



Isolation and kinetic properties of arginase in the gut of grasshopper (*Zonocerus variegatus* Linn)

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ABSTRACT

Arginase (EC 3.5.3.1) catalyzes the hydrolysis of arginine to ornithine and urea. Arginase was purified and characterized from the gut of *Zonocerus variegatus* through DEAE-cellulose and biogel-P100 gel filtration chromatography. The specific activity of the enzyme was 3.7 $\mu\text{moles}/\text{min}$ per mg of protein and a yield of 14.7%. An apparent molecular weight of 143,000 daltons was estimated by gel filtration on biogel P-100. The Michaelis constant (K_m) of the enzyme was 40 mM with arginine as substrate. The optimum pH was 8.0 and the optimum temperature was 40 °C for *Z. variegatus* arginase. The enzyme was stable up to 40 °C for 20 min and lost all of its activity at 80 °C. The enzyme was specific for arginine as substrate. The enzyme was strongly enhanced in the presence of Mn^{2+} , Na^+ , NH_4^+ and Hg^{2+} showed similar activation. Ni^{2+} and Zn^{2+} slightly inhibited *Z. variegatus*. Chelating (EDTA, citrate, ascorbic acid and urea) and thio (2-mercaptoethanol and cysteine) compounds inhibited the activity of arginase in *Z. variegatus*. While amino acids (proline, lysine, aspartate and valine) showed no inhibition on arginase activity. The presence of arginase in the gut of *Zonocerus variegatus* could be for other functions rather than urea production in urea cycle.

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Keywords: Arginase; *Zonocerus variegatus*; Distribution; Physicochemical Properties.

INTRODUCTION

The African variegated grasshopper *Zonocerus variegatus* (L.) (Orthoptera: Pyrgomorphidae), whose pest status was established about 50 years ago (COPR, 1974), feeds on numerous plant species, including the food crops banana, cassava, cocoa, citrus, cowpea and yam (Toye, 1982). However, not all the plant species it consumes contribute to the grasshopper's growth and development (Idowu and Sonde, 2004). For instance, cassava and *Chromolaena odorata* leaves support its growth and development, while *Talinum triangulare* and *Aspilla africana*

leaves do not (Toye, 1982). *Zonocerus variegatus* is a polyphagous species capable of consuming most of the plant species in its surroundings (Toye, 1982), and is reported to consume more than 250 plant species among 71 families (Chiffaud and Mestre, 1990), among which are many crops, including citrus, cocoa, banana, and vegetables, such as cassava.

Arginase (L-arginine aminohydrolase; EC 3.5.3.1) catalyses degradation of L-arginine to L-ornithine and urea, by elimination of guanidine group from arginine molecule (Hwang et al., 2001; Todd et al.,

2001; Sampaleanu et al., 2002; Ash, 2004). It is widely distributed in the biosphere and represented in all primary kingdoms (Jenkinson et al., 1996; Dabir et al., 2005). Arginase is widely distributed in insects, which are generally uricotelic. Despite this, the nutritional requirement of arginine by several species indicates that insects have a limited capacity to synthesize arginine (Pant and Kumar, 1978). Arginase plays a critical role in the hepatic metabolism of many higher organisms as a cardinal component of the urea cycle (Jenkinson et al., 1996). Additionally, it occurs in numerous organisms and tissues where there is no functioning urea cycle (Jenkinson et al., 1996; Yu et al., 2003; Ash, 2004; Mohamed et al., 2005). The detection of arginase practically in all insects, and of arginine, citrulline and ornithine in many, has been made at all stages of development (Pant and Kumar, 1978). However, the nutritional and enzymic studies hitherto made on them have not given any conclusive and unequivocal evidence regarding the operation of a Krebs-Henseleit urea cycle, and it still remains a disputed topic (Pant and Kumar, 1978). Prokaryotes and lower eukaryotes that contain arginase use it for some purpose other than as urea cycle component (Jenkinson et al., 1996). In mammalian liver, arginase functions principally in urea biosynthesis involving cyclic interconversion of ornithine and arginine (Jenkinson et al., 1996; Ash, 2004). The arginase activity present in the fat body tissue of three species of insects (the silkmoths *Hyalophora gloveri* and *Antheraea polyphemus* and the cockroach *Blaberus craniifera*) has been characterized. The aim of this study was to investigate the physicochemical properties of arginase present in the gut of *Zonocerus variegatus* with the goal of further understanding arginase physiological function in this insect.

MATERIALS AND METHODS

Materials

Trizma base, Trizma-HCl, ethylenediamine tetraacetic acid (EDTA),

DEAE Cellulose, p-dimethyl aminobenzaldelyde (Ehrlich reagent), were purchased from Sigma Chemical Company, St. Louis, Mo., USA. Sucrose, sodium chloride, ammonium sulphate (enzyme grade), glacial acetic acid, glycerol, and hydrochloric acid, were purchased from BDH Chemical Limited, Poole England. Acrylamide and N, N¹-methylene bisacrylamide (MBA), ammonium persulphate, N,N,N¹,N¹-tetramethylethylenediamine (TEMED) are from Eastman, Rochester, NY, USA. All other reagents were of analytical grade and were obtained from either Sigma or BDH. *Zonocerus variegatus* were collected from an abandoned farmland at the Ladoko Akintola University of Technology, Ogbomoso, Osun State, Nigeria, between October and November 2010.

