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Original Article

Purification and Characterization of Arginase from the Stomach of Cane Rat (*Thryonomys swinderianus,* Temminck 1827)

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Abstract

Introduction: Rodent pests are unique threats to agriculture and food security in many developing countries. Hence, this study investigated some physicochemical properties of partially purified arginase from the stomach of cane rats (*Thryonomys swinderianus)*. This was with a view to understanding the significance of the enzyme in nitrogen metabolism, which could be exploited in the control of rodent pests.

Materials and Methods: Arginase was isolated and subjected to 70% ammonium sulfate $(NH_4)_2SO_4$ precipitation and dialyzed. The dialysate was further purified using ion-exchange chromatography on CM-Sephadex C-50 matrix.

Results: Apparent K_m of the enzyme for L-arginine and V_{max} were 54 ± 0.7 mM and 0.057 ± 0.1 µmol/minute/ml, while the optimum pH and temperature obtained for the enzyme were 7 and 70 °C, respectively. NaCl, $MnCl_2$ and $HgCl_2$ activated the enzyme activity, while $FeCl_3$ totally inactivated the enzyme at tested concentrations. β-mercaptoethanol, urea and EDTA profoundly potentiated the activity of the enzyme. Enzyme activity (81%) was retained when arginine was substituted with cysteine, while histidine, proline, alanine, tyrosine and tryptophan had potent inhibitory effects on the enzyme activity.

Conclusions: The study established the presence of arginase in the stomach of cane rat and illuminated some physicochemical properties of the enzyme, which could be exploited in its deployment as a viable strategy to control *T. swinderianus*. However, further studies including structure-function investigations of the enzyme are recommended to fully exploit its potential in the control of rodent pests.

Keywords: Arginase, Arginine, Cane Rat, Enzyme

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Introduction

Agricultural practices, especially small-scale farming, contributes immensely to food security in developing countries.[1] Aside from the habitat-associated and communal problems faced by subsistence farmers, agricultural pests such as rodents, which accounts for about 42% of mammals globally, are emerging threats in food production and public health.[2-5] Meerburg et al.,[5] reported that about 280 million people in developing countries will be sufficiently fed by the accrued yield from 5% reduction in rodent-induce rice loss, making the strategic effective control of rodent pest imperative, in ensuring food security and economic development.[6]

Extensively domiciled in Western and central Africa, the rodent *Thryonomys swinderianus*, Temminck 1827 (Cane rat or grasscutter) is a major pest of many agricultural crops.[7] In Nigeria, cane rat is also known as 'oya', 'nchi', and 'gegbi' among the Yoruba, Igbo and Hausa tribes respectively [8]. Cane rats are predominantly found in humid and sub-humid African regions,[9] covered with dense thick cane-like grasses, including elephant and guinea grasses. The business of selling them could be termed lucrative because of its high price for customers who value the protein nutriments in eating cane rats.[10] Their sharp broad incisors, enable them to feed basically on reeds, roots, shoots and stems of grasses effortlessly. The havoc done by these rodent pests on crops cannot be overemphasized, as they are regarded as major pests in sugar cane, wheat and maize fields.[10]

Arginase is a ubiquitous metal-dependent enzyme with unique manganese preference, involved in the catalytic conversion of L-arginine to L-ornithine and urea.[11] The two isoforms are ARG1 and ARG2, both catalyzing the same reaction, but with different cellular environments.[11] ARG1 is cytosolic while ARG2 is a mitochondrial matrix protein. Disorders related to arginases have been noticed in patients suffering asthma, pulmonary hypertension, hypertension, T-cell dysfunction, erectile dysfunction, atherosclerosis, renal disease, ischemia- reperfusion injury, neurodegenerative disease, inflammatory disease and fibrotic disease.[12] Arginase plays a crucial role in the metabolic processes of ureotelic organisms such as *T. swinderianus*, including immune functions and synthesis of polyamines which are

needed for cell growth and development.[13-15] Alterations in the enzyme functions have been linked with several pathologies.[12]

This study therefore investigated the physicochemical properties of partially purified arginase from the stomach tissue of cane rat. This was with a view to illuminating the potential biotechnological exploitation of the enzyme in the effective control of rodent pests.

