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Properties of arginase from gut of adult cockroach (*Periplaneta americana*)

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Arginase (EC 3.5.3.1) catalyzes the hydrolysis of arginine to ornithine and urea. The role of arginase in insect has been seen in the growth of insect at various stages of development and energy requirement for flight. Here, we reported the properties of arginase in the gut of adult cockroach, *Periplaneta americana*. The enzyme was partially purified with ammonium sulphate precipitation and affinity chromatography. The specific activity of the arginase was 3.0 µmol/min/mg of protein. The Michealis (K_m) constant was 0.33 mM and the arginase preferred arginine as substrate. The optimum pH was 7.0, while the optimum temperature was 80°C. The ascorbic acid, reduced glutathione (GSH) and 2-mercaptoethanol completely inactivated the enzyme. The amino acids: lysine, valine, serine, and asparagine showed moderate inhibition (with residual arginase activities of 81.7, 96.1, 96.9 and 97.0%, respectively), while proline and cysteine (>100%, respectively) stimulated the arginase activity. The cations: Sn^{2+}, Hg^{2+}, Ni^{2+} and Co^{2+} showed slight inhibition on the enzyme. The enzyme was markedly enhanced by Zn^{2+} and Mg^{2+}.

Key words: Arginase, insect gut, cockcroach, characterization.

INTRODUCTION

Arginase (EC 3.5.3.1), a widely distributed enzyme has been studied in various organisms and is endowed with numerous functional properties (Gao et al., 1982; Lisowska et al., 1987; Jenkinson et al., 1996). In higher animals, it is the key enzyme in the urea cycle. In birds, reptiles and fishes, arginase has been shown to have different metabolic roles. In insects as well as birds which have been known to be uricotelic, the existence of urea cycle is still controversial (Gao et al., 1982; Lisowska et al., 1987; Jenkinson et al., 1996). Arginase catalyzes the hydrolysis of arginine to ornithine and urea. Two arginases A and B were reportedly produced from developing embryos of the tick *Hyalomma dromedarii* (Fahmy et al., 1994). Arginase from the flatworm *Fasciola gigantica* has been described by Mohamed et al. (2005) and two arginases: arginases I and II were reported to be present in the organism. Nagoaka et al. (2011) have also cloned two arginase cDNAs from the silkworm, *Bombyx mori*. Their work also revealed that two mRNAs named *bmarg-r* and *bmarg-f* were generated from a single gene. In plants, arginase has been reported to play various physiological and metabolic roles which include nitrogen

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mobilization during development of fruits, bulbs and tubers, seed germination, biosynthesis of glutamine and polyamines (Jenkinson et al., 1996; Dabir et al., 2005; Sempruch et al., 2008).

American cockroach, *Periplaneta americana* L., is a peridomestic cockroach. The insects are pest to human as they infest their environment (Kishore et al., 2010) can become a public health problem due to its association with human waste and disease, and its ability to move from sewers into homes and commercial establishments. *P. americana* has been extensively studied due to its economical and medical importance (Zungoli and Robinson, 1984; Fotedar et al., 1991; Smithers and Ramsey, 2001; Salehzadeh et al., 2007). The American cockroach is omnivorous eating books, putrid sake, cloth and dead insects, various products in the kitchen and many places (Kishore et al., 2010). Several control methods to eradicate cockroaches have been tried; they range from different pesticides and chemicals (Olkowski et al., 1991; Baldwin et al., 2008; Kishore et al., 2010). Ammonia was also used by Kishore et al. (2010) as a pesticide for the eradication of cockroaches. The involvement of arginase in the developmental growth and energy generation and utilization in insects has also been described (Brown, 1966; Reddy and Campbell, 1969). Therefore, the understanding of the properties of arginase in cockroach could help in the control of the insect. The present investigation describes the properties of arginase in the gut of adult cockroach, *P. americana*.

