Role of Metal Ions, Surfactants and Solvents on Enzymatic Activity of Partial Purified Glucoamylase from *Aspergillus niger* ER05

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**Abstract:** The glucoamylase requirement of different industries should vary in their physiochemical and functional properties, so the investigation of new sources for the novel enzymes is the only solution. The current study describe the production of glucoamylase from *Aspergillus niger* ER05 in a submerged fermentation. The enzyme then partially purified and then effect of thirteen different metal ions (Cs⁺, K⁺, Na⁺, Ba²⁺, Ca²⁺, Co²⁺, Cu²⁺, Hg²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Zn²⁺, Fe²⁺), surfactant as well as organic solvent on catalytic performance of glucoamylase was studied. A newly isolated *Aspergillus niger* ER05 is hyper producing strain of glucoamylase. Specific activity for the crude enzyme was found to be 6.87 KU/mg. The crude enzyme was partially purified via fractional ammonium sulphate precipitation. Ammonium sulphate saturation from 40-80% was found suitable to precipitate the enzyme. After dialyzing precipitates, the specific activities were found to be 66.33KU/mg with percent yield of 26.5. The inhibitory influence of all metal studies was interestingly found on glucoamylase activity. The strong inhibition was demonstrated in presence of Hg²⁺, Ni²⁺, Zn²⁺, Cu²⁺, Fe²⁺. Only Na⁺ ions were able to maintain the 101% relative activity at 1mM concentration. The SDS completely inhibits the enzyme activity and in presence of tween-80 and triton X-100 glucoamylase exhibited less than 45% relative activity. Furthermore, formaldehyde, isopropanol, ethanol, methanol, and DMSO stabilized the enzyme activity while chloroform inhibits enzyme activity by 48%.

**Keywords:** Activators, inhibitors, metal ions, surfactants, organic solvents, glucose, *Aspergillus niger*.

**INTRODUCTION**

Glucoamylase (α-1, 4-glucan-glucohydrolases, EC 3.2.1.3) have its own place in the catalytically active proteins family because of the broad possibilities of industrial applications [1]. Glucoamylase is an extracellular enzyme that yields β-D-glucose as the end-product after hydrolyzes α-1, 4-glycosidic bonds of starch only from the non-reducing end [2, 3]. The yield of glucose serves as a basic carbon source and driving force for fermentations, conventionally industrial production of glucoamylase was carried out by submerged fermentation [4, 5].

The industrial production of glucose after partial digested of starch with amylase majorly depends on glucoamylase. All the saccharified products from dextrin majorly required glucoamylase. Food and confectionary industries utilized high-fructose corn syrups (HFCS) produced after digestion of corn starch by glucoamylase [6]. The production of bioethanol required glucoamylase in first step to produce glucose for the fermentation. Glucose feed stocks can be further utilized in the commercial production of amino acids [7], in synthesis of drugs [8, 9] and in production of agro-chemicals [10, 11].

*Aspergillus niger* is the major glucoamylase producer from the microbial sources in enzyme production industries. The enzyme can easily recover from cell free filtrates after fermentation [12]. Glucoamylase reported from the various fungal sources widely differ from each other in their physico-chemical properties such as optimal conditions, influence of metal ions, stimulator or inhibitor specificity of different organic solvents and surfactants [13, 14]. Metals were required by some enzymes for their maximal catalytic activity [15, 16]. Metals also help in right orientation of active site of enzymes and also responsible for protein structure stabilization [17, 18]. One of the reported *A. niger* glucoamylase was found to be activated by metals. Presence of Mn²⁺ and Fe²⁺ increased the enzyme activity [19]. *Aspergillus flavus* glucoamylase was found to be activated by Mn²⁺, Co²⁺ and Ba²⁺ and was inhibited by Hg²⁺, Fe³⁺, Zn²⁺ and Cu²⁺ [20].

As the importance of glucoamylase, it is highly desirable to enhance the glucoamylase activity.

