Effect of Chemicals and Cations on the Activity of Partially Purified alpha amylase produced by *Aspergillus niger*

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Abstract: Amylases are one of the most important enzymes used in the industry. They are responsible for the hydrolysis of starch and glycogen. This research aimed to investigate the effect of cations (Ca^{2+} and K^{+}) and chemicals (EDTA and $HgCl_2$) on alpha amylase activity. Alpha amylase was produced by *Aspergillus niger* under submerged fermentation conditions in a basal salt medium. The enzyme was partially purified with activated carbon which gave a 1.57 purification fold. The effects of ethylene diamine tetratacetic acid (EDTA), mercuric chloride ($HgCl_2$), Calcium ion (Ca^{2+}) and Potassium ion (K^{+}) on the partially purified enzymes were then investigated. The enzyme was inhibited to varying degrees by EDTA and $HgCl_2$ with inhibition of approximately 62% and 52% occurred with 8mM of EDTA and $HgCl_2$ respectively but stimulated by Ca^{2+} and Potassium ion K^{+}. Activated carbon partially purified alpha amylase produced by *A. niger* with a high yield. The cations and chemicals had varying effects on the enzyme.

1. Introduction

Amylases are an important class of enzymes which are well-known and are used for the hydrolysis of starch or glycogen[1]. They are employed in starch processing industries for the breakdown of starch into simple sugars[2]. They hydrolyse α 1-4 glycosidic bonds of starch, glycogen, amylopectin and other related compounds. They can be produced either by solid state fermentation or submerged fermentation[3,4].

The use of microbes for the production of enzymes is economical. This is because microorganisms can be easily manipulated to produce metabolites e.g. enzymes[5]. Fungi are however preferred over bacteria for enzyme production because of their filamentous nature, which helps in the penetration of the mycelia through solid substrates[6]. Recent advances in enzyme technology has brought about an increased interest in the use of fungi for microbial enzyme production.

The progress in the knowledge of enzymes, has brought about an acquired importance of fungi in several industries. This is because they may serve to improve various aspects of the final industrial products. There are approximately 200 species of *Aspergillus* which are known to produce enzymes[7]. The several and diverse reactions of *Aspergillus* niger has brought about a remarkable interest in its use as a species of great commercial interest with a highly promising future. *Aspergillus niger* is also already widely applied in modern biotechnology[7,8]. Moreover, the use of *A. niger* is not only because of its production of various enzymes, but it is one of the few species of the fungus kingdom classified as GRAS (Generally Recognized as Safe) by the Food and Drug Administration (FDA).

The aim of this study was to study the effect of the chemicals (EDTA and $HgCl_2$) and cations (Ca^{2+} and K^{+}) on the activity of the partially purified enzyme.

2. Materials and methods

The fungal isolate (*Aspergillus niger)* employed for this work was isolated by exposing potato dextrose agar (PDA) plates to the air in the Microbiology laboratory of the Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria. The plates were incubated at room temperature (25 °C) for 3-5 days. Distinct colonies of the organism were sub-cultured till pure cultures were obtained. A 96-hr-old culture of the isolate was used.

Preparation and inoculation of Basal Salt medium

The basal salt medium contained the following reagents:

KH_2PO_4 (2.0g/L), $(NH_4)_2SO_4$ (1.4g /L), $MgSO_4.7H_2O$ (0.3g/L), $CaCl_2$ (0.3g/L), Urea (0.3g/L), Tween 80 (1ml/L), $FeSO_4.7H_2O$ (5mg/L), $MnSO_4$ (1.6mg/L), $ZnSO_4$ (1.4mg/L), $CoCl_2$ (2.0mg/L). One milliliter of the aqueous spore suspension containing approximately $6x10^6$ spores per ml of the fungal isolate was inoculated into conical flasks containing 1000ml growth medium. The spores were counted using the Neubauer counting chamber. One gram (1g) of substrate per 100ml of basal salt medium was used according to the method [9].

Extraction of Enzyme from Culture Media

Enzyme solution was obtained from the basal salt media after 7 days of innoculation by sieving the culture content with muslin.

Purification of the Enzymes

The extracted enzyme was purified according to the method described by [10] whereby experiments were conducted in 10 ml centrifuge tubes. Samples (10 ml) of crude enzyme (α-amylase pH 6.0) was treated with various concentrations of activated charcoal (1–4 % w/v) and incubated at 30 °C for 30 min with occasional stirring. The mixture was then centrifuged at 2500 rpm in a bench centrifuge for 10 min.

