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Response of Fungal L-glutaminase to some Anhydrides and Chelating Agents

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Abstract:

Specific activity of L-glutaminase (E.C 3.5.1.2) was determined by isolating and partially purifying it from *Penicillium chrysogenum* using ammonium sulfate (85%). Upon addition of maleic anhydride (MA) and succinic anhydride (SA) to the reaction media at different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mM), the enzyme was shown to be inhibited. The activity of the enzyme was reduced at a concentration of 10 mM by the four chelating agents ethylene glycol tetraacetate (EGTA), ethylenediaminetetraacetate (EDTA), phenannthroline, and dipyridyl, suggesting that the enzyme is a metalloenzyme. Metal cations such as $CoCl_2$, $CuCl_2$, $FeCl_3$, and $MgCl_2$ in the reaction media at a concentration of 10 mM shown to be inhibitory to the enzyme. Nevertheless, calcium chloride enhanced the enzyme activity at an equivalent concentration.

Key words: L-glutaminase, Fungi, Anhydrides, Chelating agents, Metal Cations.

1. Introduction

Biogenic catalysts are essential in industrial, medicinal, and biotechnological applications. The efficacy of enzyme utilization in any enzymatic process depends on the cost competitiveness and the presence of established chemical technology (El-Shora et al., 2024).

In the diagnosis, therapy, biochemical investigation, and monitoring of some critical diseases, microbial enzymes are of paramount

importance. The vast metabolic variety and easy genetic modification of microorganisms make them a highly valuable source of therapeutic enzymes. L-glutaminase, an amidohydrolase enzyme described in E.C 3.5.1.2, controls the first stage of the glutaminolysis pathway by transforming L-glutamine (Gln) into glutamate (Glu), followed by enzymatic processes that generate aspartate, malate, pyruvate, citrate, alanine, and lactate (Awad et al., 2021).

Microbial isolates of L-glutaminases have been obtained from fungi (El-Shora et al., 2021), yeasts (Aryuman et al., 2015), bacteria (Kumar et al., 2019), and actinomycetes (Desai et al., 2016). The successful utilization of L-glutaminase obtained from *Trichoderma koningii* and *Pseudomonas nitroreducens* in the manufacturing of nutraceuticals has shown encouraging outcomes (Alemzadeh and Sakhaei, 2017).

L-glutaminase is employed in the food industry as a flavor enhancer in fermented foods due to its catalytic function, which produces L-glutamic acid, which contributes to the umami taste (Rastogi and Bhatia, 2019). The synthesis of glutamine/glutamic acid biosensors has also employed L-glutaminases (Albayrak and Karakus, 2016).

The objective of this work was to examine the impact of anhydrides, chelating agents, and different metal ions on the functionality of partly purified L-glutaminase derived from *Penicillium chrysogenum.*

2. Materials and Methods

Experimental microorganism and production of L-glutaminase

The experimental microbe involved in the synthesis of L-glutaminase was *Penicillium chrysogenum* Thom AUMC 14100 gb (Accession No. MN219732) acquired from the Mubasher Mycological Center (AUMMC) at Assiut University, Egypt. The synthesis and isolation of L-glutaminase were conducted using a modified L-glutamine-glucose liquid medium following the method described by Saxena and Sinha (1981).

Partial purification of L-glutaminase and its assay

The L-glutaminase enzyme was partially purified by the use of 85% ammonium sulfate. The specific activity of the enzyme was then determined following the method outlined by El-Shora et al. (2021). The L-glutaminase activity was assessed using the method described by Gulati et al. (1997), by measuring the release of ammonia using spectrophotometry at 450 nm of wavelength. Quantification of protein concentration was conducted using the Bradford technique (Bradford, 1976).

Effect of some anhydrides on fungal synthesized L-glutaminase

The enzyme activity of the two anhydrides maleic (MA) and succinic anhydride (SA) was assessed at different concentrations (0.2, 0.4, 0.6, 0.8, and 1.0 mM) in the reaction mixture. The results were then reported as a percentage of the control treatment.

Effect of chelating agents on fungal synthesized L-glutaminase

An investigation was conducted to examine the impact of EGTA, EDTA, phenanthroline, and dipyridyl as chelating agents on L-glutaminase activity at a concentration of 10 mM in the reaction medium. The enzyme activity was then determined as a percentage the control.

Effect of metal cations on fungal synthesized L-glutaminase

The influence of metal cations, namely chloride salts, on enzyme activity was investigated. Tested in the reaction media were the chloride salts $CoCl_2$, $CaCl_2$, $CuCl_2$, $MgCl_2$, and $FeCl_3$.

3. Results

The current study involved the partial purification of L-glutaminase from *Penicillium chrysogenum* using ammonium sulphate (85%). The resulting concentrate had a specific activity of 12.5 units mg-1 protein. Multiple doses (0.2, 0.4, 0.6, 0.8, and 1.0 mM) of maleic anhydride (MA) and succinic anhydride (SA) were examined to determine their impact on enzyme activity. The data presented in Figure 1 demonstrate a consistent decrease in enzyme activity directly correlated with the concentration of MA. At 1 millimolar concentration, the activity reduced to 6.8 units per milligram of protein, which is 42.2% of the control value.