Therefore, for the better understanding of enzymatic reactions study of different inhibitors, destabilizers, and activators, is very significant. The objective of the present investigation is to find out the environmental contaminant influences on the activity of glucoamylase obtained from *A. niger* ER05. The metal ions, surfactants and organic solvents effect is investigate on the partially purified enzyme preparations in order to
characterize above elements as activators, inhibitors and stabilizers.

MATERIAL AND METHODS

Fungal Strain and Submerged Fermentation Conditions

Aspergillus niger ER05 has been isolated from soil and identified by sequence analysis and phylogenetic studies. Sequence with accession no: MF579602 has been submitted to NCBI GenBank. Pure cultures were maintained in PDA medium at 4°C and were repeatedly subculture after 30 days period.

The submerged fermentation has been done in optimized medium consist of 1 % soluble potato starch, 0.2% yeast extract, 0.1% peptone and 0.05% of MgSO₄ (pH 5.0). The culture was subjected to submerged fermentation for 4 days at 25±2°C. After fermentation, the mycelia were removed by filtration and then collect cell free filtrate.

Precipitation and Desalting of Proteins

The cell free fluid was subjected to fractional ammonium sulphate precipitation. First fraction of proteins was precipitate out at 40% ammonium sulphate saturation. Precipitates were separated out by centrifugation at 4000rpm. The supernatant was again saturated with ammonium sulphate till 80%. The collected precipitates were dissolved 25mM Na-acetate buffer (pH4.0). The sample was dialyzed overnight against the same buffer. Total protein was estimated by the method of Lowry [21].

Glucoamylase Assay

Glucoamylase activity was determined by using potato soluble starch (1.0%) as substrate in 25mM sodium acetate buffer (pH4.0). Dialyzed enzyme sample (0.075ml) mixed with 1.0 ml substrate and placed at 70°C for 5 min. After that, boil the reaction tubes for 5 min and then cooled to room temperature. Glucose oxidase (GOD-PAP) method was used to estimate the liberated glucose. The standard glucose was used as standard as described by Trinder [22, 23]. The absorbance of test and control were read at 546 nm using spectrophotometer.

One unit of glucoamylase is defined as “amount of enzyme that released 1.0 µM of glucose per minute under the standard assay conditions” and in the current study it is expressed as KU/ml/min. (1 kilo units/ml/min is equal to 1000 units/ml/min)

Effect of Different Metal on Glucoamylase Activity

A number of metal ions with valences from monovalent to trivalent ions were used to study their effect on the activity of glucoamylase. Chloride salts of all the metal ions were used to in this study. The concentrations, 1.0, 5.0 and 10.0 mM of three monovalent metal salts (Cs⁺, K⁺, Na⁺), nine divalent metal ions (Ba²⁺, Ca²⁺, Co²⁺, Cu²⁺, Hg²⁺, Mg²⁺, Mn²⁺, Ni²⁺, Zn²⁺) and one trivalent metal ion (Fe³⁺) were prepared in sodium acetate buffer (25 mM and pH 4.0). Each of the reaction mixtures contains of enzyme and metal ion solutions in 1:1 ration and kept at 37 °C temperatures for 60 min. After completion of incubation, enzymatic activity of the samples was conducted at 70 °C according to standard procedure describe above. The control of reaction was established without metal ion. Enzyme activity in the absence of metal ion consider as 100 percent was used to calculate percent relative activity of glucoamylase.

Effect of Surfactant on Glucoamylase Activity

Effect of several surface active agents on glucoamylase activity was determined by incubating enzyme with anionic (tween 20, tween 80, triton X-100) and nonionic surfactants such as sodium dodecyl sulfate. Glucoamylase was incubated with 10, 50 and 100 mM concentrations at 37 °C for 1 hour. Control (without surfactant) was setup and its enzyme activity was taken as 100%.

Effect of Solvents on Glucoamylase Activity

Various organic solvents exert prominent effect on glucoamylase activity. Effect of chloroform, DMSO, ethanol, formaldehyde, isopropanol and methanol was evaluated for one hour at 37 °C with three different concentrations of solvents i.e.1.0, 5.0 and 10.0 mM. Control enzyme activity (100%) was setup devoid of solvent.