Materials and Methods

Animals

Cane rat (*T. swinderianus*) were obtained from a rural settlement (Abagboro village), Ile-Ife community, Southwestern Nigeria. The animal was immediately taken to the laboratory where the stomach was excised. All experimental procedures were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health.

Chemicals

L-arginine, NaCl, CM-Sephadex C-50, ammonium sulphate, Erhlich reagent, bovine serum albumin, EDTA, β-mercaptoethanol, Iron III chloride, and manganese chloride were obtained from Sigma Chemical Company, St Louis, USA. Other used reagents were of analytical grade.

Preparation of T. swinderianus Stomach Homogenate

Approximately 50 g of the stomach tissue of *T. swinderianus* was excised and homogenized with 0.04 M Tris buffer, pH 7.2 to achieve 70% homogenate. The mixture was filtered with cheese cloth and centrifuged at 5000 ×*g* (4 ℃) for 1 h to obtain clear supernatant. The supernatant was kept frozen at -20 °C for further use.

Arginase Activity Assay

The enzyme assay was carried out according to the method of Kaysen and Strecker[16] with slight modification. The reaction mixture which contained 50 mM sodium carbonate buffer (pH 10), 1 mM $MnCl_2$, 0.33 M arginine solution and appropriate volume of enzyme preparation was added in the final volume of 1 ml. The mixture was then incubated at 37 °C for 10 minutes and the reaction was terminated by the addition of 2.5 ml of Erhlich reagent. The absorbance was taken at 450 nm after 20 minutes. The produced urea was estimated from a standard urea curve prepared by a varying concentration of urea. The unit of activity of arginase is defined as the amount of enzyme that will produce 1 µmol of urea per minute at 37 °C. Protein concentration was determined using the Bradford method.[17]

Enzyme Purification

The tissue supernatant (containing the crude enzyme) was subjected to 70% ammonium sulphate precipitation, and was left overnight at 4 ℃. The obtained precipitate was suspended in 0.04 M Tris-HCl buffer (pH 7.5) and dialysed exhaustively against the buffer for 48 h using a 3 kDa cut-off dialysis membrane. The dialysate was further purified on CM-Sephadex C-50 column (1.5 x 20 cm) previously washed and equilibrated with 0.04 M Tris-HCl buffer, pH 7.2. Fractions of 2 ml of each of them were collected at a flow rate of 20 ml/h. Bound enzyme was eluted with the same buffer containing 1.0 M NaCl, and absorbance read at 595 nm. Fractions were assayed for enzyme activity after exhaustive dialysis, and fractions in the active peak were pooled, and kept frozen at -20 ℃.

Determination of Kinetic Parameters

According to Kaysen and Strecker[16], K_m and V_{max} of the enzyme were determined by varying the concentration of arginine between 20 to 250 mM. The enzyme kinetic parameters were determined from the double reciprocal plot.

Effects of pH and Temperature

The effect of pH on arginase activity was investigated by incubating 0.05 ml enzyme aliquots at 37 ℃ for 10 minutes with buffer solutions of pH 3-10 range (5 mM Citrate, pH 3-6; 5 mM Phosphate, pH 7; 5 mM Tris-HCl, pH 8-9, and 5 mM Sodium carbonate, pH 10). The effect of temperature on enzyme activity was determined by incubating 0.05 ml enzyme aliquot at varying temperatures between 30 and 100 °C for 10 minutes.

Effect of Metal ions and Denaturing Agents

To investigate the effect of metals on enzyme activity, 0.05 ml of the enzyme was incubated differently with NaCl, $HgCl_2$, $FeCl_3$ and $MnCl_2$ at concentrations ranging from 0.1, 0.5, 1, 5 and 10 mM for 10 minutes. The effect of chemical denaturant and chelating agent on enzyme activity was investigated by incubating 0.05 ml enzyme aliquots with β-mercaptoethanol, urea and EDTA at concentrations ranging from 1, 2, 5 and 10 mM for 10 minutes.

