et al., 2016). Generally, in terms of substrate, the reactions catalyzed by ASase can be classified into 3 types (Fig. 1): sucrose as the sole substrate, sucrose and extra glycosyl acceptors as the substrate, and maltooligosaccharides as the sole substrate. With sucrose as the sole substrate, ASase can catalyze three types of enzymatic reactions: polymerization, isomerization, and hydrolysis (de Montalk et al., 2000b). The polymerization reaction, a unique characteristic of ASase, synthesizes α-glucan with only α-1,4 linkages, and has no need for any primer or nucleotide-activated sugars, such as ADP- or UDP-glucose. Simultaneously, ASase produces a certain number of sucrose isomers, turanose and trehalulose, through isomerization reactions, and catalyzes a hydrolysis reaction releasing glucose and fructose from sucrose. In addition, in the presence of sucrose and extra glycosyl acceptors, ASase has transglycosylation capacity to attach glucose molecules from sucrose to glycosyl acceptors, such as glycogen (Putaux et al., 2006), starch (Rolland-Sabaté et al., 2004), flavanone (Overwin et al., 2015b), etc., that make it a vital transglucosylation tool in producing novel polysaccharides and carbohydrate-based bioactive compounds. Additionally, in reaction with maltooligosaccharides alone, ASase can catalyze the disproportionation of it by cleaving and transferring the glucosyl unit from a maltooligosaccharide donor to another maltooligosaccharide acceptor at the non-reducing end (Albenne et al., 2002).

A series of research studies have focused on the identification, catalytic mechanism, and structure-function analyses of ASases. In 1946, ASase was found in the genus *Neisseria*, and named because its enzymatic conversion of sucrose to a glycogen- or amylopectin-like polymer (Hehre and Hamilton, 1946). Until now, ASases have been found in microorganisms belonging to diverse genera: *Neisseria* (Hehre and Hamilton, 1946), *Deinococcus* (Pizzut-Serin et al., 2005), *Alteromonas* (Ha et al., 2009), *Arthrobacter* (Seo et al., 2012a), *Methylobacillus* (Jeong et al., 2014), *Synechococcus* (Perez-Cenci and Salerno, 2014), *Methylomicrobium* (But et al., 2015), and *Cellulomonas* (Wang et al., 2017). During the 2000s, ASase was first sequenced from *Neisseria*

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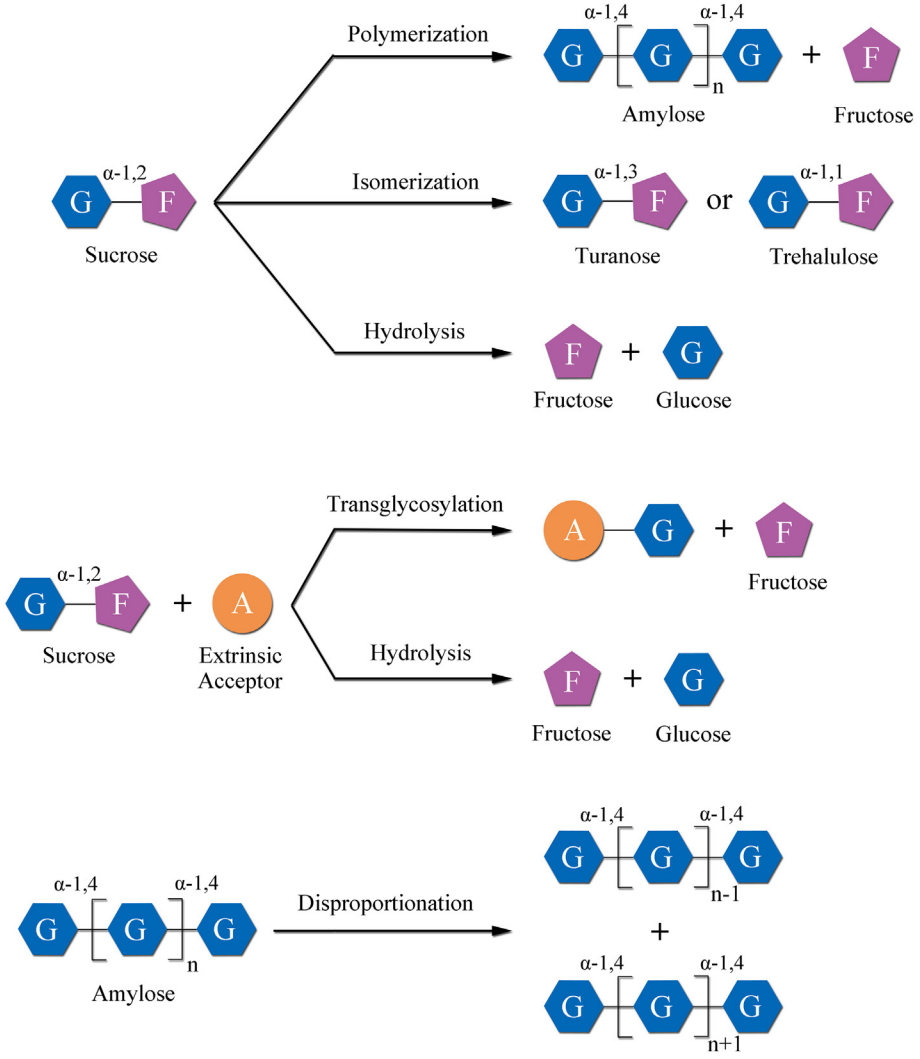