Methods

Arginase assay and protein determination

Arginase activity was determined according to the method of Kaysen and Strecker (1973). The reaction mixture contained, in final concentration, 1.0 mM Tris-HCl buffer, pH 9.5 containing 1.0 mM MnCl₂, 0.1 M arginine and 50 µl of the enzyme preparation was added in a final volume of 1.0 ml. The mixture was incubated for 10 minutes at 37 °C. The reaction was terminated by the addition of 2.5 ml Ehrlich reagent (that contains 2.0 g of p-dimethyl aminobenzaldelyde in 20 ml of concentrated hydrochloric acid and made up to 100 ml by adding distilled water).

The optical density reading was taken after 20 minutes at 450 nm. The urea produced was estimated from the urea curve prepared by varying the concentration of urea between 0.1 µmol and 1.0 µmol. The unit of activity of arginase is defined as the amount of enzyme that will produce one µmol of urea per min at 37 °C. The protein concentration was routinely determined by the method of Bradford (1976) using bovine serum albumin (BSA) as standard.

Tissue extraction and purification

All operations were carried out at temperatures between 0 and 37 °C. Centrifugations were done at 10 °C using IEC-DPR Cold Centrifuge. All buffers contained 1.0 mM MnCl₂ (Kaysen and Strecker, 1973). pH measurement was made at 25 °C using Mettler MP200 Digital pH Meter.

The frozen grasshopper was allowed to thaw at room temperature and weighed. A total of 125 g (wet weight) of tissue was used for this preparation. The weighed mass of grasshopper was minced and homogenized in a Warring Blender for 1 min in nine volumes (v/w) of the homogenization buffer containing 0.25 M sucrose and 0.01 M MnCl₂ in 0.01 M Tris-HCl buffer, pH 7.5. The homogenate obtained was stirred, occasionally, for one hour at 4 °C and then subjected to centrifugation at 6,000 rpm at 10 °C for 30 min. The supernatant was filtered through a double layer of cheesecloth. The cellular debris was resuspended in one volume of the homogenization buffer, homogenized in the Warring Blender and centrifuged under the same condition. The supernatant obtained was combined with that from the first centrifugation.

DEAE-cellulose ion exchange chromatography

A column (2.5 x 40 cm) of treated DEAE-Cellulose was packed and equilibrated with 5 mM Tris-HCl buffer, pH 7.2. The extract from the preceding step was then layered on the column. The column was first washed with 5 mM Tris-HCl buffer, pH 7.2 to remove unbound proteins, followed by elution with a 400 ml linear gradient of 0-1 M NaCl in 5 mM Tris-HCl buffer, pH 7.2. Fractions of 5 ml were collected from the column that was maintained at a flow rate of 30 ml per hour. Protein was monitored spectrophotometrically at 280 nm. The fractions were also assayed for arginase activity. The active fractions were pooled and stored in the freezer.

Gel filtration on biogel P-100

Biogel P-100 gel filtration resin was washed with several changes of 5 mM Tris-

HCl buffer, pH 7.5. This was then packed into the column (2.5 x 90) and equilibrated with 5 mM Tris-HCl buffer, pH 7.5. The enzyme fraction from the preceding step was then applied to the biogel P-100. Fractions of 4 ml were collected at a flow rate of 10 ml per hour. Protein was monitored spectrophotometrically at 280 nm. The fractions were also assayed for arginase activity. The active fractions were pooled and stored in the freezer.

Molecular weight determination

The native molecular weight was determined on a biogel P-100 column (2.5 x 90 cm). The standard proteins were α -chymotrypsinogen A (M_r 24,000; 10 mg), ovalbumin (M_r 45,000; 10 mg/ml), bovine serum albumin (M_r 67,000; 10 mg/ml), creatinine phosphokinase (M_r 88,000; 10 mg/ml) and bovine arginase (M_r 120,000; 10 mg/ml).

Determination of kinetic parameters

The kinetic parameters (V_{max} and K_m) of the enzyme were determined according to Kaysen and Strecker (1973). The K_m of arginine was determined by varying the concentration of arginine between 40 mM and 150 mM in 2 mM Tris-HCl buffer, pH 9.5, in the presence of 0.01 mM MnCl₂. The kinetic parameters were determined from the double reciprocal plot. Lines through the points were drawn by the method of regression.

Substrate specificity studies

The substrate specificity of grasshopper arginase was investigated by testing its activity towards structurally similar compounds with guanidino group. The compounds include L-arginine, L-argininemono-hydrochloride and guanidine chloride. The solutions of above compounds (30 mM) were prepared in 2 mM Tris-HCl buffer, pH 7.2. Enzyme activity was measured by spectrophotometric detection of urea as described by Kaysen and Strecker (1973). The reaction mixture contained, in final concentration, 1.0 mM Tris-HCl buffer, pH 9.5 containing 1.0 mM MnCl₂, 30 mM solution of the substrates and 50 μ l of the

enzyme preparation was added in a final volume of 1.0 ml.

Effect of temperature

The activity of arginase was assayed at temperatures between 30 °C and 100 °C to investigate the effect of temperature on the activity of the enzyme. The assay mixture was first incubated at the indicated temperature for 10 min before the reaction was initiated by the addition of 50 µl of the enzyme that had been equilibrated at the same temperature.

For the effect of heat on the stability of arginase, a 1 ml aliquot of the enzyme solution was incubated at 30 °C, 40 °C, 50 °C, 60 °C, 70 °C, 80 °C and 90 °C. 50 µl aliquot was withdrawn and assayed for residual activity at the indicated time. A plot of percentage residual activity against incubation time was made to determine the stability of the enzyme.