Effect of Amino Acids

The effect of the selected amino acids on the arginase activity was also investigated. Briefly, aliquots (0.3 M each of L-Histidine, L-Proline, L-Cysteine, L-Tyrosine, L-Alanine and L-Tryptophan) were substituted independently in place of L-arginine in the assay mixture, and the enzyme activity was measured.

Results

Purification of Arginase

Figure 1 shows the elution profile of the ion exchange chromatography of the ammonium sulphate precipitated arginase obtained from the stomach of cane rat on CM Sephadex

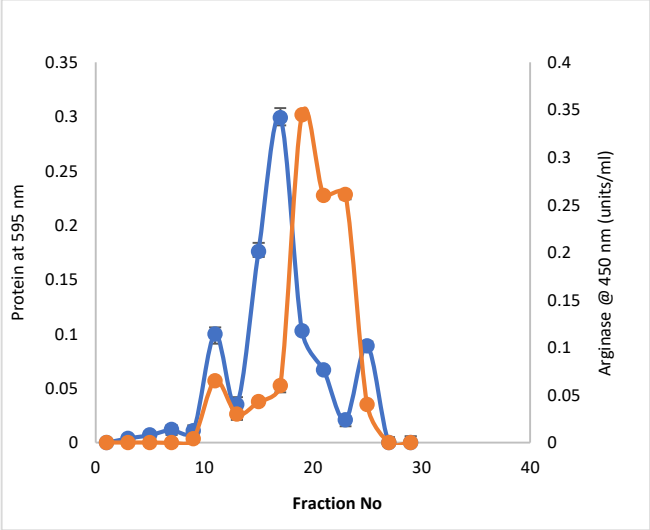

Figure 1. Ion Exchange Chromatography of Arginase (Ammonium Sulphate Dialysate) Obtained from *T. swinderianus* Stomach on CM-Sephadex C-50. Protein @595 nm (——●——), Arginase Activity@ 450 nm (units/ml) (——■——).

C-50 column. The results of the arginase purification are summarized in Table 1 with the enzyme having a yield of 33.4% after ion exchange chromatography.

Kinetic Parameters

Figure 2 shows the Lineweaver-Burk and Michalis-Meten plots used in the estimation of kinetic parameters of *T. swinderianus* arginase. The values of kinetic parameters are summarized in Table 2.

Effects of pH and Temperature

Optimum pH obtained for arginase activity in this study was pH 7 (Figure 3), as there was a loss of activity at a pH value below or above this range. The optimum temperature obtained for the enzyme activity was 70 ºC (Figure 4).

Metals and Denaturing Agents

The effect of metal ion showed that $FeCl_3$ had the highest inhibitory effect on the activity of the enzyme (Figure 5) with a loss of activity at a concentration lower than 2 mM.

Table 1. Purification Summary of Arginase Obtained from the Stomach of *T. swinderianus*

Purification step	Total Protein (mg)	Total Activity (U)	Specific Activity (U/mg)	Purification Fold	Yield (%)
Crude	259.2	70.4	0.272	1	100
Ammonium Sulphate	111.6	35.82	0.321	1.2	50.9
Ion Exchange	68.6	23.52	0.343	1.3	33.4

