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Modification of Fungal L-arginase by Some Modifiers of the active Enzyme Residues

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Abstract:

Arginase, known as L-arginine urea hydrolase or amidinohydrolase (EC 3.5.3.1), is an essential hydrolytic enzyme involved in the urea cycle catalyzing urea synthesis in the liver of mammals. The enzyme was extracted and partly purified from *Penicillium chrysogenum*, with a specific activity of 15.8 units per milligram of protein. Investigation was conducted on the impact of specific reagents, namely phenyl glyoxal (PGO), Woodward's reagent K (WRK), N-bromosuccinimide (NBS), and trinitromethane (TNM), on the active groups of L-arginase. The aforementioned chemicals exhibited a concentration-dependent inhibition of L-arginase activity at different tested concentrations (1, 2, 3, 4, and 5 M). The inhibitory effect of PGO on L-arginase at 5 M was 82.3%, whereas WRK exerted an inhibitory effect of 90.0%. Both TNM and NBS exhibited enzyme inhibition rates of 70.2% and 81.0%, respectively. The observed inhibition highlights the crucial involvement of arginyl, carboxyl, tyrosyl, and tryptophenyl residues in the process of enzyme catalysis.

Key words: Enzymes, L-arginase, *Penicillium chrysogenum.*

1. Introduction

Enzymes are ubiquitously present in all living creatures, where they expedite and regulate the essential chemical processes for sustaining life **(Nisha and Divakaran, 2014)**. Enzymes, unlike chemical catalysts, facilitate molecular changes that chemical catalysis on its own is unable to accomplish **(Liese and Hilterhaus, 2013)**. Enzymes do not able to change the equilibrium of reactions and differ from other catalysts in their specificity for the substrate. Other compounds, such as activators, which enhance enzyme activity, and inhibitors, which decrease it, can also have an effect on enzyme activity **(Bisswanger, 2014).** The amino acid L-arginine exhibits a high degree of functional diversity within physiological cells. In addition to its function as a constituent of proteins, arginine serves as a precursor for the production of polyamines, agmatine, and proline, as well as cell-signaling mediators such as glutamate, aminobutyric acid, and nitric oxide **(Siddappa *et al.*, 2018)**. Mammalian arginase is active as a trimer, however some bacterial arginases form hexmeric arginases. **(Dowling *et al.*, 2008)**. To maintain proper function, the enzyme needs a two-molecule metal cluster of manganese. These ions coordinate Mn^{2+} with water, directing the molecule and fixing it. They also permit water to act as a nucleophilic antipyretic and attack L-arginine, breaking it down into ornithine and urea (**Di Costanzo *et al.*, 2007)**. In mammals, L-arginase features two distinct isoforms, among which the urea cycle arginase (arginase I or liver arginase) is considered cytosolic, and it has been detected in erythrocytes and hepatic cells, which is responsible for catalyzing the conversion of L-arginine to L-ornithine and urea in the final step of the hepatic urea cycle **(Steggerda *et al.*, 2017)**, Another isozyme, (arginase II or kidney arginase), it is a mitochondrial enzyme with a wide range of tissue distribution, mainly found in the kidney, brain, prostate, small intestine, breast and macrophages **(You *et al.*, 2018)**. Optimal enzyme stability is of utmost significance in both fundamental and practical enzymology. Gaining insight into this stability is crucial for clarifying the mechanisms by which enzymes lose their activity and for establishing the connections between structure and stability in enzymatic molecules **(Sha *et al.*, 2021)**. Furthermore, apart from being found in mammalian tissues, L-arginase has been identified in bacteria **(Zhang*et al.*, 2013)**, yeast, actinomycetes, algae, and plants **(Winter *et al.*, 2015)**. Analysis by Alzahrani et al. (2020) revealed the presence of L-arginase in *Aspergillus nidulans*. Ammonium sulfate precipitation and DEAE-cellulose were employed to purify L-arginase from kiwi fruit **(Hale *et al.*, 1997)**. L-arginase has been employed in the management of several diseases, such as rheumatoid arthritis, neurological problems, liver cancer, and asthma. Moreover, a

lack of arginase might result in hyperargininemia, a condition that is rather a hereditary abnormality of the urea cycle **(Christopher *et al*., 1997)**. The objective of this study was to examine the impact of specific modifiers on targeted residues in the L-arginase protein on its activity.

2. Materials and Methods

Experimental microorganism and production of L-arginase

The taxonomic designation MN219732 was assigned to *Penicillium chrysogenum* Thom AUMC 14100 gb, which was acquired from the Assiut University Mubasher Mycological Center (AUMMC) located in Assiut, Egypt. The synthesis and extraction of L-arginase were conducted using a modified L-arginine-glucose liquid medium following the method described by Saxena and Sinha (1981). The medium was adjusted to pH 7.5 and incubated at 40°C.