Effect of pH on the enzyme activity

The effect of pH on arginase was studied by assaying the enzyme using the following buffers of different pH values: 5 mM citrate buffer (pH 5.0-6.5), 5 mM phosphate (pH 6.5-8.0), 5 mM Tris-HCl buffer (pH 8.0-10.0). One milliliter (ml) of the reaction mixture contained 2.5 mM of the appropriate buffer, 0.1 M arginine and 0.02 ml enzyme preparation.

Effects of inhibitors on the activity of arginase from *Z. variegatus*

The inhibitory effects of amino acids on the activity of arginase were investigated on the enzyme with arginine as substrate. The effects of five amino acids on the activity of *Z. variegatus* arginase were studied. The amino acids used for the study includes L-lysine, L-valine, L-proline, L-aspartic acid, and L-arginine. The amino acids (25 mM) were used as inhibitors with the routine assay procedure.

The inhibitory effects of chelating compounds (EDTA, citrate and ascorbic acid) were studied. Enzyme preparation was incubated with 50 mM of the required chelating compound at pH 7.5 at 37°C for 30 min and aliquot tested for arginase activity.

Also the effects of 2-mercaptoethanol and cysteine were investigated by incubating 5 µM each of the thiol compounds with 0.1 ml of the enzyme solution for 1 hr. Aliquot was taken out for arginase assay.

RESULTS

Enzyme purification

The summary of purification is shown in Table 1. The elution profiles of the chromatographic step of the procedure are shown in Figures 1 and 2. The calibration curve on biogel P-100 for native molecular weight determination is shown in Figure 3. Gel filtration on biogel P-100 resulted in a molecular weight estimate of approximately 143,000 daltons.

Kinetic parameters

The Lineweaver-Burk plot for the reciprocal of initial reaction velocity versus reciprocal of the concentration of arginine is shown in Figure 3. Figures 4 and 5 shows the Lineweaver-Burk plot for the reciprocal of initial reaction velocity versus reciprocal of the concentration of guanidine chloride and arginine monohydrochloride respectively. A summary of the true Michaelis constants, K_m obtained, as well as the maximum velocity, V_{max} values are presented in Table 2.

Effect of pH on the activity of *Zonocerus variegatus* arginase

The activity of grasshopper arginase was determined in the buffer pH range from 6 to 11 at 37 °C. The optimum pH of grasshopper arginase was at pH 8.0 (Figure 6) in the presence of $MnCl_2$.

Effect of temperature on the activity of *Zonocerus variegatus* arginase

The activity of grasshopper arginase was assayed at temperatures between 30°C and 100°C. The optimum temperature of the enzyme was found to be 40 °C at pH 9.5 (Figure 7). The effect of heat on the stability of arginase expressed as percentage is presented in Figure 8. The enzyme was

inactivated at temperature above 40 °C. The inactivation being more pronounced with increasing incubation temperature. At 80 °C, the enzyme lost about 85% of its original activity within 10 minutes of incubation (Figure 8).

Effect of metal ions on the activity of *Zonocerus variegatus* arginase

The effect of metal ions is presented in Table 3. The activity of arginase in *Z. variegatus* was strongly enhanced in the presence of Mn²⁺. Na⁺, NH₄⁺ and Hg²⁺ also showed similar activation of the enzyme. Ni²⁺ and Zn²⁺ slightly inhibited *Z. variegatus* arginase

Effect of chelating and thio compounds on the activity of *Zonocerus variegatus* arginase

Table 4 shows the effects of chelating (EDTA, citrate, ascorbic acid and urea) and thio (2-mercaptoethanol and cystein) compounds on the activity of arginase in *Z. variegatus*. The compounds showed complete inhibition of the enzyme.

Effect of amino acids on the activity of *Zonocerus variegatus* arginase

The effect of the amino acids is presented in Table 5. The activity of arginase was not affected by the two different concentrations of the amino acids.

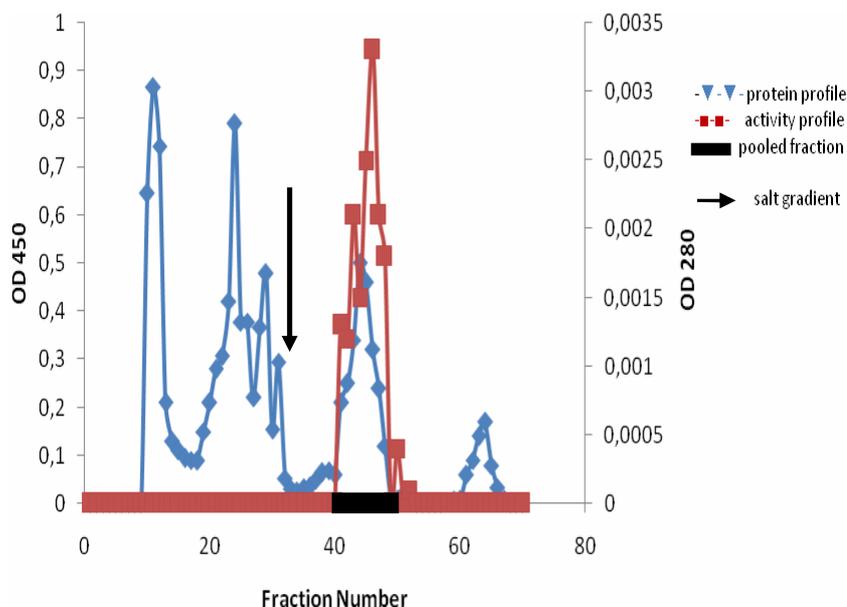


Figure 1: DEAE-cellulose ion exchange chromatography of *Z. variegatus* arginase. The column was first washed with 100 ml 0.1 M Tris-HCl buffer, pH 7.2. The enzyme solution was layered on the packed column and eluted with a 200 ml linear gradient of 0-1 M NaCl in 0.1 M Tris-HCl buffer, pH 7.2. Fractions of 5 ml were collected from the column.