MATERIALS AND METHODS

Materials

Dimethylaminobenzaldehyde, ammonium sulphate, urea, manganese (II) chloride, Tris (hydroxylmethyl) aminomethane (Tris-base) were purchased from Sigma Chemicals, USA. All reagents were of analytical grade and were obtained from Sigma Chemicals or BDH Poole House, England). Cockroaches were obtained from the Insect Physiology Laboratory of Department of Crop Protection and Production, Faculty of Agriculture, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

Methods

Collection of insect and enzyme extraction

Freshly emerged (0-3 days old) adult male and female cockroaches obtained from a colony kept in the laboratory under room temperature were used for the bioassays. The gut from the abdominal region (24.1 g) of the adult cockroach was obtained after dissecting the insect and kept in the refrigerator until required. After allowing thawing, the tissue was homogenized in three volumes of homogenization buffer containing 5.0 mM Tris-HCl pH 7.5. This was followed by centrifugation for 30 min at 4000 rpm.

Enzyme assay

Arginase activity was determined by the measurement of urea produced by the reaction with Ehrlich reagent according to the modified method of Kaysen and Strecker (1973). The reaction mixture which contained, in final concentration, 0.33 mM arginine solution, 2.0 mM Tris-Hcl buffer (pH 9.5) containing 2.0 mM manganese chloride and 0.05 ml of the enzyme preparation was added in a final volume of 1 ml. The mixture was incubated for 10 min at 37°C. The reaction was terminated by the addition of 2.5 ml of Ehrlich reagent (containing 2.0 g of p-dimethyl-amino-benzaldehyde 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 min at a wavelength of 450 nm. The urea produced was estimated from the urea curve prepared by varying the concentration of urea between 0.1 and 1.0 µmol, and a graph of optical density against urea concentration was plotted for extrapolation of the arginase activity. One unit of arginase activity was defined as the amount of arginase that will form 1 µmol of urea per unit volume per minute. The protein concentration was determined according to the method described by Bradford (1976) using bovine serum albumin (BSA) as the standard.

Ammonium sulphate precipitation

The supernatant collected above was brought to 80% ammonium sulphate saturation by the addition of appropriate amount of ammonium sulphate (472 g/L), which was added slowly with occasional stirring; this was left overnight. The precipitate was collected by centrifugation at 4000 rpm for 20 min. The precipitated protein was then dialysed against 5.0 mM Tris buffer, pH 7.2 for 4 h with hourly change of the buffer.

Reactive blue-2-agrose affinity chromatography

The column (1.5 × 10 cm) was packed with pre-treated Reactive Blue-2- Agarose resin. The column was equilibrated with the 5.0 mM Tris-HCl buffer (pH 7.2) and then 1.0 ml of enzyme solution was layered on the column. The 5 mM Tris-HCl buffer (pH 7.2) was used to elute the unbound protein before a gradient elution with 1.0 M NaCl was introduce to elute the bound protein. Fractions of 1.0 ml each were collected from the column at the rate of 20 ml per hour. The activity and protein concentration of the enzyme in the collected fractions were then assayed.

Determination of kinetic parameter

The kinetic parameters (K_m and V_{max}) of the enzyme were determined according to Kaysen and Strecker (1973). The K_m of arginine was determined by varying the concentration of the L-arginine between 45 and 300 mM. Both K_m and V_{max} were determined using double reciprocal plot of Lineweaver and Burk (1934).

Effect of pH

The effect of pH on adult cockroach gut arginase was carried out by assaying the enzyme using different pH buffers. The pH buffers used include: 0.1 M citrate buffer pH (3.0-6.0); 0.1 M Tris-HCl buffer pH (7.0-10.0) in a typical assay method.

Effect of temperature

To investigate the effect of temperature on the enzyme activity, the enzyme was assayed at the temperature ranges between 40 and 100°C. The assay mixture was incubated at the indicated temperature.

Table 1. Summary of purification of gut of *P. americana* arginase.

Fraction	Volume (ml)	Total activity (U)	Total protein (mg)	Specific activity	Yield (%)
Crude extract	73	19, 300	33, 244.2	0.46	100
80% Ammonium sulphate precipitation	64	23, 040	21, 268.7	1.08	63.98
Affinity chromatography	193	19, 300	6, 441.0	3.0	19.4

