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Research Article

Effect of temperature, pH, substrate (Starch) and glucose on stability of α-amylase from *Bacillus licheniformis* ATCC 6346

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Abstract: Thermostable α-amylases (1,4-α-D-glucan glucanohydrolase; E.C.3.2.1.1) from *Bacillus* species are of great industrial importance in the production of corn syrup or dextrose. The present study was aimed to determine the effect of temperature, pH, substrate and glucose on stability of α-amylase from *Bacillus licheniformis* ATCC 6346. To determine the effect of temperature and pH on the stability of α-amylase this was pre-incubated at different temperatures and pHs. The enzyme pre-incubated with starch and glucose separately at 85°C to determine the effect of substrate and end-product on the stability of α-amylase. Crude α-amylase retained 60% of the original activity when it was pre-incubated at 85°C for 10 min. This enzyme was more stable at pH 9.0 than other pH values (7.0 and 8.0) and no significant important was observed with addition of substrate (starch). Presence of glucose slightly increased the stability of α-amylase. There was no significant improvement in the stability of α-amylase was observed with the addition of starch however presence of glucose slightly increased the stability of the α-amylase.
Keywords: Enzyme activity, enzyme stability, α -amylase, half- life, starch

INTRODUCTION

The starch industry is one of the largest users of enzymes for the hydrolysis and modification of useful raw material [1]. The microbial amylases meet industrial demands and a large number of them are available commercially; although many microorganisms produce this enzyme and the most commonly used for their industrial application are *Bacillus licheniformis*, *Bacillus amyloliquifaciens* [2]. Weemaes *et al*. [3] reported that the stability of α-amylases produced by *B. amyloliquefaciens*, *B. licheniformis* and *B. stearothermophilus* under combined high temperature and pressure and the results indicated that α-amylase produced by *B. licheniformis* was the most stable enzyme among the three. Bacterial amylases are known to be more thermostable than fungal amylases [4]. Amylases have attracted the world's enzyme market because of their wide application in starch based industries especially food, textile, paper, detergent and baking industries [5]. They represent 25 % of the world's market of enzymes [6-7]. Most of the commercially produced amylases are of microbial origin [8]. The enzyme α-Amylase hydrolyses starch, glycogen and related polysaccharides by randomly cleaving internal α-1,4-glucosidic linkages. Amylases are most important biocatalyst due to their ability to utilize a wide spectrum of substrates, high stability towards extreme temperature, pH etc. The properties of each α-amylase such as thermostability, pH profile, pH stability, and Ca-independency must be matched to its application. For example, α-amylases used in starch industry must be active and stable at low pH, but at high pH values in the detergent industry [9]. Among microbial, plant and animal enzymes, microbial amylases have immense applications in various fields [10]. Enzymes are mostly proteins with a labile nature. Inactivating agents such as temperature, pH, chemicals, *etc*. impair the native conformation of an enzyme, thus affecting its catalytic activity. The utility of an enzyme depends mainly on its operational and storage stability [11]. Therefore, enzyme stability and its stabilization are crucial factors in the application of enzymes. Stability of enzymes can be achieved by screening intrinsically stable enzymes, adding stabilizing agents, chemical modification, immobilization, protein engineering, *etc* [12]. The objective of this study was to examine the Effect of temperature, pH, substrate (Starch) and glucose on stability of α-amylase from *Bacillus licheniformis* ATCC 6346.

MATERIALS AND METHODS

Bacterial strain and preparation of crude enzyme

Bacillus licheniformis ATCC 6346 from Heriot-Watt University U.K was used in this study. The nutrient agar medium used in the study contained (L^-) 25.0 g nutrient agar, 3.0 g soluble starch. The