Partial purification of L-arginase and its assay

L-arginase was isolated by extraction with 85% ammonium sulfate, and the enzyme was quantified using the method described by Mohamed et al. (2023) by measuring the release of ammonia using spectrophotometry at 450 nm. The specific activity was measured to be 15.7 units per milligram of protein. The Bradford method (Bradford, 1976) was employed to determine the protein content.

Effect of the various modifiers

Phenyl glyoxal (PGO), Woodward's reagent K (WRK), N-bromosuccinimide (NBS), and trinitromethane (TNM) are widely recognized as compounds that provide modifications to arginine, carboxyl, tryptophanyl, and tyrosyl residues **(El-Shora *et al*., 2008)**. Subsequently, the enzyme activity was assessed by treating the partially purified enzyme in the reaction mixture at different concentrations (1, 2, 3, 4, 5 M) as described by El-Shora et al. (2024).

3. Results

The enzyme L-arginase was extracted and partially purified from *Penicillium chrysogenum*, resulting in a specific activity of 15.8 units per milligram of protein. Variations in the concentrations (1, 2, 3, 4, and 5 M) of PGO, WRK, TNM, and NBS in the reaction mixture were used to examine the impact of different reagents on the residues inside the enzyme molecule. The four compounds exhibited different rates of inhibition of L-arginase activity, which were contingent upon the specific kind and concentration of the reagent. The inhibitor WRK (Fig. 1) shown the highest potency, effectively eliminating 90% of the enzyme's activity at a concentration of 5 M. PGO (Fig. 2) was the second most potent inhibitor, causing an 82.3% decrease in enzyme activity at a concentration of 5 M.

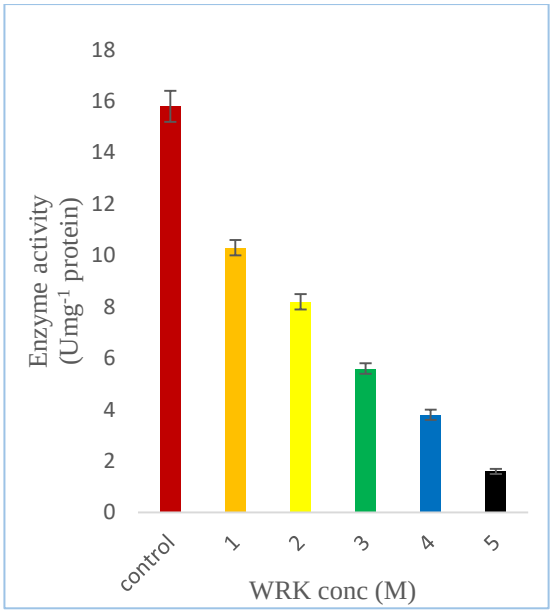

Fig. 1: Effect of woodward's reagent K (WRK) on the activity of arginase.

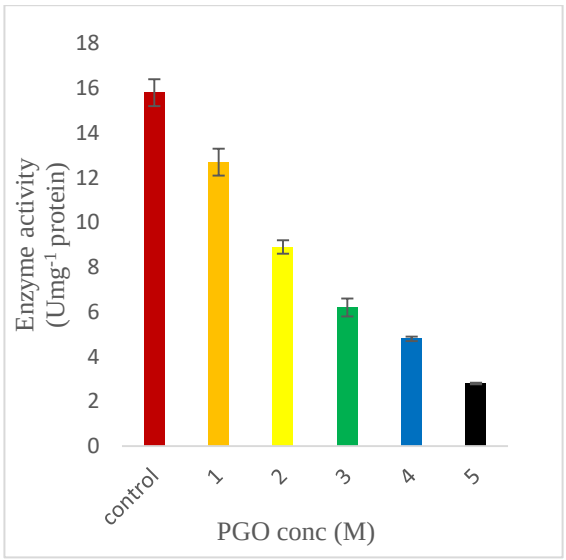

Fig. 2: Effect of phenyl glyoxal (PGO) on the activity of arginase.

NBS (Fig. 3) ranked third and inhibited 81% of the enzyme's activity at 5 M. The control sample exhibited 15.8 units mg^{-1} protein, however at 5 M the enzyme activity was 3.0 units mg^{-1} protein. Finally, TNM (Fig. 4) was the weakest inhibitor, decreasing enzyme activity by 70.2% where at 5 M the enzyme activity was 4.7 units mg^{-1} protein.

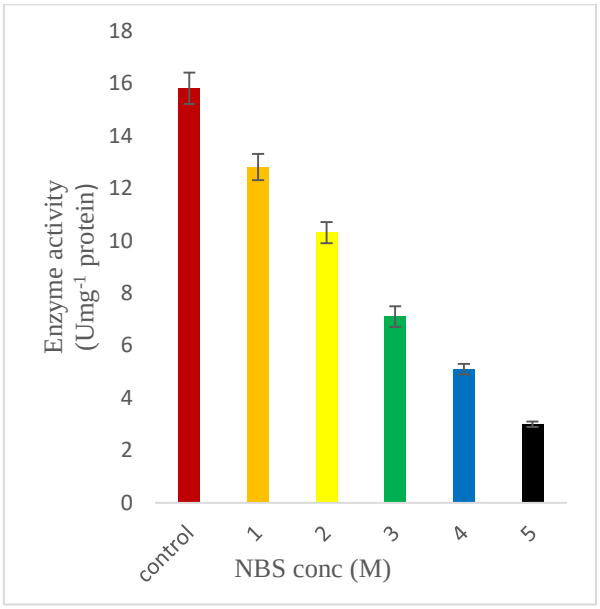

Fig. 3: Effect of N-bromosuccinimide (NBS) on L-arginase activity.

