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Research review paper

Maltooligosaccharide-forming amylase: Characteristics, preparation, and application

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ABSTRACT

As member of glycosyl hydrolase family 13, maltooligosaccharide-forming amylases (MFAses) are specific and interesting because of their capacity to hydrolyze starch into functional maltooligosaccharides, which are usually composed of 2–10 α-D-glucopyranosyl units linked by α-1,4 glycosidic linkages. MFAses have been extensively studied during recent decades, and have shown promise in various industrial applications. This review begins by introducing the potential uses of maltooligosaccharides. Then it describes the progress in the identification, assay, action pattern, structure, and modification of MFAses. The review continues with tips concerning the preparation of MFAses, which aim to improve MFAse production to meet the needs of industry. Finally, the industrial uses of MFAses are described, focusing on the production of maltooligosaccharides and application in the bread industry. Recent progress has demonstrated that the MFAses are poised to become important industrial catalysts.

1. Introduction

Maltooligosaccharides are usually composed of 2 to 10 α-D-glucopyranosyl units linked solely by α-1,4 glycosidic linkages (Min et al., 1998). They are a novel type of functional oligosaccharides with potential applications in food industry because of their mild sweetness, relative low osmolality, high water-holding capacity and suitable viscosity, as well as their ability to inhibit crystallization and delay the staling of bread (Ben Ali et al., 2001; Park, 1992). Significantly, maltooligosaccharides have potential benefits for human health. They enter the small intestine without digestion in the stomach, and then act as the major substrates for intestinal α-glucosidases derived from enterocytes (Chegeni and Hamaker, 2015), providing continuous and steady energy. Thus, they are a promising source of energy for athletes and some special patients. According to a recent report, maltooligosaccharides are also involved in glycemic control response and appear to induce the differentiation of small intestinal enterocytes (Chegeni and Hamaker, 2015).

Maltooligosaccharides can be produced by glycosyl transferases and glycosyl hydrolases, both of which have been suggested as candidate enzyme classes for use in the production of oligosaccharides (Bucke, 1996). The glycosyl hydrolases offer the advantage that they can use inexpensive and easy-to-get saccharides, such as starch, as substrates for maltooligosaccharide production. As one of the most common glycosyl hydrolases, α-amylase (1,4-α-D-glucan glucanohydrolase; EC 3.2.1.1) randomly cleaves the α-1,4 glycosidic linkages of starch to yield maltodextrin, maltooligosaccharides, or glucose. It is a member of glycosyl hydrolase family 13. Some other members of this family, including maltotriose-forming amylase (G3-amylase; EC 3.2.1.116), maltotetraose-forming amylase (G4-amylase; EC 3.2.1.60), maltopentaose-forming amylase (G5-amylase; EC 3.2.1.-), maltohexaose-forming amylase (G6-amylase; EC 3.2.1.98), and others that can specifically

Abbreviations: AmyUS100, G6-amylase from *Bacillus stearothermophilus* US100; BJAG5A, G5-amylase from *Bacillus* sp. JAMB-204; BLA, α-amylase from *Bacillus licheniformis*; CBM20, carbohydrate-binding module 20; DMSO, dimethyl sulfoxide; DNS, 3,5-dinitrosalicylic acid; G3, maltotriose; G3-amylase, maltotriose-forming amylase; G4, maltotetraose; G4-amylase, maltotetraose-forming amylase; G5, maltopentaose; G5-amylase, maltopentaose-forming amylase; G6, maltohexaose; G6-amylase, maltohexaose-forming amylase; G7, maltoheptaose; GstG6A, G6-amylase from *Geobacillus stearothermophilus*; HPLC, High-performance liquid chromatography; MFAse, maltooligosaccharide-forming amylase; TLC, thin layer chromatography

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produce maltooligosaccharides from starch are collectively known as maltooligosaccharide-forming amylases (MFAses). To this point, no review of the MFAses has become available.

Basic studies have focused on the isolation and identification of MFAses. Since the 1970s, different types of MFAses have been identified in diverse microorganisms. For example, a G3-amylase has been found in *Streptomyces* sp. (Kashiwagi et al., 2014), a G4-amylase in *Pseudomonas* sp. (Robyt and Ackerman, 1971), a G5-amylase in *Bacillus* sp. (Morgan and Priest, 1981), and a G6-amylase in *Klebsiella* sp. (Momma, 2000). Investigations conducted during recent decades have demonstrated that MFAses derived from different microorganisms exhibit distinct structures, properties, and product selectivities, which will be described in this review. Based on structure-function relationships, additional research has been devoted to modifying the enzymes to improve their stability and product selectivity, as well as other enhancements that make them more suitable for industrial applications (Doukyu et al., 2007; Khemakhem et al., 2009). This review will focus on the application of MFAses in the production of maltooligosaccharides, as well as the bread industry.

A few developed countries have monopolized the industrial production of maltooligosaccharides, leading to extremely high prices for purified maltooligosaccharides. MFAses are essential enzymes for the production of maltooligosaccharides. Thus, the potential value of MFAses has resulted in a significant amount of effort being devoted to the discovery, preparation and characterization of related amylases. The industrial production of maltooligosaccharides follows a process composed of three main steps: starch liquefaction, saccharification, and purification (Hisamatsu et al., 1979; Kimura and Nakakuki, 1990; Pankratz, 1977). Production rate, yield, product selectivity and cost should be considered when optimizing the production process, and the factors that influence these criteria will be summarized in this review.