Enzyme Assay

α-Amylase activity was determined using the method described by [11]. The reaction mixtures consisted of 2 ml of 0.05% (w/v) starch in 0.2 M citrate phosphate buffer (pH 6.0) as substrate and 0.5 ml of enzyme. Controls consisted of only 2 ml of the prepared substrate. The contents of both experimental and control tubes were incubated at 35 °C for 20 min. The reaction in each tube was terminated with 3ml of 1 N HCl. Enzyme (0.5 ml) was then added to the control tube. Two millilitre of the mixture from each of the sets of experimental and controls was transferred into new sets of clean test tubes. Three millilitre of 0.1 N HCl was added into the contents of each test tube after which 0.1 ml of iodine solution was added. Optical density readings were taken with a spectrophotometer at 670 nm. One unit of enzyme activity was defined as the amount of enzyme which produced 0.01 % reduction in the intensity of the blue colour of starch-iodine complex under conditions of the assay.

Effect of Chemicals and Cations

The effect of ethylene diamine tetraacetic acid (EDTA) and mercuric chloride ($HgCl_2$) at different molar concentrations on the activities of the enzyme was investigated. Concentrations of 0 - 8 mM, each of ethylene diamine tetraacetic acid (EDTA) and mercuric chloride ($HgCl_2$) were prepared in 0.05% (w/v) starch in citrate phosphate buffer pH 6.0. The substrate (starch) was incubated initially with each test chemical at 4 °C for 3h before being employed for the enzyme assays.

Calcium ion (Ca^{2+}) and Potassium ion (K^{+}) were also employed in this investigation. Concentrations of 0 - 40 mM were employed for the cations Ca^{2+} and K^{+}. The reaction mixtures for consisted of 2 ml substrate and 0.05 ml enzyme. These were incubated at 35 °C for 20 mins and then analysed for α-amylase activities as described above.

3. Results and Discussion

The partially purified fractions gave a purification fold of approximately 1.57 as shown in (table 1).

Table 1: Purification of partially purified amylase from *A. niger*

Fraction	Total Protein (mg)	Total Activity (Units)	Specific Activity (Units/mg)	Yield (%)	Purification fold
Crude	287	542	1.89	100	1
Activated Carbon (1%)	230	513	2.23	94.6	1.18
Activated Carbon (2%)	162	461	2.85	85.1	1.51
Activated Carbon (3%)	158	456	2.89	84.1	1.53
Activated Carbon (4%)	150	445	2.97	82.1	1.57

Effect of Chemicals and cations

Inhibition of approximately 62% and 52% occurred with 8mM of EDTA and $HgCl_2$ respectively (fig 1 and 2). On the other hand, both cations Ca^{2+} and K^{+} had a stimulation of approximately 98% and 28% respectively on α-amylase with 40mM of the cations (fig 3 and 4).