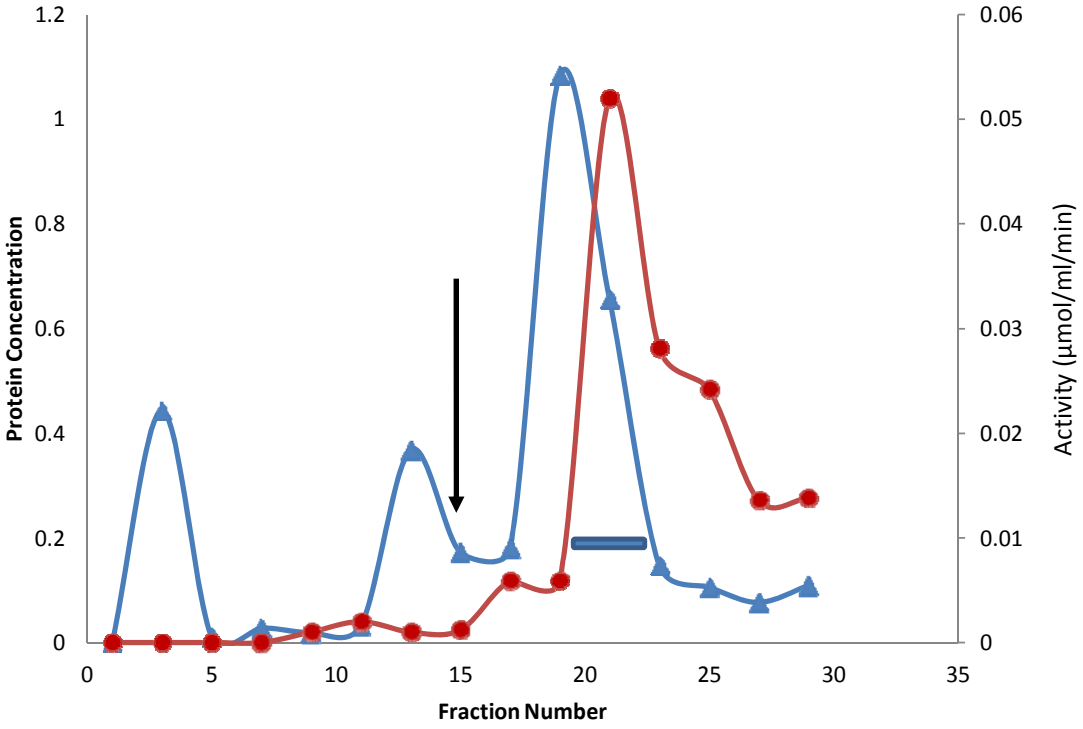

Figure 1. Affinity Chromatography of *P. americana* arginase. The column was first washed with 100 ml 0.1 M Tris-HCl buffer, pH 7.2. The enzyme solution was layered on the packed column and eluted with a 100 ml linear gradient of 0-1 M NaCl in 0.1 M Tris-HCl buffer, pH 7.2. Fractions of 1.0 ml were collected from the column. Red Line= OD 450 nm (-●-●-●-●-); Blue Line=OD 595 nm (-▲-▲-▲-▲-) ⟶ = Point of gradient elution.

The effect of divalent cations on arginase activity was determined. The cations include Zn^{2+}, Hg^{2+}, Sn^{2+}, Co^{2+}, Ni^{2+} and Mg^{2+}. The typical enzyme assay contained 0.01 mM of each cation.

Effect of reducing agents

The following reducing agents were used in the experiment: ascorbic acid, 2-mercaptoethanol and reduced glutathione. A concentration of 0.01 mM of each compound was contained in a typical assay mixture.

Effect of amino acids

The enzyme was assayed by the Kaysen and Strecker (1973) method. 0.05 mM of each of the following amino acids: valine (Val), asparagine (Asn) and cysteine (Cys), proline (Pro), serine (Ser) and lysine (Lys) were contained in the typical enzyme assay mixture.

RESULTS

Enzyme purification

The purification procedure of arginase from *P. americana* has being described previously. The procedure yielded arginase with specific activity of 3.0 µmoles/min/mg of protein and a yield of 19.4% (Table 1). The elution profile of the chromatographic step is shown in Figure 1.

Kinetic parameters

The Lineweaver-Burk plot for the reciprocal of initial reaction velocity versus reciprocal of the concentration of arginine is shown in Figure 2. The Michealis-Menton constant obtained from the curve was 0.33 mM.