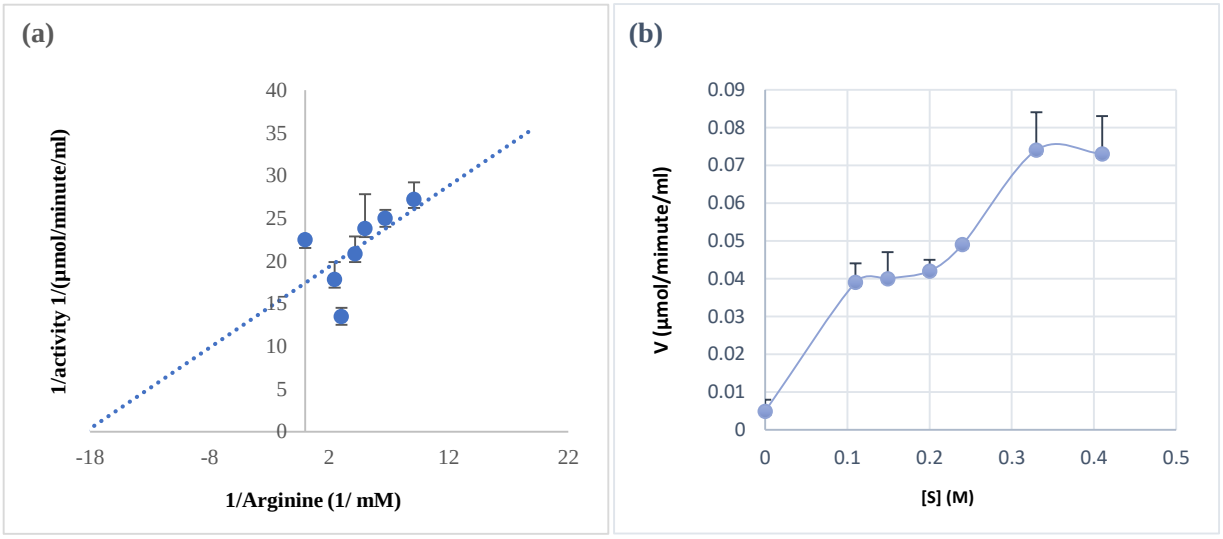

Figure 2. Graphs Showing **(a)** Lineweaver-Burk Plot of 1/V against 1/[S], and **(b)** Michaelis-Meten Plot of V against [S].

Table 2. Summary of Kinetic Parameters of *T. swinderianus* Stomach Arginase

	K_m (mM)	V_{max} (U/mg)
Arginine	54 ± 0.7	0.057 ± 0.1

Data was presented as mean ± standard error of mean (SEM)

There was an increase in enzyme activity as the concentration of NaCl increased, with gradual loss of activity after 5 mM concentration of the salt (Figure 6).

The increasing concentration of $MnCl_2$, enhanced a progressive enzyme activity (Figure 7). In Figure 8, the enzyme activity was enhanced at all $HgCl_2$ concentrations except a drop in the activity, at 1 mM concentration of the salt.

β-mercaptoethanol, urea and EDTA showed concentration dependent effects on the activity of the enzyme (Figures 9-11).

Effect of Amino Acids on Cane Rat T. swinderianus Arginase

Cysteine and proline gave a residual activity of 81% and 61% respectively. Other amino acids significantly decreased the activity (Figure 12).

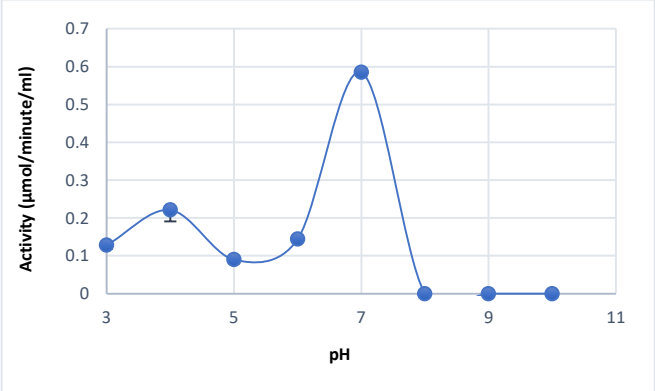

Figure 3. Effect of pH on the Activity of Arginase Purified from the Stomach of *T. swinderianus*.

