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SHORT COMMUNICATIONS

Gerald A. ROSENTHAL[1]) and Daniel H. JANZEN[2]): *Arginase and L-canavanine metabolism by the bruchid beetle,* Caryedes brasiliensis

KEY WORDS: *L*-canavanine — *Caryedes brasiliensis* — Bruchidae — *Dioclea megacarpa* — Arginase — Plant-insect interactions.

In lowland deciduous forests of Costa Rica, larvae of the bruchid beetle, *Caryedes brasiliensis* feed solely on the seeds of the leguminous vine, *Dioclea megacarpa* (Janzen, 1971). Over 95% of the free amino acid nitrogen and 55% of all nitrogen in the seed is the free amino acid, *L*-canavanine (Rosenthal, 1977).

$H_2N\text{-}C(\text{-}NH_2) = (N\text{-}O\text{-}CH_2\text{-}CH_2\text{-}CH(NH_2)COOH$
L-canavanine

The three nitrogen atoms of canavanine that constitute the terminal guanidinooxy moiety provide nitrogen for the biosynthesis of virtually every nonessential amino acid produced by this bruchid beetle Rosenthal *et al.*, 1982). Metabolism of this moiety requires arginase (EC 3.5.3.1) which splits *L*-canavanine to *L*-canaline and urea; the latter in turn is cleaved hydrolytically by urease (EC 3.5.1.5) to generate ammonia and carbon dioxide. The presence of appreciable urease activity has been demonstrated in this seed predator (Rosenthal *et al.*, 1977). Indeed, its marked ability to catabolize urea to ammonia is a major biochemical adaptation of *C. brasiliensis* to its canavanine-rich diet (Rosenthal, 1983).

L-Canavanine is not a usual substrate for arginase and this enzyme typically exhibits little activity with this nonprotein amino acid (Campbell, 1966; Reddy & Campbell, 1969). The ability of arginase to metabolize canavanine so efficiently is of critical importance in the intermediary nitrogen metabolism of this seed predator since the ammoniacal nitrogen derived from urea effectively supports the biosynthesis of nonessential amino acids (Rosenthal *et al.*, 1982). In this communication, we report the examination of the relationship of arginase to *L*-canavanine metabolism both in this seed predator and several insects that do not feed upon canavanine-containing plants.

MATERIALS AND METHODS

L-Canavanine was purified from jack bean (*Canavalia ensiformis*) seeds by the method of Rosenthal (1977) and *L*-[*guanidinooxy*-^{14}C]canavanine by radiochemical synthesis from $Ba^{14}CO_3$ (Rosenthal *et al.*, 1983). *L*-[*Guanidino*-^{14}C]arginine (53 μCi/μmole) was purchased from New England Nuclear, Boston, Mass. The remaining materials were obtained from Sigma Chemical Co., St. Louis, Mo. The bruchid beetle larvae were obtained from *Dioclea megacarpa* seeds collected in Santa Rosa National Park, Guanacaste Province, Costa Rica in 1981 and 1982. All remaining insects were obtained from colonies maintained at the University of Kentucky.

Enzyme preparation. Arginase was isolated from insects stored at −60 °C. Unless otherwise indicated, arginase assays that were conducted with *L*-arginine used 100 mM potassium glycylglycine buffer (pH 9.7); when *L*-canavanine served as the substrate, the buffer was 100 mM potassium tricine (pH 7.6). In each case, 2 mM $MnCl_2$ was present.

The frozen insects were ground either with a mortar and pestle or small glass homogenizer utilizing the above buffer; the resulting homogenate was clarified by centrifugation at 12,000 × g for 15 min. The supernatant solution was freed of floating debris by passage over several layers of cheesecloth. After adding saturated liquid ammonium sulfate (55% final vol) dropwise to the clarified insect extract, the proteins were allowed to precipitate for 45 min, and then were collected by centrifugation as above.