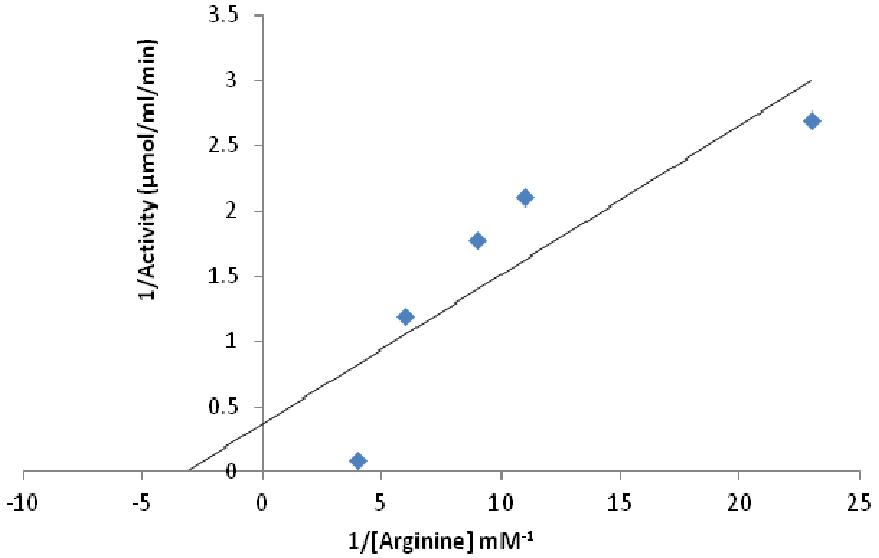

Figure 2. Lineweaver-Burk plot of *P. americana* gut arginase. The K_m of arginine was determined by varying the concentration of L-arginine between 45 and 300 mM in 2 mM Tris-HCl buffer, pH 9.5, in the presence of 0.01 mM $MnCl_2$. Enzyme activity is expressed in micromole per minute.

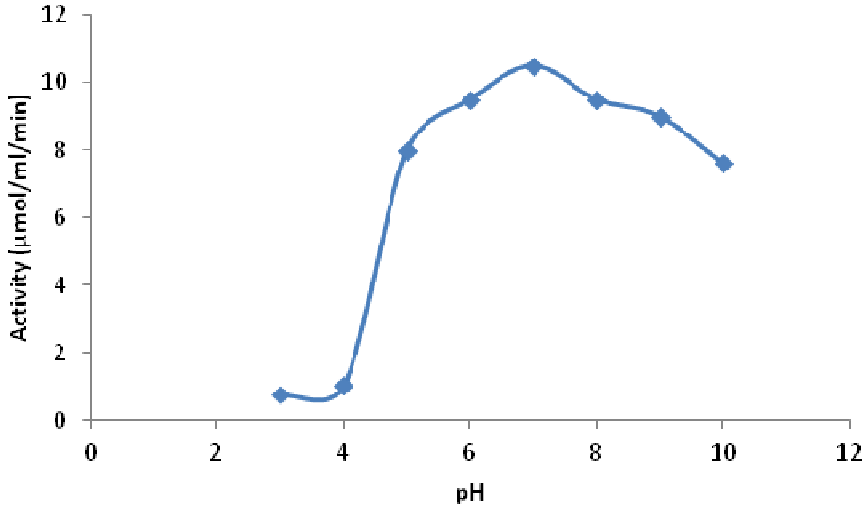

Figure 3. Effect of pH on *P. americana* arginase activity. One ml of the reaction mixture contained 2.5 mM of the appropriate buffer, 0.1 M arginine and 0.02 ml enzyme preparation.

Effect of pH

The activity of *P. americana* arginase was determined in the assay buffer pH range from 6 to 10 at 37°C. The optimum pH of *P. americana* arginase was at pH 7.0 (Figure 3) in the presence of $MnCl_2$.

Effect of temperature

The activity of *P. americana* arginase was assayed at temperatures between 40 and 100°C. The optimum temperature of the enzyme was found to be 80°C at pH 9.5 (Figure 4).