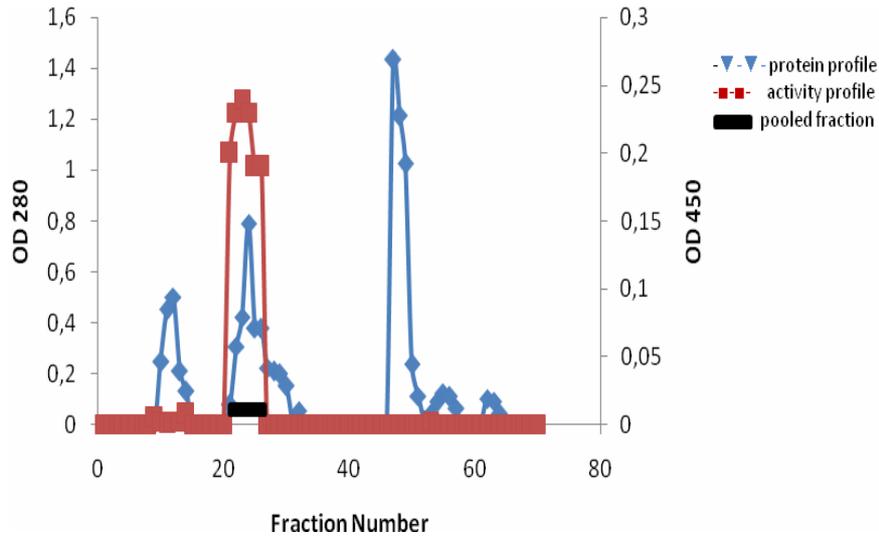


Figure 2: Gel filtration chromatography on biogel P-200 of *Z. variegatus* arginase. The column (2.5 × 90) was first equilibrated with 5 mM Tris-HCl buffer, pH 7.5. The enzyme fraction from the preceding step was then applied to the column. Fractions of 4 ml were collected at a flow rate of 10 ml per hour.

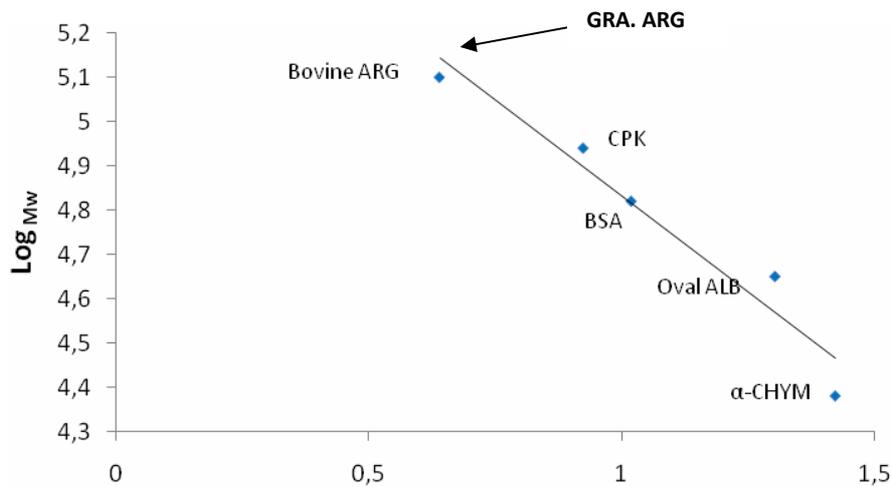


Figure 3: Calibration curve on biogel P-100 for native molecular weight determination of *Z. variegatus* arginase. The native molecular weight was determined on a Biogel P-100 column (2.5 × 90 cm). The standard proteins as indicated on the curve are α-CHYM (α-chymotrypsinogen A), Oval ALB (ovalbumin), BSA (bovine serum albumin), CPK (creatinine phosphokinase) and Bovine ARG (bovine arginase). Grasshopper arginase (GRA. ARG) is indicated by the arrow.