After dissolving the protein in a minimum volume of appropriate buffer, the protein solution was desalted on a 20 × 440 mm column of Sephadex G-25. All insect extracts were prepared daily.

Enzyme assay. To conserve the costly radioactive amino acids which were required in high concentrations, a micro radiometric arginase assay was developed. The appropriate substrate was placed in the small chamber of the 25-ml Erlenmeyer flask depicted in Fig. 1. The vessel was sealed with a rubber septum that supported a plastic center well (Kontes Glass

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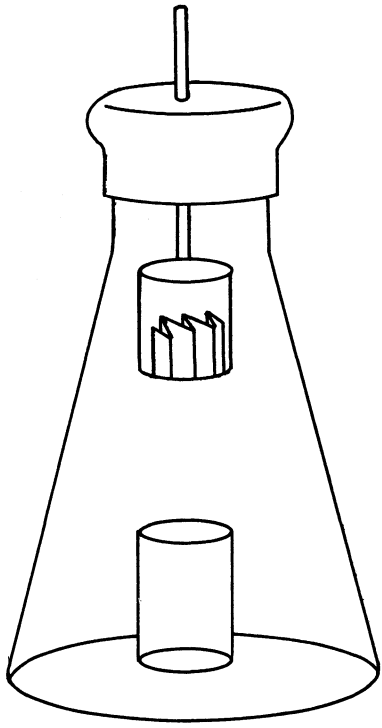

Fig. 1. Apparatus for the micro radiometric assay of arginase.

Co.) containing a folded piece of filter paper impregnated with 5 drops of Hydroxide of Hyamine. The reaction was commenced by gently injecting 0.10 ml of enzyme solution supplemented with 0.4 mg of commercially prepared urease (Sigma type III). Enzyme assays were terminated by the addition of 0.20 ml of 2N HCl. Carbon dioxide evolution was enhanced by gentle agitation of the reaction vessel overnight at room temperature. Controls consisted of appropriate samples in which acid was added prior to the enzyme.

Protein assay. Protein content was determined with bovine serum albumin as the standard by the method of Lowry *et al.*, (1951).

RESULTS AND DISCUSSION

The arginase of various larvae have been compared kinetically by focusing on two parameters: the apparent Michaelis-Menten (K_m), which is an inversely related approximation of the affinity of an enzyme for its substrate, and the maximum velocity of the reaction (V_{max}). The latter is a measure of product formation under substrate saturation conditions. Kinetic determinations of the apparent K_m value for arginine indicates a range from a low of 23 ± 1 in *Heliothis virescens* to 52 ± 11 in *Musca domestica* (Table I). Larval arginases possess much less affinity for canavanine than for arginine since the apparent K_m for canavanine ranges from 176 ± 14 in *H. virescens* to 357 ± 17 in *M. domestica.*

Comparable evaluations of the arginase of *C. brasiliensis* disclosed an apparent K_m for canavanine of 43 ± 11. This is only 1.6 times greater than the arginine value. The free canavanine content of the larvae of the bruchid beetle is about 2.6 times greater than that of free arginine (Rosenthal & Janzen, 1983). Thus, the slightly greater apparent K_m value for canavanine as compared to arginine is balanced by the greater canavanine concentration within the bruchid beetle larvae.

When arginine functions as the substrate, the V_{max} is 1.1 to 4.8 μmoles min^{-1} mg^{-1} of soluble protein (Table I). Canavanine, which is not the natural substrate for this enzyme, is a less effective substrate than arginine and it sustains product formation that varies from 0.15 to 0.48 μmoles/min/mg protein. Comparison of the ratio of the V_{max} value for arginase when arginine is the substrate rather than canavanine reveals a value of 1.3 for *C. brasiliensis.* This compares to 6.0 to 28.2 for the other tested insects (Table I). These kinetic values provide a quantitative measure of the significantly greater ability of the arginase from *C. brasiliensis* to catabolize canavanine than that observed in the other tested insects.