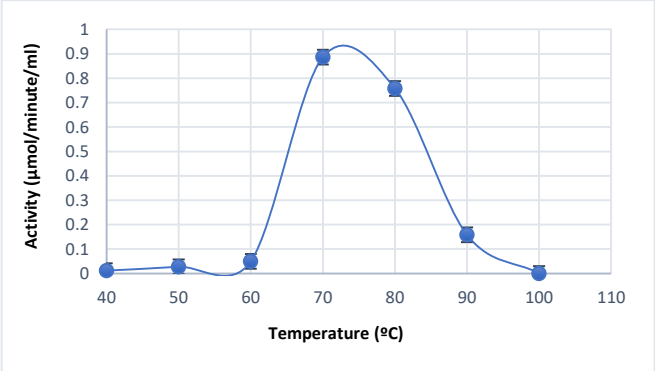

Figure 4. Effect of Temperature on the Activity of Arginase Partially Purified from the Stomach of *T. swinderianus*.

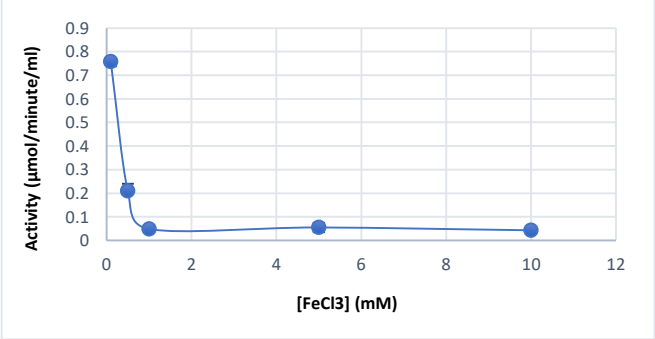

Figure 5. Effect of $FeCl_3$ on the Activity of Partially Purified Arginase from the Stomach Tissue of *T. swinderianus*.

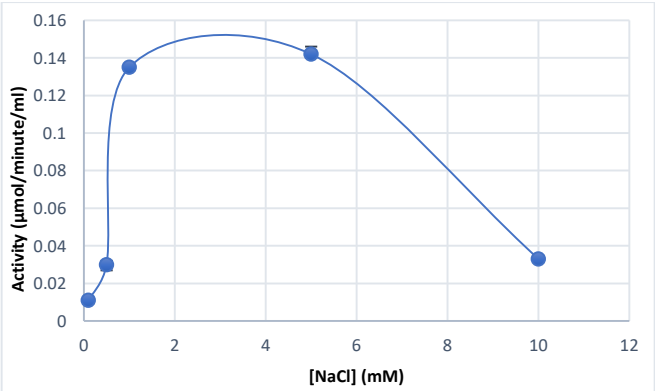

Figure 6. Effect of NaCl on the Activity of Partially Purified Arginase from the Stomach Tissue of *T. swinderianus*.

Discussion

The specific activity of arginase from the stomach of *T. swinderianus* was 0.343 µmol/minute per mg of protein. Different K_m values for arginases from different sources have been reported. It has been observed that the K_m values of ureotelic organisms fall between 1 -20 mM, while uricotelic ranges from 40-200 mM.[18] The K_m value of arginase from the stomach of cane rat for L-arginine was 54 mM. The obtained K_m value, compared well with arginases from other sources, which is a bit higher than the values given to ureotelic arginases. However, it falls between the overall K_m values for arginases. Several studies have reported a low K_m for uricotelic arginases. Okonji et al.,[19] reported a K_m of 0.33 mM for arginase purified from the gut of *Periplaneta americana* (cockroach). Also, Okonji et al.,[20] reported a K_m of 40 mM for *Z. variegatus* (grasshopper) arginase. Furthermore, Okonji et al.,[19] working reported a K_m of 11.6 mM for L-arginine. Ehigie et al.,[21] also reported a K_m of 12.5 mM for L-arginine by *M. rosenbergii* (giant freshwater prawn) arginase, which also compares well to K_m of 7.1 mM in camel liver arginase.[20] The effect of temperature has been investigated on arginase activity and various optimum temperatures have been reported. Therefore, the effect of temperature was carried out on arginase activity from the stomach of cane rat with an optimum temperature of 70 °C, similar to the optimum temperature of camel liver arginase reported by Maharem et al.[11]

