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Studies on Antifungal and Antibacterial Activity of Glucose Aero dehydrogenase

Muhammad Anjum Zia, Mehreen Ishaq, Ishtiaq Ahmed and Hafiz Muhammad Nasir Iqbal

Enzyme Biotechnology Labortary, Department of Chemistry and Biochemistry,
University of Agriculture, Faisalabad, Pakistan

Abstract: The biotechnological potential of glucose aerodehydrogenase enzymes from microorganisms has pinched a great deal of attention for use of their antimicrobial activity in a variety of industrial processes. By keeping in view the wide range applications of glucose aerodehydrogenase in industrial and clinical sectors, the study was planned to produce, purify the glucose aerodehydrogenase from *Aspergillus niger* under pre-optimized SmF using easily available agricultural waste material, Corn steep liquor and to check the antimicrobial activity of indigenous enzyme. A purification fold of 7.23 with activity 12.94 U mL^{-1} and 40.56 U mL^{-1} specific activity were achieved respectively after chromatographic purification technique. Antimicrobial activity of purified glucose aerodehydrogenase was determined by disc diffusion method. Glucose aerodehydrogenase inhibited the growth of *S. aureus* only while reduced activity against *B. subtilis* and two fungal strains *A. niger* and *Rhizopus Stolonifer* was observed. It can be concluded that glucose aerodehydrogenase has poor antimicrobial activity against the investigated organisms.

Key words: Antimicrobial Activity · Glucose Aerodehydrogenase · *Aspergillus niger* · *Rhizopus stolonifer*

INTRODUCTION

Oxido-reductases a large group of enzymes containing glucose oxidase is also called glucose aerodehydrogenase [1]. Glucose aerodehydrogenase (beta-D-glucose: oxygen-1-oxidoreductase) also called as glucose oxidase, catalyzes the oxidation of beta-D-glucose to gluconic acid, with simultaneous production of hydrogen peroxide by utilizing molecular oxygen as an electron acceptor.

Glucose aerodehydrogenase is being produced mostly by microorganisms such as *Aspergillus niger, Botrytis cinerea* and *Penicillium sp.* [2]. The mycelium of the organism exhibited glucose aerodehydrogenase which is released from the mycelium by means of cell disruption techniques as an intracellular enzyme produced from *Aspergillus niger* [3]. X-ray crystallography was used to determine the tertiary structure of glucose oxidase fabricated from *A. niger* and electrical communication between the enzyme and the electrode was assembled to drawn the results [4]. Glucose aerodehydrogenase has an activation pH 6.0 that is highly specific for β-D-glucose, molecular weight 6688 Daltons, but immunoblotting and SDS–PAGE demonstrated that the enzyme formed aggregates [5].

Microbial glucose aerodehydrogenase is presently paid much attention due to exceptional applications in industrial sector (chemical, pharmaceutical, food, beverage, clinical chemistry and biotechnology) [6]. On commercial scale production glucose aerodehydrogenase enzyme works as biosensor to check the presence of glucose in fermentation broth. It has a striking effect in food industry such as improving color, flavor, texture and shelf life of various products by removal of glucose and oxygen. The antibacterial properties of glucose aerodehydrogenase containing hydrogen peroxide which effectively kills bacteria and is characterized by an antioxidant power need to be validated using model food system [7]. In this study we intended to produce and purify glucose aerodehydrogenase from *Aspergillus niger* to present prospective application for medical purposes.

MATERIALS AND METHODS

The research work was carried out in the Enzyme Biotechnology Lab., Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad.

Corresponding Author: Muhammad Anjum Zia, Enzyme Biotechnology Lab., Department of Chemistry & Biochemistry, University of Agriculture, Faisalabad, Pakistan. E-mail: rmazia@hotmail.com.

Isolation of Glucose Aerodehydrogenase

Chemicals and Spore Suspension: All the chemicals and reagents used were of analytical grade and the fungal strains were obtained from the Enzyme Biotechnology Laboratory, Department of Chemistry and Biochemistry, University of Agriculture, Faisalabad and preserved at 4°C [8]. The fungal culture was used for glucose aerodehydrogenase production by continuous shaking. The spores of *Aspergillus niger* were incubated in a conical flask having 50 mL of Vogel's media (pH 5.5), which was kept on a rotatory shaker at 120 rpm for 36 hrs and temperature was adjusted at 30°C [9]. This 36 hours suspension was used as inoculum and added to each flask for production of glucose aerodehydrogenase. The counting of spores was carried out by haemocytometer using the low power of microscope [10].