Effect of metal ions

Arginase has been reported to be a metalloenzyme. Mn^{2+} satisfied the metal ion requirement of *P. americana* gut arginase. The effect of metal ions is presented in Table 2. The activity of arginase in *P. americana* was strongly

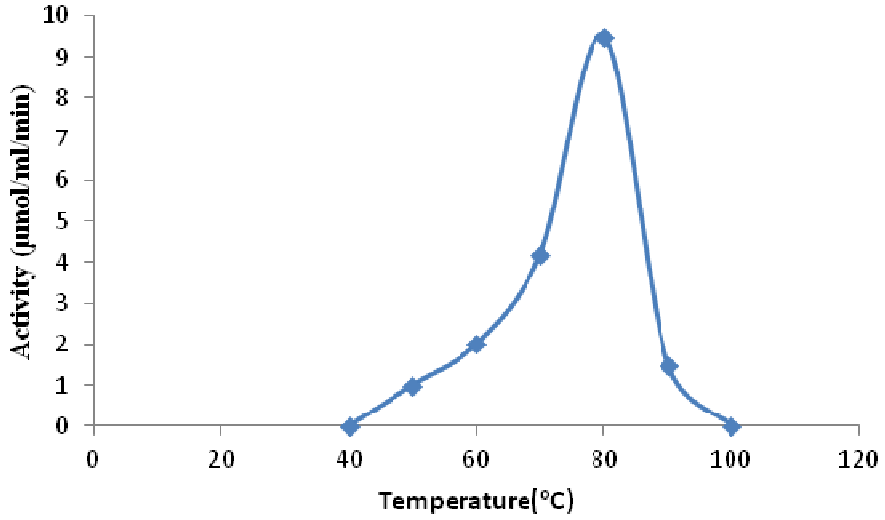

Figure 4. Effect of temperature on *P. americana* arginase activity. The activity of *P. americana* arginase was assayed at temperatures between 40 and 100°C.

Table 2. Effect of Metal ions on the activity of *P. americana* gut arginase.

Cation (10.0 µM)	% Residual activity
Co^{2+}	84.6
Hg^{2+}	85.1
Ni^{2+}	79.0
Mg^{2+}	>100
Zn^{2+}	>100
Sn^{2+}	89.9

> 100% strongly enhanced the activity of the enzyme

Table 3. Effect of reducing agents on the activity of *P. americana* gut arginase.

Reducing agent (10.0 µM)	% Residual activity
Ascorbic acid	26.3
GSH	34.9
2-Mercaptoethanol	1.47

Table 4. Effects of amino acids on activity of *P. americana* gut arginase.

Amino acid (0.01 mM)	% Residual activity
Lysine	81.7
Cysteine	>100
Proline	>100
Valine	96.1
Serine	96.9
Asparagine	97.0

> 100% strongly enhanced the activity of the enzyme

enhanced by Mg^{2+} and Zn^{2+}, but was inhibited by Co^{2+}, Hg^{2+}, Ni^{2+} and Sn^{2+}.

Effect of ascorbic acid, GSH and 2-mercaptoethanol

Table 3 shows the effects of ascorbic acid GSH and 2-mercaptoethanol on the activity of arginase in *P. americana*. The compounds completely inhibited the enzyme.

Effect of amino acids

The result of the effect of various amino acids on the activity of *P. americana* gut arginase is presented in Table 4. The amino acids: lysine, valine, serine and asparagine showed moderate inhibition (with residual arginase activities of 81.7, 96.1, 96.9 and 97.0%, respectively), while proline and cysteine (>100%, respectively) stimulated the arginase activity.

DISCUSSION

Arginases are broadly metaloenzymes distributed in nature. These enzymes catalyze the arginine hydrolysis to ornithine and urea (Lisowska et al., 1987; Jenkinson et al., 1996; Okonji et al., 2011). In this study, we reported the properties of arginase in the gut of adult cockroach, *P. americana*. The arginase was partially purified with ammonium sulphate precipitation and affinity chromatography. The specific activity of the arginase was 3.0 µmol/min/mg of protein. Lisowska et al. (1987) reported very low activity of arginase in intestine of 31 different species of organisms that includes: Annelides, Arthropoda