Efficient mobilization of the nitrogen sequestered in the canavanine molecule is made possible by the production of arginase and urease that effectively metabolizes *L*-canavanine. In the case of urease, the larvae rely upon extraordinarily elevated levels of urease activity. It is not known if this reflects its high content in the insect, its large turnover number, or both. In the case of arginase, the larvae rely upon an enzyme that exhibits marked substrate affinity for and efficient substrate utilization of *L*-canavanine. This finding adds a further dimension to the emerging picture of the numerous biochemical adaptations of this seed predator (and perhaps its microbial flora) which enable its effective use of an otherwise poisonous seed resource.

TABLE I

Arginase of various insects[1]

Insects[2]	Kinetic parameters			
	K_m		V_{max}	
	Arginine	Canavanine	Arginine	Canavanine
	(mM)		(μmol/min/mg)	
Drosophila melanogaster (L)	29 ± 7	276 ± 10	1.1 ± .1	0.15 ± .05
Musca domestica (L)	52 ± 11	357 ± 17	4.8 ± .2	0.17 ± .05
Tribolium castaneum (A)	36 ± 8	196 ± 11	3.6 ± .1	0.41 ± .05
Callosobruchus maculatus (A)	32 ± 3	186 ± 12	2.1 ± .1	0.12 ± .05
Manduca sexta (L)	46 ± 2	232 ± 12	2.9 ± .1	0.48 ± .1
Heliothis virescens (L)	23 ± 1	176 ± 14	3.2 ± .2	0.32 ± .1
Caryedes brasiliensis (L)	27 ± 3	43 ± 11	2.8 ± .2	2.10 ± .1

[1] Arginase activity determined as described in text. K_m and V_{max} values were obtained from [S]/v vs. [S] plots determined by regression analysis, lines having a r value of less than 0.999 were discarded. Values represent mean and range of 3 determinations.

[2] (A): use of adult insects; (L): terminal stadium larvae.

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REFERENCES

Campbell, J. W. (1966). A comparative study of molluscan and mammalian arginases. *Comp. Biochem. Physiol.* **18**: 179—199.

Janzen, D. H. (1971). Escape of juvenile *Dioclea megacarpa* (Leguminosae) vines from predators in a deciduous tropical forest. *Amer. Nat.* **105**: 97—112.

Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951). Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **193**: 265—275.

Reddy, S. R. R. & Campbell, J. W. (1969). Arginine metabolism in insects: properties insect fat body arginase. *Comp. Biochem. Physiol.* **28**: 515—534.

Rosenthal, G. A. (1977). Nitrogen allocation for *L*-canavanine synthesis and its relationship to chemical defense of the seed. *Biochem. Syst. Ecol.* **5**: 219—220.

—— (1983). Biochemical adaptations of the bruchid beetle, *Caryedes brasiliensis* to *L*-canavanine, a higher plant allelochemical. *J. Chem. Ecol.* **9**: 803—815.

Rosenthal, G. A., Downum, K. R. & Mattler, J. E. (1983). Radiochemical synthesis of *L*-[*guanidinooxy*-^{14}C]canavanine. *Anal. Biochem.* **133**: 277—282.

Rosenthal, G. A., Hughes, C. G. & Janzen, D. H. (1982). *L*-Canavanine, a dietary nitrogen source for the seed predator, *Caryedes brasiliensis* (Bruchidae). *Science* **217**: 353—355.

Rosenthal, G. A. & Janzen, D. H. (1983). Avoidance of nonprotein amino acid incorporation into proteins by the seed predator, *Caryedes brasiliensis* (Bruchidae). *J. Chem. Ecol.* **9**: 1353—1361.

Rosenthal, G. A., Janzen, D. H. & Dahlman, D. L. (1977). Degradation and detoxification of canavanine by a specialized seed predator. *Science* **196**: 658—660.

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