Figure 2 shows that treating glutaminase with succinic anhydride (SA), another anhydride, constantly decreased glutaminase activity to 3.3 units mg^{-1} protein, or 20.6% of the control.

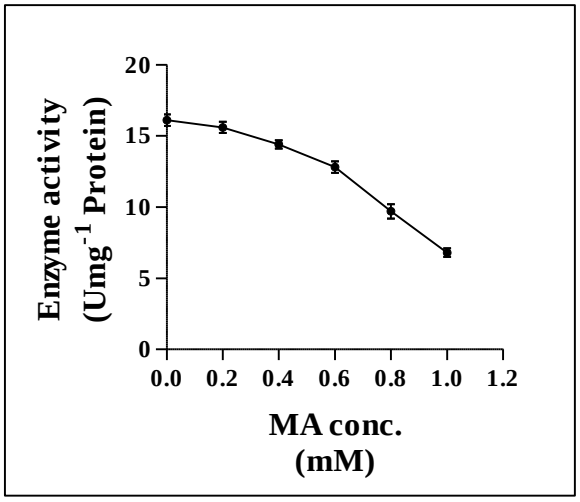

Fig. 1: Effect of maleic anhydride (MA) on the activity of glutaminase.

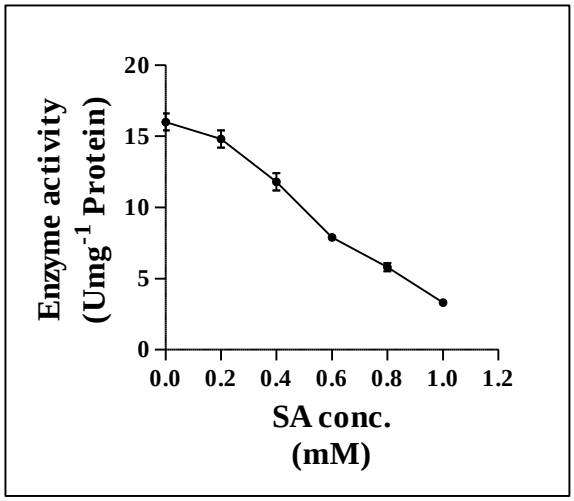

Fig. 2: Effect of succinic anhydride (SA) on the activity of glutaminase.

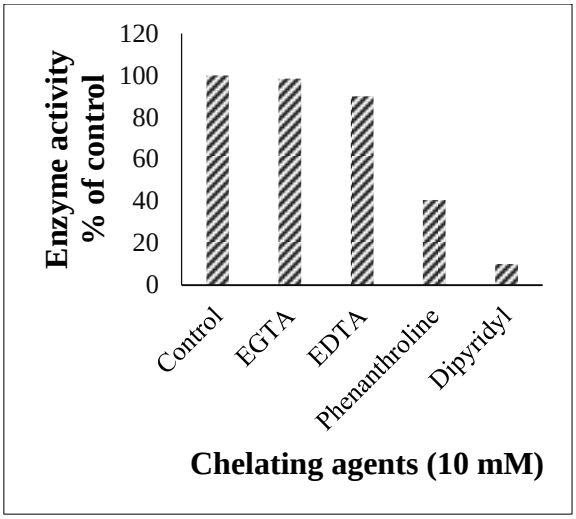

Fig. 3 Effect of various chelating agents on the activity of L-glutaminase.

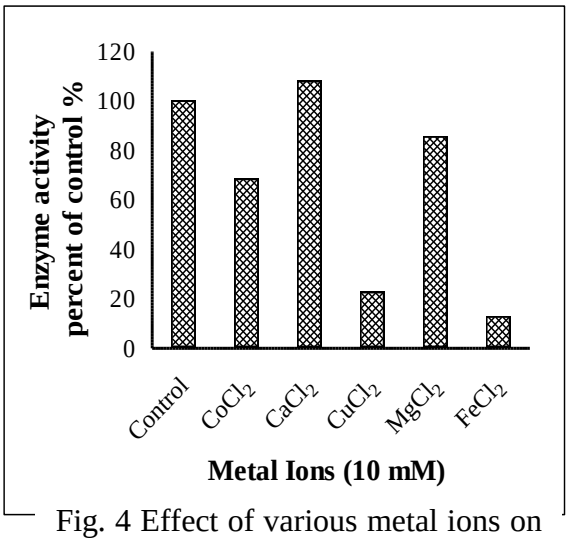

Fig. 4 Effect of various metal ions on the activity of glutaminase.

The results indicated in Figure 3 illustrate the impact of several chelating agents at a concentration of 10 mM in the glutaminase reaction mixture. Among the four substances tested, phenanthroline and dipyridyl were shown to be the most powerful inhibitors, inhibiting 59.5% and 90% of the enzyme activity, respectively. Nevertheless, EGTA and EDTA only achieved a 1.6% and 10% inhibition of the enzyme activity, respectively.

