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Research review paper

Fungal glucoamylases

Dariush Norouzian [b,*], Azim Akbarzadeh [a], Jeno M. Scharer [b], Murray Moo Young [b]

[a] *Pilot Biotechnology Department, Pasteur Institute of Iran ,Tehran 13164, Iran*
[b] *Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1*

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Abstract

Fungi are employed to produce industrially important glucoamylases. Most glucoamylases are glycosylated. Glycosylation enhances the enzyme stability. Glucoamylases contain both starch binding and catalytic binding domains, the former being responsible for activity on raw (insoluble) starch. Proteases may act on this domain causing the enzyme to lose its activity on insoluble starch. Optimal activity is observed at pH 4.5 to 6.5 and 50 to 70 °C. Glucoamylases contain up to 7 sub-sites with highly varying affinity. They can be produced by different methods including submerged, solid state and semi-solid state fermentation processes.

Keywords: Glucoamylase function; Fungal fermentation; Fungal enzymes; Biochemical properties; Kinetic characteristics; Industrial production

Contents

1. Introduction

Glucoamylase (GA), also known as amyloglucosidase or γ-amylase (EC 3.2.1.3), is a biocatalyst capable of hydrolyzing α-1,4 glycosidic linkages in raw (sparse-

* Corresponding author. Present address: Pilot Biotechnological Department, Pasteur Institute of Iran, Tehran 13164, Iran.
E-mail address: danorouzian@yahoo.com (D. Norouzian).

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ly soluble) or soluble starches and related oligosaccharides with the inversion of the anomeric configuration to produce β-glucose. In addition to acting on α-1,4 linkages, the enzyme slowly hydrolyzes α-1,6 glycosidic linkages of starch (Weil et al., 1954; Pazur and Ando, 1960; Koshland, 1953; Fierobe et al., 1998). The specific activity (K_{cat}/K_m) towards the α-1,6 linkage is only 0.2% of that for the α-1,4 linkage (Hiromi et al., 1966; Sierks and Svensson, 1994; Frandsen et al., 1995; Fierobe et al., 1996). This adversely affects the yield in industrial applications of saccharification (Sauer et al., 2000). An increase of the glucose yield in saccharification beyond the present 96% level can be achieved by suppressing the activity of GA on the α-1,6 linkages present in starchy materials (Sauer et al., 2000).

The widely accepted mechanism of hydrolysis involves proton transfer from the catalyst to the glycosidic oxygen of the scissile bond. A general acid–base catalyst (McCarter and Withers, 1994; Sinnot, 1990; Konstantinidis and Sinnot, 1991; Tanaka et al., 1994) donates hydrogen to the glucosidic oxygen and a catalytic base guiding the nucleophilic attack by a water molecule on the C-1 carbon of the glucose moiety. The amino acid residue, Glu 179 of glucoamylase produced by *Aspergillus niger* has been identified as the general acid catalyst, and Glu 400 as the probable catalytic base group (Harris et al., 1993; Sierks et al., 1990; Frandsen et al., 1994). Glucoamylases are industrially important biocatalysts and have extensive uses in the manufacture of crystalline glucose or glucose syrup either as soluble or immobilized enzymes (Abraham et al., 2004; Torres et al., 2004; D'Souza and Kubal, 2002; Ruadze et al., 2001). Efforts are being made to produce and study the characteristics, structure, and function of this valuable biocatalyst from different primarily fungal sources.

2. Fungi-producing GA

Many fungal species are capable of producing GA under different fermentation conditions and techniques. Most attempts involved seeking fungal species capable of hydrolyzing raw starch at elevated temperatures. The various fungi synthesizing GA that is active at higher temperatures include *Aspergillus awamori*, *Aspergillus foetidus*, *Aspergillus niger*, *Aspergillus oryzae*, *Aspergillus terreus*, *Mucor rouxians*, *Mucor javanicus*, *Neurospora crassa*, *Rhizopus delmar*, *Rhizopus oryzae* (Pandy et al., 2000) and *Arthrobotrys amerospora* (Jaffar et al., 1993; Norouzian and Jaffar, 1993). However, the industrial focus has been on GA from *Aspergillus niger* and *Rhizopus oryzae*. The employment of GAs from these sources in the starch processing industries is due to their good thermostability and high activity at near neutral pH values (Frandsen et al., 1999; Reilly, 1999).

3. Enzyme production

Glucoamylases can be produced by submerged, solid state and semi-solid state fermentation using stirred tank vessels, airlift reactors or stacked trays. GA production is influenced by bioreactor design and operating mode. Bioreactors that had been employed to study GA production included flasks, trays, rotary reactors, columns (both horizontal and vertical) bioreactors (Pandy and Radhakrishna, 1992). Bo et al. (1999) investigated the production of glucoamylase in an air-lift bioreactor employing *Rhizopus oligosporus*. Glucoamylase was produced by a recombinant *Aspergillus niger* in glucose limited chemostat supplemented with various organic nitrogen sources (Richard et al., 2000). It was observed that cultures supplemented with various organic nitrogen sources including L-alanine, L-methionine, casamino acids, or peptone had shown reduced specific glucoamylase production. Fujio and Morita (1996) and Ramadas et al. (1996) employed *Rhizopus* SP.A-1 and *Aspergillus niger* to produce GA by solid state and semi-solid state fermentation. They found higher GA titer in the semi-solid fermentation system. Pedersen et al. (2000) compared GA production in batch, continuous and fed batch operations and found maximum production during batch cultivation.

4. Enzyme characteristics

Glucoamylases of fungal origin usually occur in multiple forms (Manjunath et al., 1983; Miah and Ueda, 1977; Pazure et al., 1971) and these multiplicities may be related to either the activity of protease produced along with GA concomitantly or, as pointed out by Svensson et al. (1986), the forms may be derived by different secondary processing. Fungal glucoamylases have two domains, namely a catalytic domain and a starch binding domain. The two domains are connected by an *O*-glycosylated polypeptide linker located at the N-terminus. The starch binding domain of GA plays an active role in hydrolyzing raw starch and supports the enzyme adsorption to the cell wall where local increase of enzyme concentration may result in enhanced glucose flow to the cell (Kaneko et al., 1996; Neustroev et al., 1993). Partial or total