RESULTS AND DISCUSSION

Glucoamylase from Aspergillus niger ER05 was produced under optimized culture conditions in submerged fermentation. After fermentation, cell free filtrate collected and proteins was precipitated out in two step ammonium sulphate precipitation. First fraction of 0 - 40% saturation was removed and CFF again precipitated till 80% saturation. The precipitate then dialyzed and the enzyme activity was determined in collected precipitates. Glucoamylase was partially
purified up to 26.5 fold with specific activity of 66.33KU/mg (Table 1).

**Effect of Several Metals on Glucoamylase Activity**

The interactions of several metal ions to glucoamylase from *Aspergillus niger* ER05 was studied by incubating enzyme with metal ion solutions. Three different concentrations of each metal ion were investigated. The relative activity was measured with reference to the enzyme activity present in the absence of any metal ion. In enzyme substrate reactions, metal ions have ability to influence the rate of reaction as they bind on active site of enzyme and can be grouped into inhibitors, activators and stabilizers at appropriate concentration [18].

Results outcomes presented that three monovalent ions i.e. Cs⁺, K⁺ and Na⁺ exhibited inhibitory effect on enzyme activity at 10mM concentration as the relative activity was 61%, 67% and 57% respectively (Table 2). Subsequently, the relative activity of Na⁺ increases to 101% when the ten times decrease concentration was tested. Hua et al., 2014 has been found that glucoamylase activity from *Bispora* sp. MEY-1 has been increased by monovalent cations i.e. Li⁺, Na⁺, K⁺ and 5 mM Na⁺ increased relative activity of glucoamylase to 140%.

In the presence of all nine divalent metal ions, inhibition in the glucoamylase activity was represented in Figure 1. Ba²⁺, Ca²⁺, Co²⁺, Mg²⁺ and Mn²⁺ acted as inhibitors as they decreased glucoamylase activity via 44%, 46%, 38%, 48% and 52% respectively at 10 mM concentration.

While, a higher inhibitory effect has been noticed with 10mM Cu²⁺ (40% relative activity), Zn²⁺ (33% relative activity) and Ni²⁺ (32% relative activity). Similar results have been reported by Hua et al., 2014 with Cu²⁺ at same concentration. However, Ni²⁺ and Zn²⁺ increased the enzyme activity [24]. One of the amyloglucosidase reported by Gudi et al. in 2013, showed the reduction in enzyme activity by following

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**Table 1:** Partial Purification Procedure of Glucoamylase from *Aspergillus niger* ER05

<table>
<thead>
<tr>
<th>Purification step</th>
<th>Total Enzyme (KU)</th>
<th>Total Protein (mg)</th>
<th>Specific activity (KU/mg)</th>
<th>Fold purification</th>
<th>Yield (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude CFF</td>
<td>2828.7</td>
<td>411.7</td>
<td>6.87</td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>40% NH₄SO₄</td>
<td>31.1</td>
<td>4.4</td>
<td>7.02</td>
<td>1.02</td>
<td>1.1</td>
</tr>
<tr>
<td>80% NH₄SO₄</td>
<td>802.7</td>
<td>13.98</td>
<td>57.41</td>
<td>8.35</td>
<td>28.3</td>
</tr>
<tr>
<td>Dialyzed PPT (80%)</td>
<td>751.1</td>
<td>11.3</td>
<td>66.33</td>
<td>9.65</td>
<td>26.5</td>
</tr>
</tbody>
</table>

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**Table 2:** Effect of Different Metal Ions on Glucoamylase from *Aspergillus niger* ER05