Fermentation Methodology and Enzyme Extraction: Fermentation process was carried out in 250 mL conical flasks containing working volume of 50 mL of fermentation media (Corn steep liquor; 2 g $100mL^{-1}$, Glucose; 4 g $100mL^{-1}$, Urea; 3 g $100mL^{-1}$, KH_2PO_4; 0.6 g $100mL^{-1}$, $CaCO_3$; 0.04 g $100mL^{-1}$, The pH 5.5 was adjusted and medium was autoclaved at 121 °C for 15 minutes and allowed to cool at room temperature. The medium was inoculated with spores of *Aspergillus niger* for production of glucose aerodehydrogenase [11]. After growth up to 36 hours, the sample of each flask was filtered. The filtrate was used for enzyme activity determinations and also for purification purposes.

Enzyme Assay: The enzyme activity was determined by the method of Worthington [12]. One unit (U) of enzyme activity was defined as amount that produced 1 µmol of H_2O_2 per minute at 30°C. The protein content of the sample was estimated by Biuret method [13].

Purification of Glucose Aerodehydrogenase: The intracellular purification of glucose aerodehydrogenase enzyme was carried out by ammonium sulfate precipitation, ion exchange and gel filtration chromatography. The crude enzyme was purified by ammonium sulfate precipitation using the method of Iqbal *et al.* [14] with minor modifications. Further purification of the partially purified glucose aerodehydrogenase was carried out by DEAE-cellulose and Sephadex-G-200 column (Sigma) gel filtration chromatography using the method previously described by Ahmed *et al.* [15] with minor modifications. The flow rate was maintained at 2mL min^{-1} and up to 25 fractions were collected, each of 2mL and both the enzyme activity and the protein content was analyzed for each separate fraction.

Antimicrobial Activity of Glucose Aerodehydrogenase: Antimicrobial activity of glucose aerodehydrogenase was determined by using disc diffusion method [16]. Antimicrobial activity of glucose aerodehydrogenase was determined against bacterial (*Staphylococcus aureus* and *Bacillus subtilus*) and fungal (*Aspergillus niger* and *Rhizopus stolonifer* strains. The paper disc was laid on microbial colonies exhibited plates containing 100 µL of glucose aerodehydrogenase. The inhibition of bacterial growth and formation of clearance zones were measured using zones reader [17].

RESULTS AND DISCUSSION

Aspergillus niger was cultured in fermentation medium containing (2%) corn steep liquor as growth support substrate under optimum conditions (Inoculum size, 5 mL; Fermentation period, 36 hrs) for the production of glucose aerodehydrogenase.

The activity and protein contents of the crude enzyme extracted from *A. niger* were observed as 21.04 U mL^{-1} and 3.75 mg mL^{-1} respectively having 5.61 U mg^{-1} of specific activity. The fraction of 60% supernatant and sediments of *Aspergillus niger* contained the activity of 19.06 and 19.21 U mL^{-1} respectively. While the 85% supernatant and sediments of *Aspergillus niger* showed the activity of 30.29 and 36.27 U mL^{-1} respectively (Fig. 1).

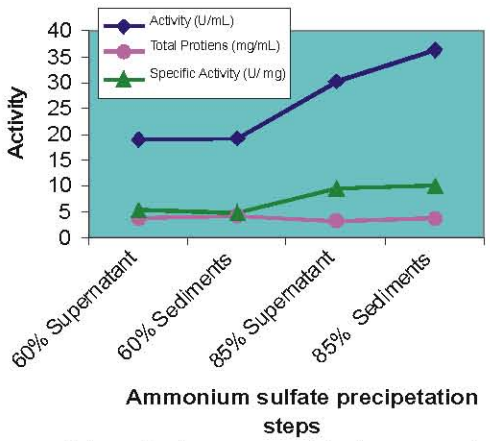

Fig. 1: Activity of glucose aerodehydrogenase isolated from *Aspergillus niger* in crude and partially purified extracts.

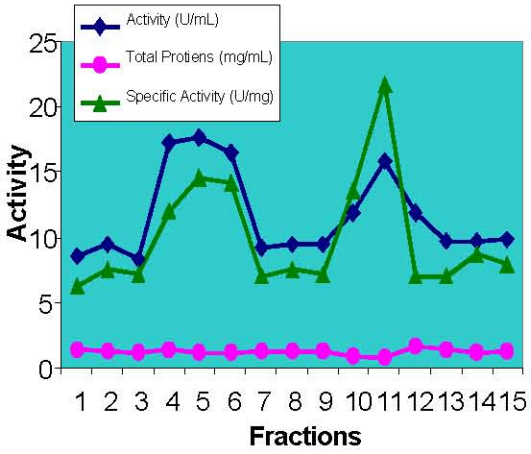

Fig. 2: Activity of glucose aerodehydrogenase obtained after an ion exchange.