Fig. 1. The reactions catalyzed by ASase, including (A) sucrose as the sole substrate, (B) sucrose and extra glycosyl acceptors as the substrate, and (C) maltooligosaccharides as the sole substrate.

polysaccharea, successfully cloned and heterologously expressed in *Escherichia coli* (Büttcher et al., 1997), followed by reports of its three-dimensional structure (Skov et al., 2000). A decade later, a dimeric structure was reported (Guerin et al., 2012), and revealed that dimerization can greatly contribute to the thermostability of ASase. Based on the sequence-structure-function relationships, a number of rational and semi-rational engineering were conducted to enhance the transglucosylation capabilities of ASase, especially the transglucosylation capabilities toward non-natural accepters (Daude et al., 2014).

Based on extensive enzymology research, a wide range of applications of ASase have come to light, such as a soluble multi-enzyme system for synthesizing cyclodextrins (Koh et al., 2016), cycloamyloses (Kim et al., 2011b), and trehalose (Jung et al., 2013); the high-yield production of turanose (Park et al., 2016); the biological preparation of amylose microparticles (Lim et al., 2014), aglycone compounds (Jung et al., 2009), and resistant starch (Ryu et al., 2010). As a multifunctional enzyme of great industrial potential, it is worth systematically summarizing recent studies on the characteristics, structure, and catalytic mechanism of ASase, and present its extensive applications in the biosynthesis of sugars, active compounds, and materials.

2. Biochemical characteristics

Until now, ASases have been characterized from several different bacteria as follows: *N. polysaccharea* (NP-ASase) (Büttcher et al., 1997), *Deinococcus radiodurans* (DRd-ASase) (Pizzut-Serin et al., 2005), *Deinococcus geothermalis* (DG-ASase) (Emond et al., 2008b; Seo et al., 2008), *Alteromonas macleodii* (AM-ASase) (Ha et al., 2009), *Arthrobacter chlorophenolicus* (AC-ASase) (Seo et al., 2012a), *Deinococcus radiopugnans* (DRp-ASase) (Kim et al., 2014c), *Methylobacillus flagellates* (MF-ASase) (Jeong et al., 2014), *Synechococcus* sp. (SS-ASase) (Perez-Cenci and Salerno, 2014), *Methylomicrobium alcaliphilum* (MA-ASase) (But et al., 2015), *Cellulomonas carbonis* (CC-ASase) (Wang et al., 2017), and *Neisseria subflava* (NS-ASase) (Park et al., 2018a).

2.1. Role in microbial metabolism

In most investigated microorganisms, ASase contributes to the synthesis of glycogen and other polysaccharides (Okada and Hehre, 1974). In many bacterial species, glycogen is one of the storage polysaccharides synthesized in the presence of nucleotide sugars and enzymes including ADP-glucose pyrophosphorylase, glycogen synthases, and branching enzymes (Preiss and Romeo, 1994). However, among some bacteria, such as the genus *Neisseria* (Mackenzie et al., 1978) and the genus *Methylomicrobium* (But et al., 2015), they have another pathway of glycogen elongation through ASase from sucrose other than ADP-glucose. Apart from glycogen synthesis, ASase transfers the glucose moiety to a maltooligosaccharide or α-1,4-glucan in an oxygenic