<table>
<thead>
<tr>
<th>Metal ions</th>
<th>1mM</th>
<th>5mM</th>
<th>10mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CsCl</td>
<td>76</td>
<td>69</td>
<td>61</td>
</tr>
<tr>
<td>KCl</td>
<td>72</td>
<td>69</td>
<td>67</td>
</tr>
<tr>
<td>NaCl</td>
<td>101</td>
<td>63</td>
<td>57</td>
</tr>
<tr>
<td>BaCl₂</td>
<td>50</td>
<td>56</td>
<td>56</td>
</tr>
<tr>
<td>CaCl₂</td>
<td>51</td>
<td>53</td>
<td>54</td>
</tr>
<tr>
<td>CoCl₂</td>
<td>56</td>
<td>58</td>
<td>62</td>
</tr>
<tr>
<td>CuCl₂</td>
<td>48</td>
<td>44</td>
<td>40</td>
</tr>
<tr>
<td>HgCl₂</td>
<td>32</td>
<td>30</td>
<td>16</td>
</tr>
<tr>
<td>MgCl₂</td>
<td>42</td>
<td>46</td>
<td>52</td>
</tr>
<tr>
<td>MnCl₂</td>
<td>47</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>NiCl₂</td>
<td>48</td>
<td>40</td>
<td>32</td>
</tr>
<tr>
<td>ZnCl₂</td>
<td>28</td>
<td>33</td>
<td>33</td>
</tr>
<tr>
<td>FeCl₃</td>
<td>32</td>
<td>38</td>
<td>41</td>
</tr>
</tbody>
</table>
heavy metals at 10 mM concentration i.e. Fe$^{2+}$, Hg$^{2+}$, Cu$^{2+}$, Zn$^{2+}$ and Ni$^{2+}$ reduced by 60%, 40%, 30%, 20% and 11% respectively [13].

The highest inhibitory effect was showed by Hg$^{2+}$ on glucoamylase activity, 32%, 30% and 16% relative activity at 1 mM, 5 mM and 10 mM concentration. As, 10 mM Hg$^{2+}$ ion concentration abolish 84% of enzyme activity, so emerged as the strong inhibitor [13, 20, 24].

One of the trivalent ions Fe$^{3+}$ was studied to detect its impact on activity of glucoamylase. Results exhibited that Fe$^{3+}$ has been inhibited the activity of enzyme by 41% at 10 mM concentration [20]. However, the Hua et al. reported the stimulatory impact of Fe$^{3+}$ with the same concentration [24].

**Effect of Surfactant on Glucoamylase Activity**

The surface active agents were recognized as a major ingredient of detergents and well-known factor for the alterations in the activity of enzymes. In this current investigation, glucoamylase of *Aspergillus niger* ER05 was subjected to the effect of nonionic and anionic detergents (Figure 2). According to the results, both tween-80 and triton X-100 exhibited pronounced inhibitory activity on glucoamylase at 1 mM concentration i.e., 45% and 35% relative activity respectively. It is also noted that with the increase in surfactant concentration that relative activity further declined. Gudi et al., 2013 reports the detergent-mediated production of Glucoamylase, which showed surfactant tolerance. The enzyme retains 90% of its activity in the presence of high concentration of detergent [13].

Sodium dodecyl sulfate as an anionic detergent exhibited complete inhibition of glucoamylase activity at all concentrations tested. Hu et al. [24] showed strong inhibition of glucoamylase activity i.e. 86-87% by SDS. It has been reported that the most of the anionic detergents have the ability to absorb on the surface of proteins, denature their native structure, thus eventually hinders the function of proteins [25, 26].

**Effect of Solvents on Glucoamylase Activity**

Incubating glucoamylase in 1 mM, 5 mM and 10 mM concentrations of organic solvents for an hour and afterward checking the influences on catalytic performance of enzyme was investigated. The glucoamylase of *Aspergillus niger* ER05 is extraordinarily stable in the presence of all organic solvents under study. It was observed that except, chloroform and DMSO (1 mM) presence of other solvents stabilized the activity (Figure 3). These results were in accordance to the glucoamylase of
Halolactibacillus sp. SK71 which showed good stability in organic solvents [27]. The interface provided by nonpolar solvents helped the enzyme to be in exposed confirmation thus stimulating the enzyme activity [28].

CONCLUSION

Our current investigation on glucoamylase of Aspergillus niger ER05 showed the influences of different metal chlorides, surface active agents and solvents of organic nature on the enzyme activity. It was concluded that glucoamylase was inhibited by most of the metals, in which the Hg^{2+} showed the maximum inhibition, SDS, tween-80, triton X-100, chloroform and DMSO also showed inhibitory effect.

REFERENCES


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