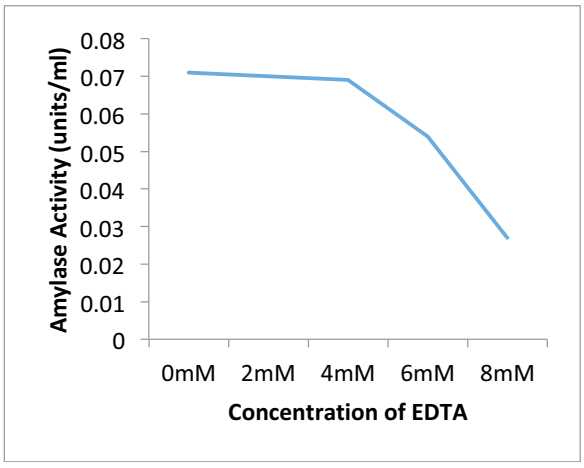

Fig 1: Effect of EDTA on partially purified α-amylase from *A. niger*

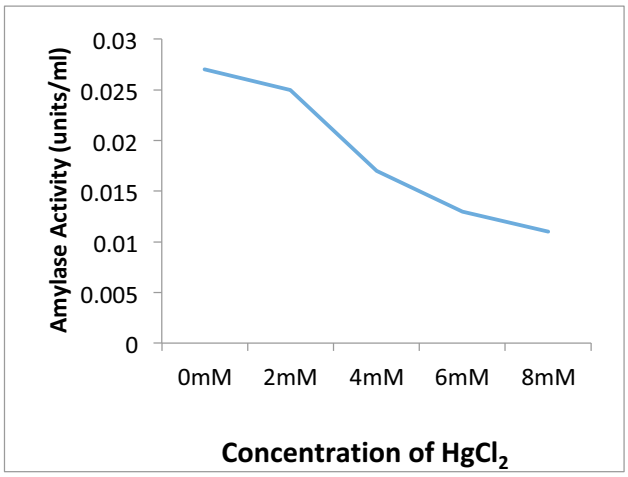

Fig 2: Effect of $HgCl_2$ on partially purified α-amylase from *A. niger*

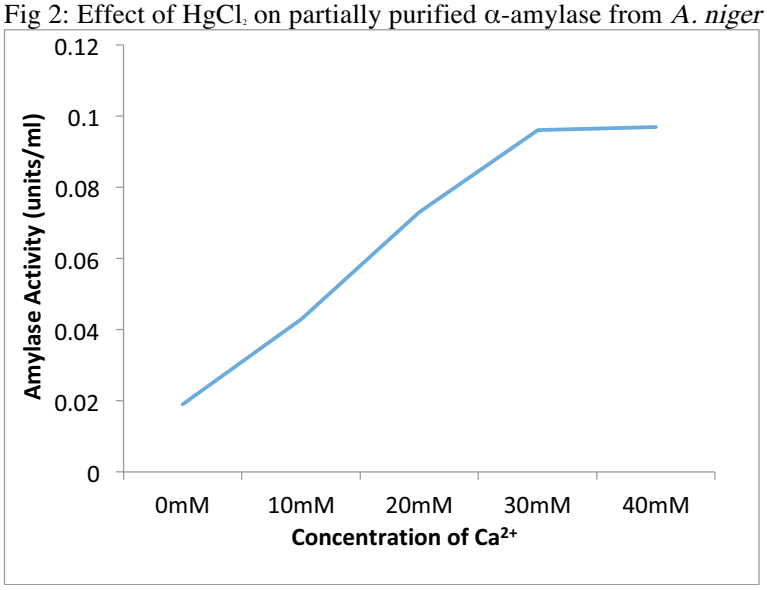

Fig 3: Effect of Ca^{2+} on partially purified α-amylase from *A. niger*

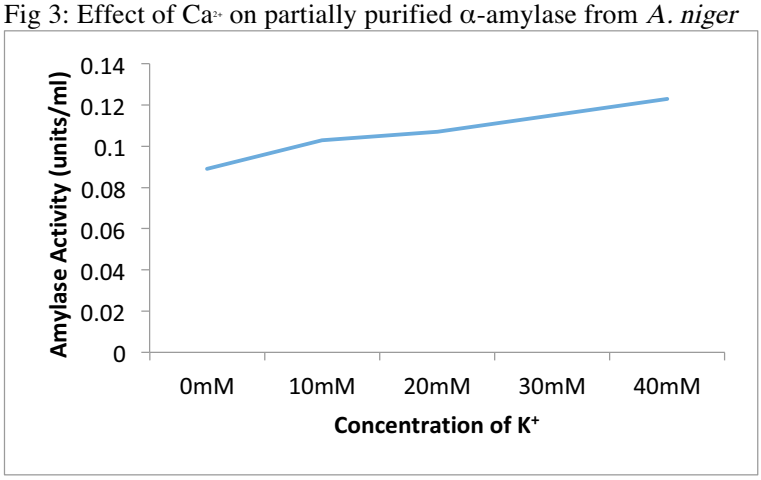

Fig 4: Effect of K^{2+} on partially purified α-amylase from *A. niger*

Production of alpha amylase under submerged fermentation conditions was employed in this study. This method has been widely employed for production of amylases and result obtained are in agreement with previous authors[12,13]. The cations employed in this work enhanced the activities alpha amylase under investigation. It was observed that Ca^{2+} and K^{+} had a stimulation of about 98% and 28% increase in activity respectively. This agrees with the work of previous authors[14-16]. The activation of enzymes by cations is usually through a variety of mechanisms. The metal ion may produce direct activation of enzymes in a reaction whereby the reaction is affected by a change of surface charge on the enzyme protein, particularly in the case of polyvalent cations, where they may produce very marked changes in the electro-kinetic potential of proteins[17]

The chemicals EDTA and Hg^{2+} were inhibitory to the enzymatic activities of the alpha amylase under investigation. Similar inhibition of enzymatic activities by metal ions have been reprted[18,19]. EDTA is a metal chelating agent that forms a complex with an inorganic group and it consequently reduces the affinity of the enzyme for its substrate[17].

4. Conclusion

The results suggest that Ca^{2+} and K^{+} serve as cofactors which will help to enhance the enzyme activity. This research will recommend that reactions set up in bioreactors with enzyme use should avoid the use of chemicals such as EDTA and Hg^{2+}. This is because they will interfere with the activity of the enzymes thereby slowing down the rate of reaction.

References

[1]. Krishna, P. R., Srivastava, A. K., Ramaswamy, N. K., Suprasanna, P. and S.F.D Souza (2011). Banana peel as substrate for alpha-amylase production using *Aspergillus niger* NCIM 616 and process optimization. *Indian J Biotechnol* 11: 314-319.

[2]. Alva, S., Anumpama, J., Savla, J., Chiu, Y. Y., Vyshali, P., Shruti, M., Yogeetha, B. S., Bhavya, D., Purvi, J., Ruchi, K., Kumudini, B. S. and Varalakshmi, K. N. (2007). Production and characterization of fungal amylase enzyme isolated from Aspergillus sp. JGI 12 in solid state culture. *Afr.* J. *Biotechnol.* **6**: 576-581.