activation medium used in the study contained (L^-) 25.0 g nutrient broth, and 3.0 g soluble starch at pH 7.0. The fermentation medium contained (L^-) 4.0 g soluble starch, 5.0 g $(NH_4)_2SO_4$, 6 g peptone; 0.01g $FeCl_3$; 0.01 g $MgCl_2.6H_2O$; 0.01g $CaCl_2.2H_2O$; 4.0 g of KH_2PO_4 and 7.5 g of K_2HPO_4 at pH 7.0 [13]. A loopful of *Bacillus licheniformis* ATCC 6346 grown in nutrient agar slants with 0.3 % soluble starch at 37 ^{o} C for 24 h was transferred to 10 mL activation medium and incubated at 42 ^{o} C in a rotary shaker (100 rpm) for 12 h and used as the inoculum. The fermentation medium was inoculated with 20% (v/v) inoculum and the inoculated flasks were incubated for 48 h at 42 ^{o} C and spun at 100 rpm. The crude enzyme was prepared by centrifuging the inoculated fermentation medium, after incubation for 48h at 42^{o}C, at 10000 × g in a refrigerated centrifuge for 10 min at 4^{o}C. The supernatant was then taken in a tube and stored at 4^{o}C for further analysis.

Effect of temperature on the stability of α-amylase

To determine the effect of temperature on the stability of α-amylase, α-amylase in pH 7.0 buffer was pre-incubated at 75 and 85^{o}C and the enzyme activity was monitored [14]. The half-life of the enzyme was taken as the time taken for its activity to be reduced to half of the original activity.

Effect of pH on the stability of α-amylase

To determine the effect of pH on the stability of α-amylase samples were pre-incubated at 85^{o}C in pH 7.0, 8.0 and 9.0 separately. At different time intervals and the activity of α-amylase was monitored [14]. The half-life of the enzyme was taken as the time taken for its activity to be reduced to half of the original activity.

Effect of substrate (starch) on the stability of α-amylase

To determine the effect of starch on the stability of α-amylase samples, 20gL^{-1} starch-α-amylase solution was pre-incubated at pH 7.0 (0.01M phosphate buffer) and 85^{o}C in a water bath. At different time intervals and the activity of α-amylase was monitored [14]. The half-life of the enzyme was taken as the time taken for its activity to be reduced to half of the original activity.

Effect of glucose on the stability of α-amylase

To determine the effect of glucose on the stability of α-amylase samples, 0.5M glucose-α-amylase solution was pre-incubated at pH 7.0 (0.01M phosphate buffer) and 85^{o}C in a water bath. At different time intervals and the activity of α-amylase was monitored [14]. The half-life of the enzyme was taken as the time taken for its activity to be reduced to half of the original activity.

RESULTS AND DISCUSSION

Bacillus licheniformis ATCC 6346 has produced α-amylase (44.1UmL^{-1}) with 21.18 Umg^{-1} protein-specific activities in crude enzyme extract. The optimum temperature and pH of the crude enzyme was 85^{o}C and pH 7.0 [15].

Effect of temperature on the stability of α-amylase

The thermostability of α-amylase from *B.licheniformis* ATCC 6346 was studied at different temperatures. When the crude α-amylase was pre-incubated at 85^{o}C for 10 min it retained 60 % of the original activity and at 60 min all its activity was lost (Figure 1). When the crude α-amylase was pre-incubated at 75^{o}C, 88 % of the activity was retained at 10 min (Figure 1) and 81 % of its original activity remained at 60 min. Half-life of the α-amylase at 75 and 85^{o}C were 203.15 and 13.75 min respectively at pH 7.0. Stability of the α-amylase was longer at 75^{o}C than 85^{o}C because high temperatures denature the α-amylase. *Bacillus subtilis* AX20 α-amylase showed 60 and 35 % of maximum activity at 40 and 70^{o}C, respectively, and the amylase showed stability at 50^{o}C for 45 min [16]. Temperature stable α-amylase from *Bacillus licheniformis* 584 was reported by Saito and Yamamoto [17], that this enzyme rapidly lost activity at temperature above 76^{o}C. Since the *B.licheniformis* ATCC 6346 α-amylase was not sable at 75^{o}C beyond 5 min.