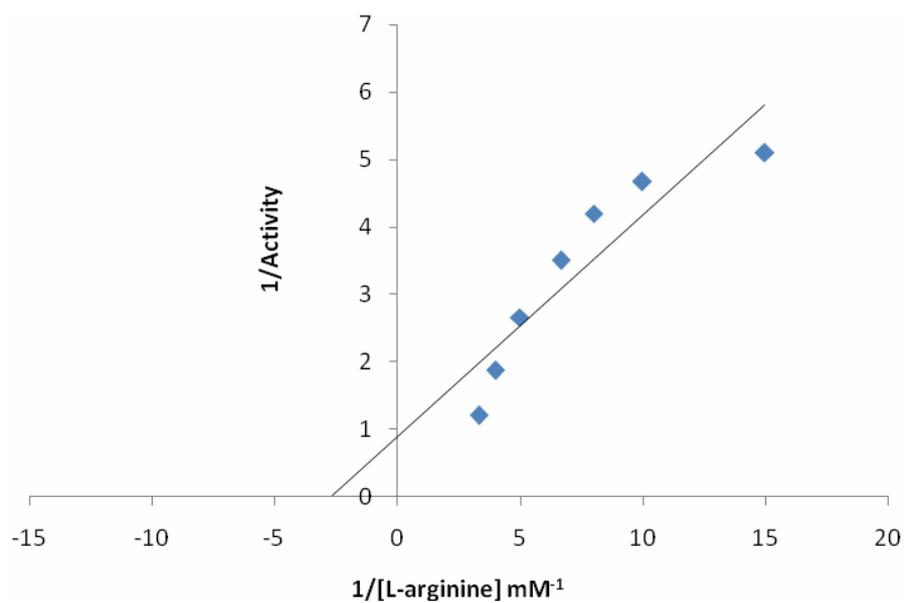


Figure 4: Lineweaver-Burk Plot. The K_m of arginine was determined by varying the concentration of L-arginine between 40 mM and 150 mM in 2 mM Tris-HCl buffer, pH 9.5, in the presence of 0.01 mM $MnCl_2$.

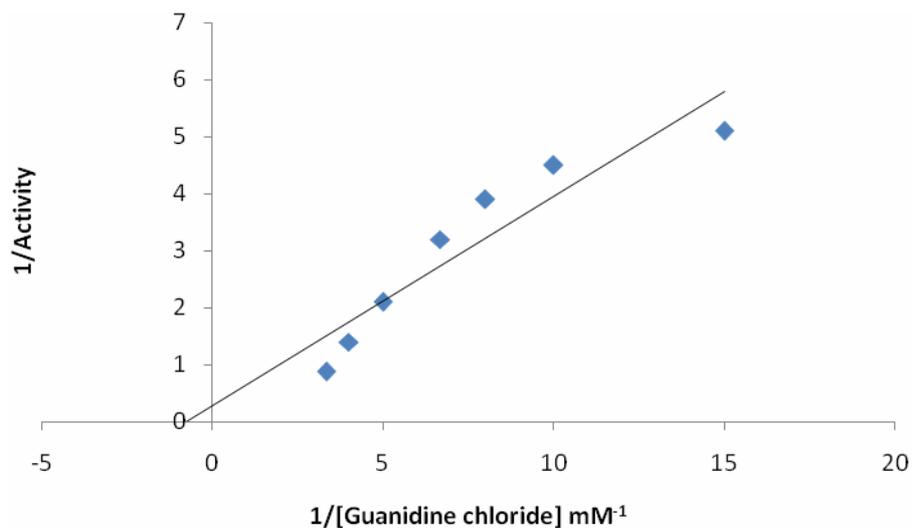


Figure 5: Lineweaver-Burk Plot. The K_m of arginine was determined by varying the concentration of guanidine chloride between 40 mM and 150 mM in 2 mM Tris-HCl buffer, pH 9.5, in the presence of 0.01 mM $MnCl_2$.

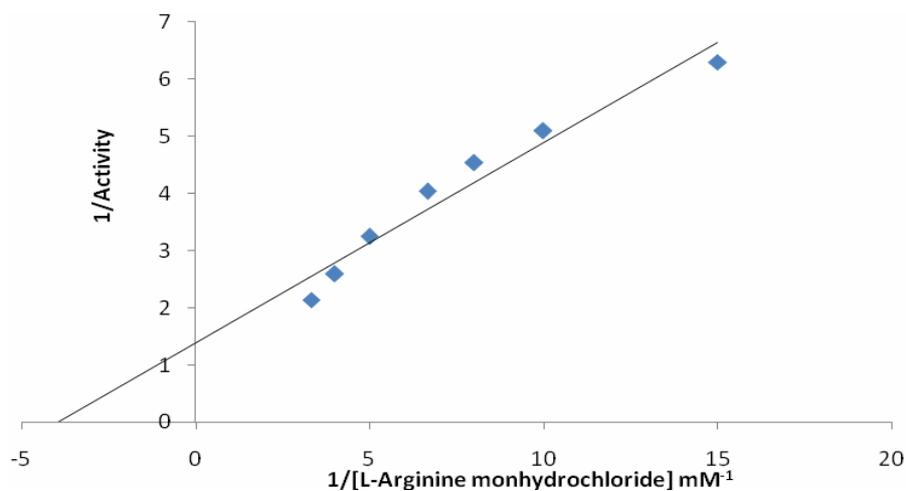


Figure 6: Lineweaver-Burk Plot. The K_m of arginine was determined by varying the concentration of L-arginine monohydrochloride between 40 mM and 150 mM in 2 mM Tris-HCl buffer, pH 9.5, in the presence of 0.01 mM $MnCl_2$.