and Chordata. They observed large variations of the enzyme activities tested and a very low arginase activity up to 0.20 mU/ mg of protein for cockroach, locust and other lower animals was obtained. Rodrigues et al. (2010) reported specific activities of foot muscle, gills and pool of other tissues in the cytosol of *Connicum* to be 87.0, 15.1, 9.8 and 3.8 mU/mg of protein, respectively. The Michealis Menten (K_m) constant of *P. americana* was 0.33 mM and the arginase showed preference to arginine as substrate. This value falls in the range of the k_ms of invertebrate arginases which varies widely between 2 and 158 mM (Porembska, 1973; O'Malley and Terwilliger, 1974; Mohamed et al., 2005). Carvajal et al. (1988) reported K_m values of 25 and 3.0 mM at pH 7.5 and 9.5, respectively for gills and foot muscle of marine mollusc *Chiton latus*. K_m of 6.0 mM was reported for *F. gigantica* arginase type II (Mohamed et al., 2005).

Optimum pH of ~7.0 was observed for *P. americana.* Different pH values have been reported for the enzyme in different species of organisms (Jenkinson et al., 1996). Mohamed et al. (2005) reported a maximum activity for *F. gigantica* arginase II at pH 9.5. Mammalian arginase appears to show more basic pH optima between 9.0-10.5 (Jenkinson et al., 1996), while McGee et al. (2004) reported an acidic pH optimum value of 6.1 for *Helicobacter pylori*. An optimum temperature of 80°C was obtained for *P. Americana.* Arginases from different sources have shown relatively high optimum temperature values. This in most cases has been attributed in part to the metal content of the enzyme (Green et al., 1991; Jenkinson et al., 1996). *Helix pomata* and *Helix aspersa* showed optimum temperature between 60 and 65°C (Baret et al., 1972). *Pista pacifica* was reported to have an optimum temperature around 60°C (O'Malley and Terwilliger, 1974). Lavulo et al. (2001) also reported an optimum temperature of 70°C for wild type arginase of rat liver. Dabir et al. (2005) reported 35 and 45°C as optimum temperature values for *Vigna catjang* cotyledon and buffalo liver arginases respectively.

The effects of ascorbic acid, reduced glutathione and 2-mercaptoethanol showed complete inactivation of *P. americana* arginase. Reports have shown that these compounds have varying effects on arginases from different sources (O'Malley and Terwilliger, 1974; Jenkinson et al., 1996). O'Malley and Terwilliger (1974) reported a stimulatory effect on *P. pacifica* arginase, while an inhibitory effect was observed by Reddy and Campbell (1969) on insect arginase. On the effect of metals on arginase activity, the *P. americana* arginase was slightly inhibited by Sn^{2+}, Hg^{2+}, Ba^{2+}, Ni^{2+} and Co^{2+} but was significantly enhanced by Zn^{2+} and Mg^{2+}. Arginase is a metaloenzyme that shows preference for Mn^{2+} (Jenkinson et al., 1996; Dabir et al., 2005; Mohamed et al., 2005). Zn^{2+} and Ca^{2+} were reported to inhibit the arginase of mollusc *Chiton latus* (Carvajal et al., 1988). Cd^{2+} has been found to activate and inhibit arginases from different species of organisms (Tormanen, 2006).

In our study, lysine, valine, serine and asparagine showed slight inhibition (with residual arginase activities of 81.7, 147, 96.1, 96.9 and 97.0%, respectively), except cysteine and proline that did not inhibit the arginase activity (Table 4). The role of proline in insect metabolism especially in energy generation has been reported (Reddy and Campbell, 1969). Reddy and Campbell (1969) have also shown that the arginase present in *H. gloveri* fat-body functions as a catabolic enzyme for the conversion of arginine into proline. Carvajal et al. (1988) working on arginase from gill and foot muscle tissues of the marine mollusc *Chiton latus* observed significant inhibition of ornithine, lysine and branched-chain amino acids. The conversion of arginine into proline by intact fat-body tissue was used to show that the enzymes in insect fat body also function in this capacity (Reddy and Campbell, 1969).

Conclusion

The metabolic patterns of the various species of insects with their diverse living environments must be extremely varied (Gao et al., 1982). It is possible therefore, to suggest that arginase activity in the gut of *P. americana* might play a role in the development of the insect and energy utilization through the arginase - proline metabolism.

Conflict of Interests

The authors declared that there is no conflict of interests.

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