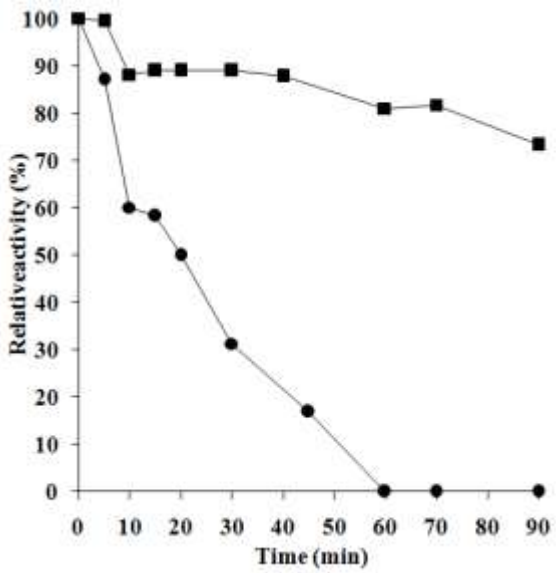

Figure 1: Stability of crude α-amylase at (■), 75; (●), 85^{o}C and at pH 7.0. α-Amylase activity was measured at 85^{o}C using 20gL^{-1} starch as substrate by incubating for 5 min at pH 7.0.

Effect of pH on the stability of α-amylase

The effect of different pH on the stability of α-amylase from *B.licheniformis* was studied. pH is known to affect the synthesis and secretion of α-amylase just like its stability [18]. Crude α-amylase was pre-incubated at different pH values (7.0, 8.0 and 9.0) and the activity of α-amylase at different time intervals were measured and presented in Figure 2. When the crude α-amylase

was pre-incubated at pH 7.0 and 8.0, the activity was totally lost at 60 and 50 min respectively at 85°C. It retained 40% of the initial activity when pre-incubated at pH 9.0 and at 85°C for 60 min. α-Amylase was stable at pH 9.0 than that at pH 7.0 and 8.0. The half-life of the α-amylase from *B.licheniformis* ATCC 6346 at pH 7.0 (0.01M phosphate buffer), 8.0 (0.01M Tris buffer) and 9.0 (0.01M Glycine buffer) were 13.75, 12.8 and 43.09 minutes respectively at 85°C. The optimum pH for the reaction of crude α-amylase was exhibited at pH 7.0 in 0.01M phosphate buffer.

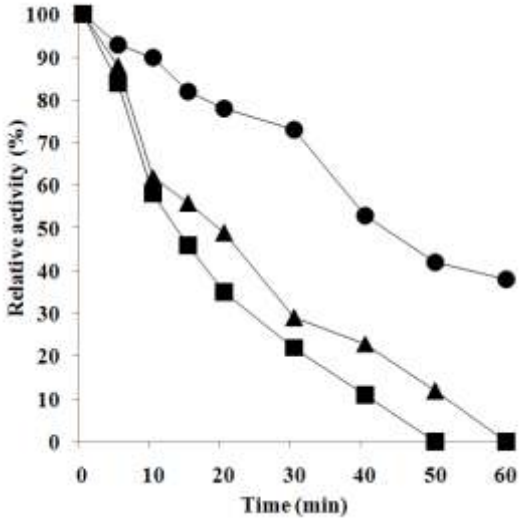

Figure 2: Stability of crude α-amylase at different pH values of (▲), 7; (■), 8 and (●), 9. α-amylase activity was measured at 85°C, using 20gL^{-1} starch as substrate by incubating for 5 min.