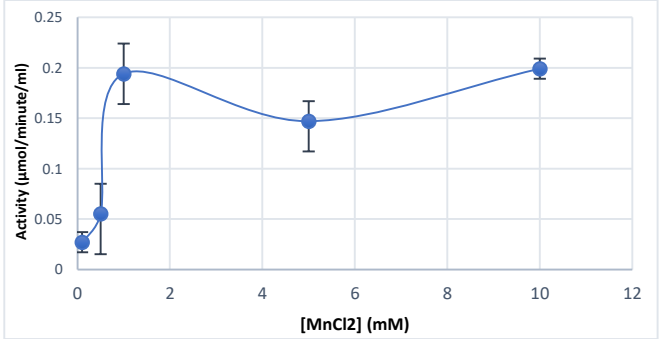

Figure 7. Effect of $MnCl_2$ on the Activity of Partially Purified Arginase from the Stomach Tissue of *T. swinderianus*.

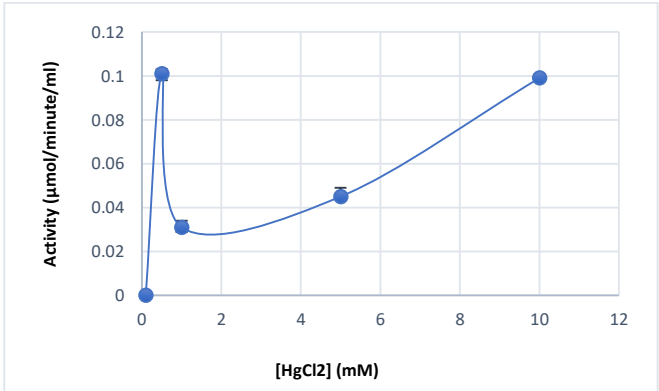

Figure 8. Effect of $Hgcl_2$ on the Activity of Partially Purified Arginase from the Stomach Tissue of *T. swinderianus*.

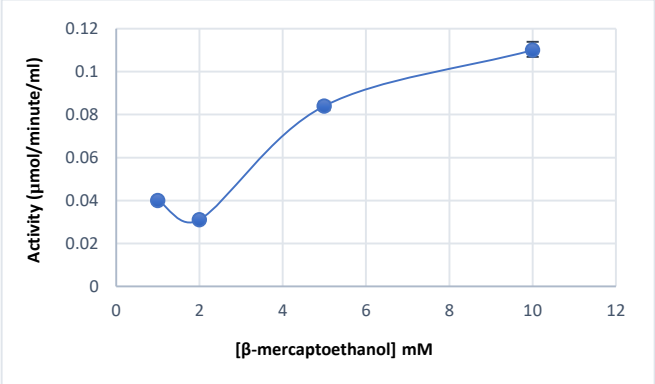

Figure 9. Effects of β-mercaptoethanol on the Activity of Partially Purified Arginase from the Stomach Tissue of *T. swinderianus* arginase.

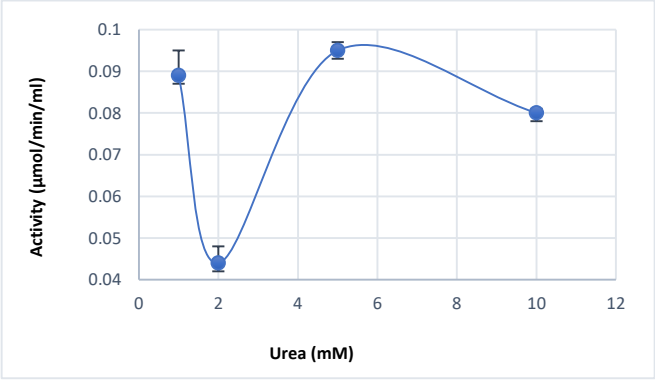

Figure 10. Effect of urea on the Activity of Partially Purified Arginase from the Stomach of *T. swinderianus*.