[3]. Egas, M. C., Da Costa, M. S., Cowan, D. A. and E.M. Pires (1998). Extracellular alpha-amylase from *Thermus filiformis* Ork A2: Purification and biochemical characterization, *Extremophiles* 2: 23-32.

[4]. Khan, J. A. and Yadav, S. K. (2011). Production of alpha amylases by *Aspergillus niger* using cheaper substrates employing solid state fermentation. *Int. J. Pl.An and Env.Sci.* **1**(3): 100-108.

[5]. Aiyer, P. V. (2005). Amylases and their applications. *Afr. J. Biotechnol.* **4**(13): 1525- 1529.

[6]. Ramachandran, S., Patel, A. K., Nampoothiri, K. M., Francis, F. and Nagy, V. (2004). A potential raw material for the production of alpa-amylase. *Bioresour. Technol.* **93:** 169-174.

[7]. Soares I., Távora Z., Barcelos R.P. and Suzymeire B (2012). Microorganism Produced Enzymes in the Food Industry, Scientific, Health and Social Aspects of the Food Industry, Dr. Benjamin Valdez (Ed.), ISBN: 978-953-307-916-5, InTech, Available from: http://www.intechopen.com/books/scientific-health-and-social-aspects-of-the-food-industry/microorganismproduced-enzymes-in-the-food-industry

[8]. Andersen M.R., Nielsen M.L. and Nielsen J. (2008). Metabolic model integration of the bibliome, genome, metabolome and reactome of *Aspergillus niger*. *Mol. Sys. Biol.* **4(178):** 1-13.

[9] Peter-Albert, C.F .,Ajayi, A.A ., and Awosika, F.A . (2015). Studies on Xylanase Production by *Aspergillus niger* on Tomato Pomace Medium. In: Mojekwu, J.N., Ogunsumi, L.O., Ojigi, L. M. Atepor, L., Thwala, D.W., Sackey, S. Awere E., and Bamfo-Agyei, E. (Eds) African Journal of Applied Research .(AJAR) Journal, Vol.1,No.1 ISSN 2408-7920 January 2015, Cape Coast, Ghana. 286-298.

[10]. Kareem, S.O., Akpan, I, Popoola, T.O.S. and Sanni L.O. (2011). Activated Charcoal—A Potential Material in Glucoamylase Recovery. *Enzyme Res.* http://dx.doi.org/10.4061/2011/483943

[11]. Adejuwon, A. O., Adejuwon, M.A., Ajayi, A.A., Bamkefa, B.A., Omololu-Aso, J., Alao, O.O., and Adesina, F.C. (2013). Effect of some nitrogen sources of growth medium on α-amylase production by *Penicillium solitum* and *Aspergillus rubrum* isolated from yam (*Dioscorea alata*). *Researcher* **5**(2):1-4.

[12]. Vidyalakshmi R., Paranthaman R. and Indhumathi J. (2009). Amylase Production on Submerged Fermentation by Bacillus. *Wor.J. Chem.* **4(1):** 89-91.

[13]. Mathew J. J., Vazhacharickal P.J., Sajeshkumar N.K. and Ashokan A (2016). Amylase production by *Aspergillus niger* through submerged fermentation using starchy food byproducts as substrate. *Int. J. Herb.***4(6):** 34-40.

[14]. Ahvazi B., Boeshans K. M., Idler W., Baxa U. and Steinert P.M. (2003). Roles of calcium ions in the activation and activity of the transglutaminase 3 enzyme. *J. Biol. Chem.* **278** (26): 23834 – 23841

[15]. Ajayi, A.A., Adejuwon, A.O., Awojobi, O.K. and Olutiola, P.O. (2007). Effect of Cations and Chemicals on the Activity of Partially Purified Cellulase from Tomato (Lycopersicon esculentum Mill) Fruits Deteriorated by Aspergillus flavus Linn. *Pak. J. Nutr.* **6** (2): 198-200.

[16]. Gohara D.W. and Di Cera E. (2016). Molecular Mechanisms of Enzyme Activation by Monovalent Cations. *J. Biol. Chem.* **291**: 20840–20848.

[17]. Dixon M. and Webb E.C (1971). Enzymes. William Clowes and Son. Great Britain pp 950.

[18]. Naika G.S. and Tiku P.K. (2011). Influence of ethylenediaminetetraacetic acid (EDTA) on the structural stability of endoglucanase from *Aspergillus aculeatus*. *J. Agric. Food Chem.* **59** (13): 7341-7345

[19]. Adejuwon A. O., Ajayi A.A. and Ladokun O.A. (2015). Alpha amylase from *Rhizopus arrhizus* Fisher. *J.f Pla. Sci..* **10**: 99-107.