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ORIGINAL ARTICLE

Purification of an alpha amylase from *Aspergillus flavus* NSH9 and molecular characterization of its nucleotide gene sequence

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Abstract
In this study, an alpha-amylase enzyme from a locally isolated *Aspergillus flavus* NSH9 was purified and characterized. The extracellular α-amylase was purified by ammonium sulfate precipitation and anion-exchange chromatography at a final yield of 2.55-fold and recovery of 11.73%. The molecular mass of the purified α-amylase was estimated to be 54 kDa using SDS-PAGE and the enzyme exhibited optimal catalytic activity at pH 5.0 and temperature of 50 °C. The enzyme was also thermally stable at 50 °C, with 87% residual activity after 60 min. As a metalloenzymes containing calcium, the purified α-amylase showed significantly increased enzyme activity in the presence of Ca^{2+} ions. Further gene isolation and characterization shows that the α-amylase gene of *A. flavus* NSH9 contained eight introns and an open reading frame that encodes for 499 amino acids with the first 21 amino acids presumed to be a signal peptide. Analysis of the deduced peptide sequence showed the presence of three conserved catalytic residues of α-amylase, two Ca^{2+}-binding sites, seven conserved peptide sequences, and several other properties that indicates the protein belongs to glycosyl hydrolase family 13 capable of acting on α-1,4-bonds only. Based on sequence similarity, the deduced peptide sequence of *A. flavus* NSH9 α-amylase was also found to carry two potential surface/secondary-binding site (SBS) residues (Trp 237 and Tyr 409) that might be playing crucial roles in both the enzyme activity and also the binding of starch granules.

Keywords α-Amylase · *Aspergillus flavus* NSH9 · Characteristic · cDNA · Nucleotide sequence

Introduction

Alpha amylase (α-1, 4 glucan-glucanohydrolase, EC 3.2.1.1) belongs to a family of endo-acting amylases that hydrolyses α-1,4 glycosidic bonds randomly throughout the starch molecule producing oligosaccharides and monosaccharides including maltose, glucose, and alpha limit dextrin at α-anomeric configuration (Bhanja et al. 2007). Most α-amylases are metalloenzymes, which require calcium ions (Ca^{2+}) as co-factor for their activity, structural integrity, and stability. Ever since the establishment of a sequence-based classification of all glycoside hydrolases in 1991, the α-amylases family has been known as family 13 of glycoside hydrolases (GH) (Henrissat 1991). GH13 is the largest member of the GH-H clan which also contains GH-70 and GH-77 (MacGregor 2005). In 2006, Stam et al. further divided members of GH13 into 35 subfamilies based on their sequence similarity and phylogenetic reconstruction criteria. To date, there are up to 42 subfamilies in GH13 and the number is still being updated (http://www.cazy.org/Glycoside-Hydrolases.html) (Valk et al. 2016). Fungal α-amylases are mainly classified into subfamilies of GH13_1 and GH13_5 with members in subfamily GH13_1 being extracellular and fungal specific, while those in subfamily GH13_5 are intracellular and have high sequence similarities to the bacterial α-amylases (Stam et al. 2006; van der Kaaij et al. 2007). A more recent study by Da Lage et al. (2013) reported on an additional family of GH13_32 for Basidiomycetes α-amylase which originated from Actinobacteria.

Having approximately 25% of the world enzyme market, amylase such as α-amylase is one of the most popular and important forms of industrial amylases due to its ability to

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