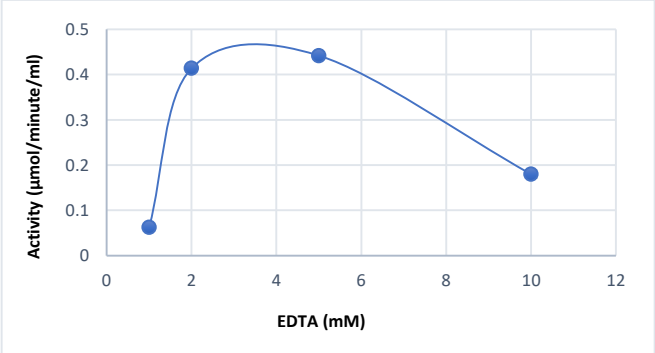

Figure 11. Effect of EDTA on the Activity of Partially Purified Arginase from the Stomach of *T. swinderianus*.

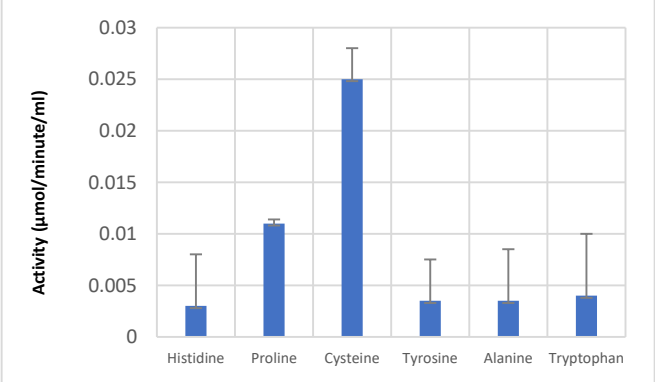

Figure 12. Effects of Selected Amino Acids on the Activity of Partially Purified Arginase from the Stomach Tissue of *T. swinderianus*.

Fitzpatrick et al.,[22] reported that *S. monsoni* arginase has an optimum temperature of 37 °C. Patchett et al., [23] obtained an optimum temperature of 60 °C from the extreme thermophile *Bacillus caldovelox*. Vitreous humor arginase was found to have maximum activity at a temperature of 40 °C [24]. Lavulo et al.,[25] demonstrated that rat stomach arginase variants are characterised by decreased temperature stability (63.6-65.5 °C) relative to the wild-type enzyme (70 °C).

One of the most investigated factors influencing arginase activity is the effect of pH. Several investigators have reported varied optima pH for arginases from different sources. The effect of pH was carried out on cane rat stomach arginase and an optimum pH 7 was obtained. Okonji et al.,[20] reported an optimum pH of 8 for grasshopper arginase, while the pH of 7 from this study, compared adequately with arginases from various sources. Also, Ehigie et al., [21] reported an optimum pH 8.5 for arginase from the hepatopancreas of giant freshwater prawn. Furthermore, Vitreous humor arginase showed most activity at pH 8.8.[24] On the other hand, it was revealed that arginases in mammals had basic optima pH 9.5-10.1 though exceptions have also been noted.[18] McGee et al.,[26] reported an optimum pH 6.0 and 6.1 for *Bacillus anthracis* and *Helicobacter pylori* arginase respectively. Mahareem et al.,[11] reported an optimum pH 9.0 for camel liver arginase. Plant arginases have been reported to have an optimum pH 9.4.[18] Arginase is a metalloenzymes in which manganese acts as a co-factor, activator and a stabilizing molecule. This requirement of a divalent cation has been reported to be a feature of arginase activity for eukaryotes and prokaryotes origin.[16,18,27] For this study, the effect of cations (Fe^{3+}, Na^{+}, Mn^{2+} and Hg^{2+}) was studied on arginase activity and it was observed that the presence of Fe^{3+} showed a decreasing effect on the activity of the enzyme as the concentrations increased. However, the inhibition showed the least effect at 10 mM, while a drastic effect was noticed at 0.1 mM. The interaction of Na^{+} showed a concentration dependent effect on the stomach arginase with the lowest and highest inhibitions at 5 and 0.1 mM respectively. Conversely, the interaction of manganese (II) chloride and mercury chloride showed increasing effects on the enzyme activity with the lowest and the highest inhibitions at 10 and 0.1 mM, 0.5 and 0.1 mM respectively. This is while, Mn^{2+} has been reported to be a physiological activator of arginase. Okonji et al,.[19] reported that the liver arginase activity from *Heterotis niloticus* showed an increase activation with Mn^{2+}, Zn^{2+}, Mg^{2+}, Hg^{2+}, Co^{2+} and Na^{2+}, while other metals such as Sn^{2+}, Ni^{2+} and Ba^{2+} showed a slight inhibition. Also, Okonji et al.,[20] reported that grasshopper gut arginase activity was strongly enhanced in the presence of Mn^{2+}, Na^{2+}, NH_4^{+} and Hg^{2+} while Mn^{2+} and Zn^{2+} slightly inhibited the enzyme. Furthermore, Okonji et al.,[19] reported that Zn^{2+} and Mg^{2+} strongly enhanced the activity of *Periplaneta americana* gut arginase while Sn^{2+}, Hg^{2+}, Ni^{2+}