Figure 4 demonstrates that all the cations examined suppressed glutaminase activity, with the exception of calcium ions which increased enzyme activity by 7.9% compared to the control. The activity was suppressed by 77.4% and 87.2%, respectively, by cupper chloride and ferrous chloride.

4. Discussion

The enzyme L-glutaminase is of primary importance and has considerable potential uses as antioxidant and anticancer agent and in L-theanine synthesis. (Chitanand and Shete, 2012). The parameters affecting enzyme synthesis not only augment the amount but also enhance the quality of the

enzyme, rendering it more appropriate for particular uses. Furthermore, the optimization of these factors can have a direct influence on enzyme synthesis and cell yield (Ibrahim et al., 2015).

The stability, affordability, and manufacturability of fungal L-glutaminase make it highly useful as a pharmaceutical target. Furthermore, it has considerable promise in the field of anticancer treatment (Amobonye et al., 2019).

The present study demonstrated that maleic anhydride and succinic anhydride deactivated L-glutaminase. Their inactivation of other enzymes, including ribonuclease, was ascribed to cis-aconitic anhydride, which is regarded a reversible blocking agent for amino groups in proteins (Takahashi, 1977).

The chelating chemicals EGTA, EDTA, and dipyridyl demonstrated inhibitory effects on L-glutaminase activity, indicating that L-glutaminase is a metalloenzyme. Turk et al. (2018) demonstrated that EDTA effectively inhibited L-glutaminase from *Streptomyces pratensis.* The inhibitory effect of EDTA arises from its capacity to complex with the metal ions necessary for enzyme function, therefore causing the enzyme to become inactive. However, EDTA had no substantial impact on the activity of L-glutaminase from *A. flavus* (Abu-Tahon and Issac, 2019).

The activity of purified L-glutaminase obtained from Bacillus sp. B12 was decreased when incubated with 10 mM of Hg^{2+}, Cu^{2+}, Fe^{2+}, and Zn^{2+}, whereas Ca^{2+} ions were observed to enhance its enzymatic activity. (Abdelhameed et al., 2020). A potential explanation for the higher L-glutaminase activity seen in the presence of Ca^{2+}, as compared to the control without metal ions, is the capacity of this metal ion to inhibit enzyme denaturation (Fujiwara et al., 1993). The presence of –SH groups in the enzyme, seen from its inhibition by Cu^{2+} and Mg^{2+}, is essential for its catalytic activity (El-Shora and Taha, 2010).

Moreover, Ca^{2+} ions stimulated the activity of additional enzymes, including phytase (El-Shora and Abo-Kassem, 2001). The enzyme activation induced by Ca^{2+} may be attributed to the enhancement of interactions within the enzyme molecules and the binding of Ca^{2+} to the autolysis site (Harris and Davidson, 1994).

Ultimately, the inhibitory effects of anhydrides and chelating compounds on L-glutaminase from *Penicillium chrysogenum* suggest that it is a metalloenzyme. Furthermore, calcium chloride stimulated the activity of L-glutaminase while heavy metals produced inhibitory effects.

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الملخص العربي

استجابة إنزيم الجلوتامينيز الفطري لمركبات الأنهيدريد والمركبات المخلبية والكاتيونات المعدنية

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تم عزل إنزيم الجلوتامينيز ((EC 3.5.1.2) وتنقيته جزئيا من فطر بينسيليوم كريزيجونم بنشاط 5و12وحدة لكل مللجم بروتين بواسطة كبريتات الأمونيوم (85٪). تم تثبيط الإنزيم بواسطة أنهيدريد المالئيك (MA) وأنهيدريد السكسينيك (SA) وذلك في وسط التفاعل بتركيزات مختلفة (0.2و 0.4و 0.6و 0.8 و1.0 مللي مولار). أدت المعاملة بهذين المركبين الي تثبيط تدريجي للنشاط الإنزيمي معتمدا على التركيز المستخدم. ثبطت العوامل المخلبية الأربعة إيثيلين جليكول رباعي الأسيتات (EGTA) وإيثيلين داي أمين رباعي الأسيتات (EDTA) والفينانثرولين وداي بيريديل النشاط الإنزيمي عند تركيز 10 مللي مول وبالتالي تشير هذه النتائج إلى أن الإنزيم يحتوي على ذرة إحدى العناصر ويسمى ميتالوانزيم. تم تثبيط الإنزيم بواسطة الكاتيونات المعدنية في صورة املاح الكلوريد والتي شملت كلوريد الكوبلت وكلوريد النحاس وكلوريد الحديد وكلوريد الماغنسيوم في وسط التفاعل عند تركيز 10ملي مولار. وقد أظهرت النتائج أن كل الكاتيونات المستخدمة في صورة كلوريد ثبطت النشاط الانزيمي بينما حفز كلوريد الكالسيوم النشاط الانزيمي عند بنفس التركيز.