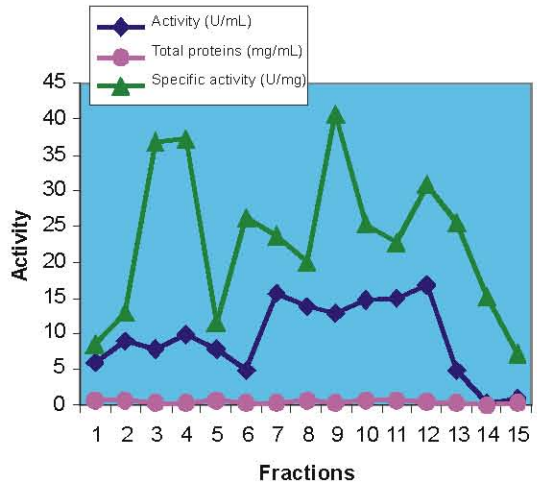

Fig. 3: Activity of glucose aerodehydrogenase obtained after the gel filtration.

The results clearly showed that the specific activity, recovery and purification fold in the precipitates had reached their maximal values at 85% ammonium sulphate concentration as reported earlier. El-Sherbeny *et al.* [18] reported that desalted sample of glucose aerodehydrogenase showed the activity and specific activity of 18.18 U mL^{-1} and 7.73 U mg^{-1} correspondingly. In ion exchange chromatography the glucose aerodehydrogenase from *Aspergillus niger* had the maximum activity 15.82 U mL^{-1} with 21.67 U mg^{-1} specific activity at 11th fraction (Fig. 2). Glucose aerodehydrogenase from *Aspergillus niger* was partially purified to a yield of 28.43% and specific activity of 13.5 U mg^{-1} through ammonium sulfate precipitation, anion exchange and gel filtration chromatography [19]. Kelly and Reddy [20] reported that

Table 1: Antimicrobial activity of glucose aerodehydrogenase against bacterial strains

Sample	*B. subtilis*	*Staphylococcus aureus*
Glucose aerodehydrogenase from *Aspergillus niger*	-	+

(+) (-): + sign indicates that the glucose aerodehydrogenase contained the strong antimicrobial activity while, - sign shows glucose aerodehydrogenase has no antimicrobial activity/ or to some extent.

Table 2: Antimicrobial activity of glucose aerodehydrogenase against fungal strains

Sample	*A. niger*	*Rhizopus stolonifer*
Glucose aerodehydrogenase from *Aspergillus niger*	-	-

(+) (-): + sign indicates that the glucose aerodehydrogenase contained the strong antimicrobial activity while, - sign shows glucose aerodehydrogenase has no antimicrobial activity/ or to some extent.

after DEAE-sephadex treatment of *P. chrysosporium* glucose aerodehydrogenase, 60% of the enzyme was recovered with 5.4 fold purification. The purification fold and increase in specific activity showed that enzyme has better purification trend after this treatments.

Purification of the enzyme by gel filtration technique on sephadex G-200 column at pH 5.6 increased the specific activity of the enzyme 40.56 U mg^{-1} (Fig. 3). Glucose aerodehydrogenase from *A. niger* in 9th fraction showed the maximum activity that was 12.94 U mL^{-1} while 40.56 U mg^{-1} was its specific activity with 7.23 fold purification.

The increasing trend in specific activity was observed with marching forward each purification step as shown in figures (1-3). The fractions having peak values were arranged for the enzymatic activity against microbial strains.

Antimicrobial activity of glucose aerodehydrogenase was checked against a panel of microorganisms. The possible application of glucose aerodehydrogenase was inhibiting the growth of organisms [21]. The antimicrobial effect of glucose aerodehydrogenase consequences the high acidity and its property to produce H_2O_2 [22]. *In vitro* antimicrobial activity of the culture extracts was tested against various clinical microbial isolates namely, *Bacillus subtilis*, *Staphylococcus aureus*, *A. niger* and *Rhizopus stolonifer* (Tables 1 and 2). Glucose aerodehydrogenase produced from *A. niger* showed activity against *Staphylococcus aureus* while negative results was found against *Bacillus subtilis*, *A. niger* and *Rhizopus stolonifer*. Onyegeme-Okerenta *et al.* [23] reported that

glucose aerodehydrogenase inhibits the growth of *B. subtilis* and *A. niger;* the potency varied with carbon source [23]. At the end of this work it can be concluded that glucose aerodehydrogenase has poor activity against *Bacillus subtilis*, *A. niger* and *Rhizopus stolonifer*.

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