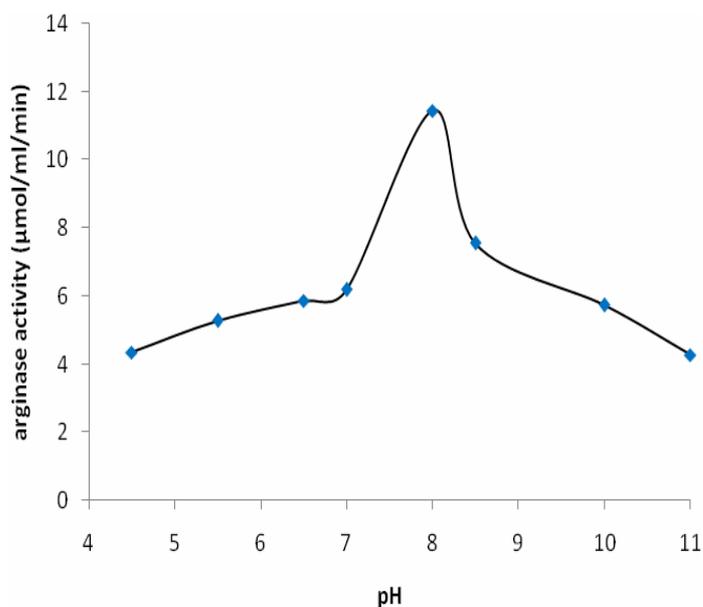


Figure 7: Effect of pH on the *Z. variegatus* arginase activity. One ml of the reaction mixture contained 2.5 mM of the appropriate buffer, 0.1 M arginine and 0.02 ml enzyme preparation. The following buffers of different pH values were used for the pH determination: 5 mM citrate buffer (pH 6.0-6.5), 5 mM phosphate (pH 6.5-8.0), 5 mM Tris-HCl buffer (pH 8.0-11.0).

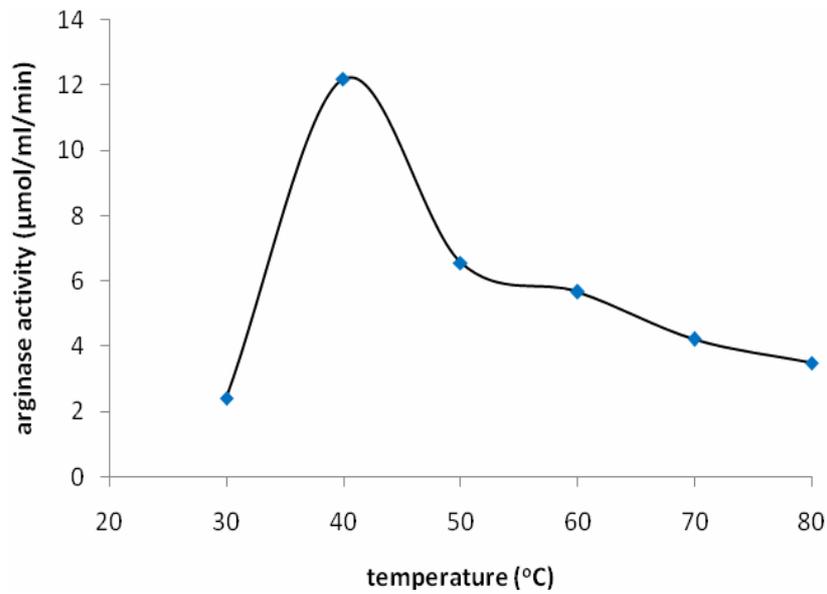


Figure 8: The activity-temperature profile showing the optimum temperature. The activity of *Z. variegatus* arginase was assayed at temperatures between 30°C and 100°C. The assay mixture containing 50 µl was first incubated at the indicated temperature for 10 min before initiating the reaction by the addition of an aliquot of the enzyme.

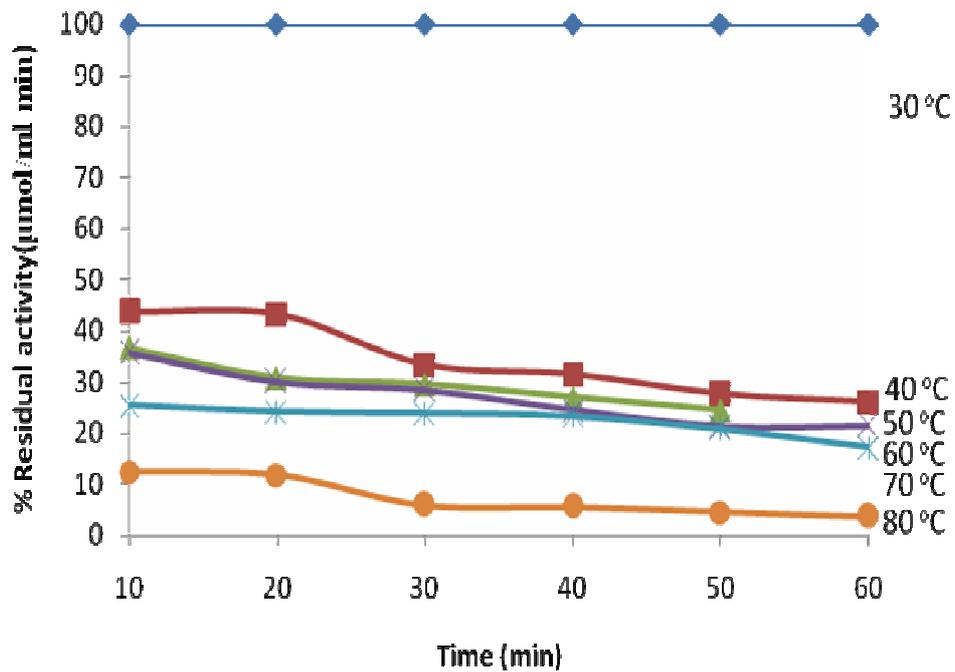


Figure 9: Effect of temperature on the stability of *Z. variegatus* arginase. An enzyme solution was incubated at the desired temperature and aliquots were withdrawn and assayed for residual activity at the various indicated time. The activities were expressed as percentage.