2. Identification of MFAses

MFAses were originally identified by observing an unusual hydrolase activity in some amylase preparations. This hydrolase activity produced considerable amounts of maltooligosaccharides from starch. A G6-amylase firstly found by Kainuma et al. in 1972 (Kainuma et al., 1972) was separated from a pullulanase preparation obtained from an *Aerobacter aerogenes* culture. During the past few decades, MFAses expressed by bacteria, especially *Bacillus* sp., have been extensively studied, while some mesophilic and thermophilic fungi have also been reported to produce MFAses. The microbial strains that have been recognized as sources of MFAses are identified in Table 1. In several instances, these amylases have excellent product selectivity; thus, they can be named after their major maltooligosaccharide product, such as the G5-amylase from a deep-sea *Bacillus* sp.(Hatada et al., 2006) and the G4-amylase from *Pseudomonas stutzeri* (Maalej et al., 2014). The latter was found to catalyze the formation of extremely high levels of maltotetraose (G4) from starch (98%, w/w). In other instances, the amylases produce multiple major products. For example, the MFAse from *B. mojavensis* was reported to produce a mixture of maltotriose (G3), maltopentaose (G5) and maltohexaose (G6) from starch (Hmidet et al., 2010).

The available research has shown that a single genus, even a single species, may contain more than one putative MFAse, as shown in Table 1. Since the 1970s, G4-amylases have been widely reported from *P. stutzeri* strains. Robyt and Ackerman (1971) identified seven G4-amylases varying in molecular mass and isoelectric point from a *P. stutzeri* strain. Strain MS300 was reported to secrete four G4-amylases (two major forms, A and B, and two minor forms, C and D) (Kobayashi et al., 1998). Nakada et al. (1990) identified two active G4-amylases, G_4-1 and G_4-2, from *P. stutzeri* MO-19. These two enzymes had identical N-terminal sequences, but different C-terminal sequences. G_4-2 was shown to arise from the limited proteolysis of G_4-1.

As for other bacteria that produce MFAses, three amylases were found in the culture filtrate of alkalophilic *Bacillus* sp. H-167. All of these enzymes produce G6 in the initial stage of hydrolysis, with the maximum yield of 25–30% (Hayashi et al., 1988). Murakami et al. (2008) purified and characterized five alkaline, thermotolerant G4-amylases from *B. haloduans* MS-2-5. Proteolytic degradation and internal amino acid sequence analyses confirmed that all these amylases are produced from a single polypeptide. The gene encoding this polypeptide, named *amyA*, was cloned and expressed, in *Escherichia coli* for further study. Kashiwagi et al. (2014) reported two G3-amylases from *Streptomyces* species and analyzed their starch degradation profiles. Recently, a halotolerant G6-amylase was found from *Corallococcus* sp. EGB (Li et al., 2015), while two novel G3-amylases were purified from *Kitasatospora* sp. MK-1785 (Kamon et al., 2015) and *Microbulbifer thermotolerans* DAU221 (Lee et al., 2015), respectively.

As the number of sequenced microbial genomes has increased, the number of open reading frames annotated as MFAses has also increased. The Genbank accession numbers of the genes that have been shown to encode MFAses are presented in Table 1.

3. Assays of MFAse activity and products

Most of the methods used to determine MFAse activity are based on one of two phenomena observed in the degradation of starch: (a) change in the iodine-staining properties of the starch solution; or (b) an increase in the reducing power of the enzymatic reaction system. Both phenomena are characteristic of the action of α-amylases. The method based upon the former phenomenon, which has been called the blue value method (Fuwa, 1954), has been performed, with slight modification, using soluble starch as the substrate (Kanai et al., 2004).

The other type of method, measuring changes in the reducing power of starch, has been used more extensively.There are three methods with different sensitivities based on this principle: (a) The Somogyi-Nelson method (Somogyi, 1952) is relatively more complex in operation, but because it is more sensitive, it can be used to measure low levels of activity; (b) The Bernfeld method (Bernfeld, 1955) uses 3,5-dinitrosalicylic acid (DNS) as a color reagent; (c) A more widely used modified DNS method advanced by Miller in 1959 (Miller, 1959) employs the optimal composition of the DNS reagent. However, the assay methods described above are applicable to all α-amylases, not just MFAses. The common limitation of these methods is that the activity does directly represent the capacity for producing maltooligosaccharides. Hence, further measurements are needed to analyze the hydrolysis products of the enzymatic reaction.

The original method used to identify the individual products was paper chromatography (Kainuma et al., 1972; Okemoto et al., 1986), which was soon replaced by thin layer chromatography (TLC) (Wagner and Wiemken, 1987). TLC is still the simplest chromatographic technique currently used to analyze maltooligosaccharides. By analyzing the starch hydrolysis reaction after it has been underway for different amounts of time, the product components present during each stage can be preliminarily determined (Maalej et al., 2014; Murakami et al., 2008). Another important use of TLC is to explore the action pattern of the MFAse by using maltooligosaccharides (maltose–maltoheptaose (G7)) as substrates. Although useful and convenient in many cases, TLC shows limited application because it is easily influenced by the environment and not so sensitive and reliable as other methods. Besides, it is difficult to quantify the hydrolysate using TLC.

High-performance liquid chromatography (HPLC) has been widely used to quantify the product components. HPLC analysis is carried out on an NH_2 analysis column or a saccharide analysis column, using water or a mixture of acetonitrile and water as the mobile phase. The products are usually detected by using a refractive index detector or by using an evaporative light scattering detector as a sensitive and accurate substitute (Ben Ali et al., 2001; Candussio et al., 1990; Hayashi et al., 1988; Kashiwagi et al., 2014). Recently, a more efficient method was used to measure the maltooligosaccharides in samples.