Effect of starch (substrate) on the stability of α-amylase

The effect of substrate (20gL^{-1} starch) on the stability of crude α-amylase from *B.licheniformis* ATCC 6346 was studied at 85°C. To improve the stability of the α-amylase the crude α-amylase was incubated with starch. The enzyme lost 40% of its initial activity at 10 min and all its activity at 60 min at pH 7.0 (Figure 3). When the enzyme was incubated with 20gL^{-1} of soluble starch, 55% of its initial activity was retained at 10 min at 85°C and at pH 7.0 (0.01M phosphate buffer) and 1.2% of initial activity at 60 min of incubation. Half-life of the α-amylase in the presence of 20gL^{-1} was 11.62 min at 85°C and pH 7.0. There was no significant improvement in the stability of α-amylase was observed with the addition of starch. In the presence of substrate (starch) α-amylase from *Thermomonospora curvata* showed no significant protective effect on the stability [19]. For *Bacillus* sp. WN11 α-amylase 2% starch was a better stabilizer than 5 Mmol Ca^{2+}. α-Amylase stability was improved with increasing starch concentration [20]. α-Amylase from *Aspergillus tamarii* showed, thermostability in presence of starch [21].

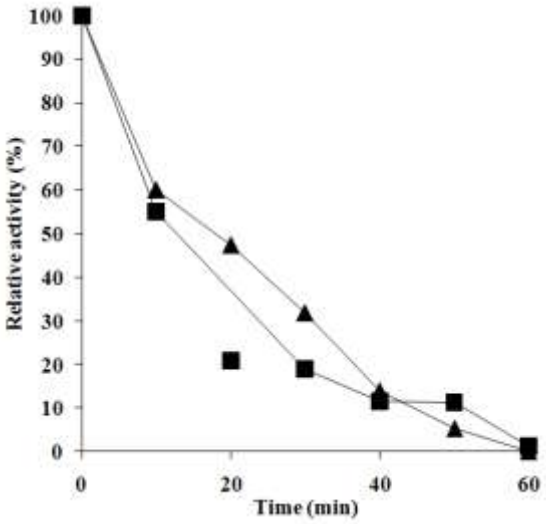

Figure 3: Stability of crude α-amylase (▲), without starch and (■), with 20gL^{-1} starch. α-Amylase activity was measured at 85°C, using 20gL^{-1} starch as substrate by incubating for 5 min at pH 7.0.

Effect of glucose on the stability of α-amylase

The effect of glucose (0.5M) on the stability of α-amylase by *Bacillus licheniformis* ATCC 6346 was studied at 85°C. When the crude α-amylase from *B.licheniformis* ATCC 6346, was incubated at 85°C, it lost 40% of its initial activity at 10 min and lost all its total activity at 60 min of incubation at pH 7.0 (Figure 4). When the α-amylase was incubated with 0.5M glucose 66 and 4% of its initial activities were retained at 10 and 60 min respectively.

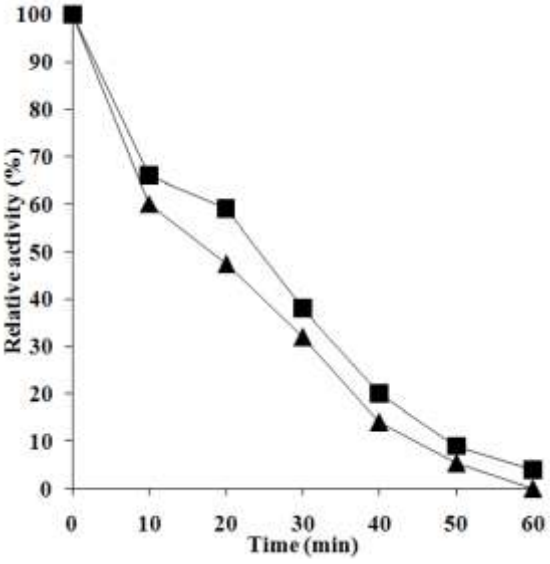

Figure 4: Stability of crude α-amylase (▲), without glucose and (■), with 0.5M glucose. α-amylase activity was measured at 85°C, pH 7.0 and using 20gL^{-1} starch as substrate for 5min of incubation

Therefore glucose slightly increased the stability of the α-amylase. Half-life of the α-amylase in

the presence of 0.5M glucose was 16.69 min at 85^{o}C. The temperature stability of α-amylase was slightly improved by the addition of glucose.

CONCLUSION

Findings of the present study revealed that the presence of glucose slightly increased the stability of the α-amylase from *Bacillus licheniformis* ATCC 6346.

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