Table 1: Summary of purification of *Z. variegatus* arginase.

Fractions	Total Volume (ml)	Total Activity (Unit)	Total protein (mg)	Specific Activity (Unit/mg)	Purification Fold	% Yield
Crude Extract	320	12,410	9546.0	1.3	1	100
Ion exchange Extract	175	8692	5216.8	1.7	1.3	54.6
Gel Filtration	65	5211	1403.4	3.7	3.0	14.7

Table 2: Summary of kinetic properties of different substrate for *Z. variegatus* arginase.

	Arginine	Guanidine Chloride	L-Arginine monohydrochloride
K_m	40	100	250
V_{max}	11	25	6.7

Table 3: Effect of metal ion on *Z. variegatus* arginase activity.

Metal ions	500 μ M
Zn ²⁺	65.2
NH ⁴⁺	100
Hg ²⁺	97.6
Na ⁺	100
Ni ⁺	80.5
Mn ²⁺	100

Table 4: Inhibition effects of chelating and thio compounds on *Z. variegatus* arginase.

Chelating/Thio Compounds	% Residual activity
EDTA	20.1
Citrate	17.3
Ascorbic acid	14.8
Urea	22.7
2-Mercaptoethanol	31.5
Cystein	28.2

Table 5: Effects of amino acids on the activity of *Z. variegatus* arginase

Amino acids	% Residual Activity	
	50 mM	100 mM
Proline	98	95
Lysine	91	91
Aspartate	93	90
Valine	86	79
Arginine	100	100

DISCUSSION

Arginase is an enzyme that is ubiquitous in nature and has been identified and studied for several decades in a large number of organisms (Jenkinson et al., 1996; Ash et al., 2000; Dabir et al., 2005; Mohamed et al., 2005; Scaraffia et al., 2008). Traniello et al. (1975) found two arginases in chicken kidney while Herzfeld and Raper (1976) reported the heterogeneity of arginase in rat tissues. A variety of functions has been proposed for the extrahepatic Type 2 arginase which includes the synthesis of ornithine for the production of glutamate and proline, control of cellular level of arginine and ornithine and synthesis of polyamines. New functions are being proposed for the arginase in different environments and organisms. Examples include its involvement in bulb growth and sprouting in plants, normal mammary gland development in rat, ammonia detoxification, hormone secretion, immune modulation and cellular replication in humans (Jenkinson et al., 1996; Shi et al., 2001; Todd et al., 2001). In vertebrates, as well as in insects, uric acid is produced by degradation of purines (Scaraffia et al., 2008). The production of urea in insects has been attributed to arginase, which catalyzes the hydrolysis of arginine to form urea and ornithine. However, unlike in vertebrates, where arginine is generated in the urea cycle, the action of arginase in insects is limited to arginine from dietary sources or from endogenous protein turnover (Scaraffia et al., 2008). This is because insects lack one or

more genes encoding enzymes required for the urea cycle (Jenkinson et al., 1996; Scaraffia et al., 2008).

The specific activity of the arginase from *Z. variegatus* was 3.4 $\mu\text{mole}/\text{min}/\text{mg}$ of protein. The enzyme was purified through DEAE-Cellulose ion exchange chromatography and biogel P-100. Different specific activity values have been reported for non hepatic type 2 arginase from different sources. In *Fasciola gigantical*, arginase had very high activity compared to the other urea cycle enzymes (Mohamed et al., 2005). Molecular weight of *Z. variegatus* arginase was 143,000 dalton as determined by gel filtration. The molecular weight of yeast arginase was estimated to be 120,000 by Sephadex G-200 gel filtration (Chan and Cossins, 1973). The molecular weight of arginase II in *F. gigantical* as determined by gel filtration and SDS-PAGE, was 92,000 (Mohamed et al., 2005). Reddy and Campbell (1970) used gel filtration and density gradient centrifugation to estimate the molecular weight of arginase from the land planarian *Bipalium kewense*, land snail *Helix aspersa* and the laughing gull *Larus atricilla*. The molecular weight of arginases from land planarian and land snail was approximately 240,000, while that from the gull was 120,000. Invertebrate arginases occur as large multimers (Mohamed et al., 2005). Arginase from human malaria parasite *Plasmodium falciparum* is a homotrimer of 160,000 dalton. Dialysis of the arginase against EDTA results in monomers of approximately 48,000 dalton

(Muller et al., 2005). In contrast, small monomeric arginases of about 27,000 dalton have been encountered in the gut of the earthworms *Lumbricus terrestris*. A consideration of the known molecular weights of arginases from different species indicates there is no general correlation between the molecular weight of arginase from a given species and that species' excretory nitrogen metabolism.