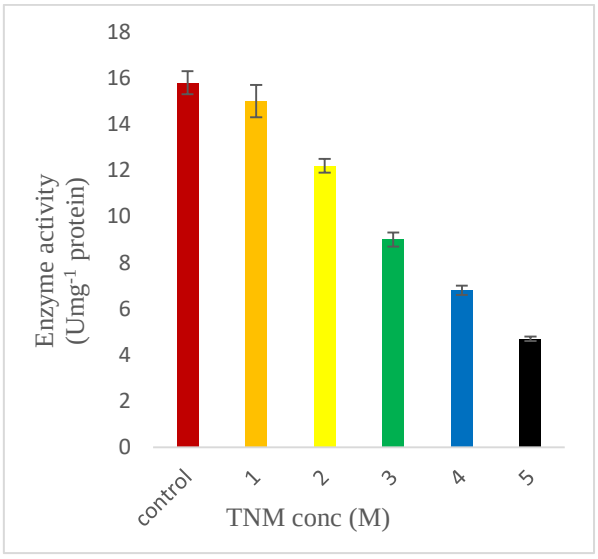

Fig. 4: Effect of trinitromethane (TNM) on L-arginase activity.

4. Discussion

This fundamental amino acid is essential in humans, as it contributes to the recovery of liver illness, improves cardiac function, facilitates weight loss, and strengthens immunity. Owing to these substantial advantages, L-arginine is highly sought after in the healthcare and pharmaceutical sectors. Therefore, this particular research area has attracted significant interest because of its immense business potential **(Bagi *et al*., 2013).**

Ornithine, which is the immediate to arginine, is involved in the maintenance of proline and glutamate biosynthesis, as well as polyamines generation. Furthermore, urease catalyzes the further breakdown of urea into ammonia and carbon dioxide. It is hypothesized that L-arginase and urease directly recycle urea nitrogen to meet the metabolic requirements of growing seedlings **(Siddappa *et al*., 2018).**

The current study involved the chemical alteration of L-arginase using PGO, WRK, TNM, and NBS. The enzyme was rendered inactive by different concentrations of each reagent. The obtained results are consistent with the conclusions made by Zhang et al. (2013), who documented the indispensability of these residues for the catalytic activity of L-arginase derived from *Bacillus subtilis*.

Chemical modification is essential for investigating the mechanics of enzyme activity. This methodology facilitates the identification of the precise amino acid residues that are accountable for the catalytic characteristics of the protein. Altered enzymatic specificity has been detected in chemical modification studies **(Kaiser *et al*., 1985).**

Conclusively, the enzyme protein L-arginase from *Penicillium chrysogenum* was found to be inhibited by several modifiers of the crucial residues, including PGO, WRK, TNM, and NBS. WRK exhibited the highest inhibitory potency, while TNM demonstrated the lowest effectiveness.

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الملخص العربي

تحوير إنزيم الأرجينيز الفطري بواسطة بعض المحورات لمجموعات الأنزيم الفعالة

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الأرجينيز (أميدو هيدروليز EC 3.5.3.1) هو إنزيم مائي يلعب دورًا حاسمًا في دورة اليوريا، حيث يحفز إنتاج اليوريا في كبد الثدييات. تم عزل الإنزيم وتنقيته جزئيًا من فطر بنيسليوم كريزوجيونم، مع نشاط نوعي قدره 15.8 وحدة لكل مللي جم بروتين. تمت دراسة تأثيرات بعض الكواشف على المجموعات النشطة في إنزيم الأرجنيز، بما في ذلك فينيل جليوكسال (PGO)، وكاشف وودوارد ك (WRK) ، و كاشف ن-بروموسكسينيميد (NBS) وثلاثي نيتروميثان (TNM) . ثبطت هذه المركبات نشاط إنزيم الأرجينيز عند التركيزات المختلفة والمختبرة (1، 2، 3، 4، و5 مولار) بطريقة تعتمد على التركيز. عند 5 مولار، ثبط مركب الجليوكسال نشاط الأرجينيز بنسبة 82.3 %، بينما ثبط كاشف ووداورد ك الإنزيم بنسبة 90.0%. بينما ثبط كل من ثلاثي نيتروميثان و ن-بروموسكسينيميد الإنزيم بنسبة 70.2% و81.0% على التوالي. يشير هذا التثبيط إلى أهمية الدور الأساسي لمجموعات الأرجينيل والكربوكسيل والتيروزيل والتريبتوفينيل في عمل إنزيم الأرجينيز.