and Co^{2+} showed a slight inhibition of the enzyme. Also, Kaysen and Strecker[16] report, which is contrary to Maharem et al.,[11] report of Fe^{2+} and Co^{2+} as good camel liver arginase activators, Sr^{3+} and Zn^{2+} as inhibitors, submitted that Hg^{2+}, Fe^{2+}, $Co^{2+}$and Ni^{2+} were acting as inhibitors, decreasing the rat kidney arginase activity. The effects of other compounds (β-mercaptoethanol, urea and EDTA) were also studied on the cane rat stomach arginase activity. The presence of β-mercaptoethanol showed a decreasing effect on the enzyme activity with the lowest and highest inhibitions at 10 and 0.1 mM respectively. The interaction of urea and EDTA showed a concentration dependent effect on the enzyme activity with the lowest and highest inhibitions at 1 and 2 mM and 5 and 1 mM respectively. EDTA is a chelating compound and has been shown to completely inhibit arginase.[28] The results are similar to Okonji et al.,[20] who reported that grasshopper arginase was strongly inhibited by EDTA. Also, Green et al.,[29] who have worked on *Saccharomyces cerevisiae* arginase reported a strong inhibition of the enzyme by EDTA. In another report, Ehigie et al.,[21] reported that EDTA, citrate and urea strongly inhibit arginase activity from the hepatopancreas of giant freshwater prawn (*Macrobrachium rosenbergii*). The effect of amino acid on arginase from the stomach of cane rat showed that L-histidine, L- proline, L-tyrosine, L-alanine and L-tryptophan had significant percentage inhibitions (89%, 61%, 87%, 87% and 86% respectively) while L-cysteine had moderate inhibition (19%). In explaining the inhibition of arginase activity by amino acids, the liver arginase of teloest fish (*Marluccius gayi)* was also found to be markedly inhibited by branch chain amino acid and especially by isoleucine, leucine, valine, lysine, and proline where they all caused 50% inhibition.[28] Okonji et al.,[30] also reported that L-proline, L-lysine, L-glutamic acid, L-Aspartic acid, L-aspartate, L-Serine and L-valine caused less than 50% inhibition on arginase activity from the liver of *Kinixy erosa* (tortoise). Also, the liver arginase of *H. nitoticus* was slightly inhibited by L-Aspartate and L-Proline[19] as camel liver arginase was inhibited by ornithine, lysine, valine, leucine and isoleucine.[11]

Conclusion

In conclusion, the study established the presence of active arginase in the stomach of cane rat. The overall results from this study provided information on the physicochemical properties of the enzyme, hinting the basic mechanism of its catalytic mechanism. This information could be exploited via enzyme biotechnology in the development of effective control strategy of rodent pests. However, further study including structure function investigations of the enzyme are recommended to fully exploit the potential of this enzyme.

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Authors' Contributions

All the authors have equally contributed to the success of the present work. All authors contributed to the drafting, critical review and editing of the manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflicts interest.

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