The Michealis constant for arginine was 40 mM for *Z. variegatus* gut arginase. This value was in the range of the K_m s of invertebrate arginases which vary widely between 2 mM in silkmoths and crayfish and up to 158 mM in a polychaete annelid (Porembaska, 1973; O'Malley and Terwilliger, 1974; Jenkinson et al., 1996; Dabir et al., 2005). The apparent Michaelis constant of 12.5 mM and 250 mM were obtained for arginine in two buffer systems (glycinate and phosphate buffers respectively) (Chan and Cossins, 1973). Mohamed et al. (2005) reported a K_m of 6 mM for *F. gigantical*. The K_m value of *Vigna catjang* cotyledon arginase was 42 K_m and is in the range of other reported K_m (40-83 mM) for plant arginases (Dabir et al., 2005). Arginine is a common substrate for the enzyme arginase (Jenkinson et al., 1996; Ensunsa et al., 2004). The activity of *Z. variegatus* arginase was highly specific for arginine. Studies have shown different arginine analogs to be good substrates for arginase; most arginases reported show preference for arginine as substrates (Jenkinson et al., 1996). Reczkowski and Ash (1992) reported the activity of guanidino compounds as alternate substrates for rat liver arginase and its dependent on the length of the amino acid side chain. The arginase present in the livers of uricotelic and ureotelic animals has similar substrate specificity (Mohamed et al., 2005).

The *Z. variegatus* enzyme exhibited a maximal activity at pH 8.0. *F. gigantical* showed maximum activity at pH 9.5 (Mohamed et al., 2005) while the pH optimum of *H. niloticus* was 5.5, the purified arginase

of *Helicobacter pylori* had an acidic pH optimum of 6.1 (McGee et al., 2004). A pH of 10.0 was reported for *Vigna catjang* cotyledon arginase (Dabir et al., 2005). Acid-base status influences many aspects of insect biology, including insect distributions in aquatic systems, insect-plant and insect-pathogen interactions, membrane transport phenomena, and the mode of action of pesticides. Acid-base status in the hemolymph and gut lumen of insects is generally well regulated. The pH of the midgut lumen varies with the phylogeny and feeding ecology (Harrison, 2001). Insect fluids have buffer values similar to those of vertebrates. Evidence to date suggests that the dominant mechanisms for control of renal acid-base excretion involve hormonal regulation of H^+ -V-ATPase activity (Punzo, 1988; Harrison, 2001).

Optimum temperature was recorded for *Z. variegatus* arginase at 40 °C. Similar result was reported for *F. gigantical* (Mohamed et al., 2005). They further reported the stability of the enzyme at 40 °C for 15 min and at 50 °C, the activity of the enzyme was completely lost. Similarly, arginase from fresh water teleostean fish *Clarias batrachus* was found to be stable between 10 and 37 °C, its activity was decreased at higher temperature and showed no activity at 80 °C (Singh and Singh, 1988). For mammals, the optimum temperature for human vitreous humor arginase was 40 °C (Gursu, 2001). Furthermore, the enzymes in ectothermic animals such as *Z. variegates* and *Periplanata americana* have been adapted through natural selection to function at the range of temperatures experienced by these organisms. In general, the reaction rate of an enzyme catalyzed reaction increases by a factor of 2-3 for each 10 °C rise in temperature. Above about 40 degrees Celsius, enzymes will start to denature (unfold) and become non-functional (Diekman and Ritzmann, 1987).

Arginase is a metalloenzyme in which manganese acts as a cofactor as well as activator in almost all reported arginases (Ash, 2004; Dabir et al., 2005). Most arginases are

activated by manganese (Jenkinson et al., 1996; Ensunsa et al., 2004; Mohamed et al., 2005). The effect of metals on *Z. variegatus* was similar to most of the inhibitory effects reported for arginases. Mn^{2+} , N^{+} and NH_4^{+} strongly activated the *Z. variegatus* enzyme where Hg^{2+} , Ni^{2+} and Zn^{2+} showed slight inhibition on the enzyme. Dabir et al. (2005) reported that replacement of Mn^{2+} ion with other metal ions such as Mg^{2+} , Co^{2+} , Ni^{2+} and Fe^{2+} restored $\geq 50\%$ activity, whereas Ca^{2+} , Zn^{2+} and Cd^{2+} inhibited *Vigna catjang* cotyledon arginase. In buffalo liver arginase, Mg^{2+} activated the enzyme, but was inhibited by Ca^{2+} , Ni^{2+} , Co^{2+} , Cd^{2+} , Fe^{2+} and Zn^{2+} (Dabir et al., 2005). In *F. gigantical*, arginase enzyme was activated by Mn^{2+} , but was inhibited by Fe^{2+} , Ca^{2+} , Hg^{2+} , Ni^{2+} , Co^{2+} and Mg^{2+} (Mohamed et al., 2005). Arginase activity was completely inhibited by the presence of EDTA, citrate, ascorbic acid, and urea. Thio compounds (2-mercaptoethanol and Cystein) also inhibited the enzyme.

In our study, amino acids did not show any inhibitory effect, rather the arginase activity in the gut of *Z. variegatus* was enhanced. The presence of arginase and its high activity in the gut of *Z. variegatus* may suggest a major role for arginase in proline formation. Our finding is not uncommon with results obtained for the enzyme in other sources. Arginase was reported to have increased during adult development in winged adult moth; this was associated with increase in proline concentration, a major substrate utilized in insect flight metabolism (Reddy and Campbell, 1969). Proline has also been ascribed another function as an energy reservoir in insect flight muscle, where it is rapidly utilized during flight (Pant and Kumar, 1978, Matthias and Gerd, 2009).

In conclusion, the presence of arginase in the gut of *Zonocerus variegatus* could be for other functions rather than urea production in urea cycle. Insect arginase shows most of the properties characteristic of arginases from

other animal and plant species. Both differences and similarities exist between it and the arginase present in uricotelic vertebrates.

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