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NONHEMOCYTE SOURCES OF SELECTED LYSOSOMAL ENZYMES IN *BIOMPHALARIA GLABRATA, B. TENAGOPHILA* AND *B. STRAMINEA* (MOLLUSCA: PULMONATA)

GARY E. RODRICK*
W. MONTEIRO**
W. A. SODEMAN, JR.*

The specific activities of acid phosphatase, alkaline phosphatase, β-glucuronidase, lysozyme, glutamate-oxalacetate transaminase and glutamate-pyruvate transaminase were determined in the head-foot and digestive gland of Brazilian Biomphalaria glabrata *(Touros),* B. tenagophila *(Caçapava) and* B. straminea *(Monsenhor Gil).*

All six enzymes were detected in the 3000g supernatant. Both cytoplasmic enzymes, glutamate-oxalacetate and glutamate-pyruvate transaminase exhibited the highest specific activities. In the case of the four hydrolytic enzymes assayed, β-glucuronidase exhibited the highest specific activity while lysozyme showed the lowest activity.

All six enzymes are thought to be produced by cells within the head-foot and digestive gland of B. glabrata, B. tenagophila *and* B. straminea.

It is well established that molluscan hemocytes, especially granulocytes, are the sites of lysosomal enzyme synthesis (Cheng, 1975; Yoshino & Cheng, 1976a). Furthermore, it is known that when challenged with foreign materials, there is an increased synthesis of at least certain lysosomal hydrolases within these cells and that the enzymes are selectively released into the serum (Cheng et al, 1975; Yoshino & Cheng, 1976a, b; Cheng et al, 1977; Cheng, 1978). In addition, it has also been demonstrated that these enzymes can degrade certain bacteria (McDade & Tripp, 1967; Rodrick & Cheng, 1974; Cheng, 1980).

In order to determine whether there exist other sources of lysosomal enzymes, besides that of the hemocytes, from which serum lysosomal enzymes could have originated, we investigated the levels of activities of additional lysosomal enzymes associated with the headfoot and digestive gland tissues of *Biomphalaria glabrata, B. tenagophila* and *B. straminea.*

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*University of South Florida, College of Medicine, Department of Comprehensive Medicine, Box 12, 12901 North 30th Street, Tampa, Florida 33612, USA.

**Universidade de Brasília, Departamento de Biologia Animal, 70910 Brasília, DF Brazil.

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MATERIALS AND METHODS

Biomphalaria glabrata was collected from Touros, Rio Grande do Norte, *B. tenagophila* from Caçapava, São Paulo, and *B. straminea* from Monsenhor Gil, Piauí, Brazil. They were cultured at the Department of Malacology, Instituto Oswaldo Cruz, in aquaria containing *Elodea canadensis,* and fed with lettuce leaves. Each group consisted of 15-20 snails, with a mean shell diameter of 15±3mm, 10±2mm, and 8.5±2mm respectively. The head-foot and digestive gland of each snail of all three groups were dissected and perfused for 120 minutes in circulating dechlorinated water in order to remove residual serum and hemocytes at 5-8°C. Tissues were weighed and homogenized using a Potter-Elvehjem glass homogenizer in 0.25M sucrose plus 1mM EDTA. The resulting crude homogenate was then centrifuged at 3000g for 15 minutes at 5° to 7°C. The resulting supernatants from each species of snails were maintained in a frozen state (−15°C) until assayed for acid and alkaline phosphatases, β-glucuronidase and lysozyme. In addition to the four lysosomal enzymes, the specific activities of two transaminases, glutamic-pyruvic transaminase (GPT) and glutamic-oxalacetic transaminase (GOT) were determined.

Protein was determined by the method of Lowry et al (1951). Lysozyme (EC 3.2.1.17, *N*-acetylmuramide glycanohydrolase) was determined by a modification of the method of Shugar (1952) as described by Rodrick & Cheng (1974). The methods of Fishman & Lerner (1953) and Bessey et al (1946) were used to determine the activity of acid phosphatase (EC 3.1.3.2., orthophosphoric monoester phosphohydrolase) and alkaline phosphatase (EC 3.1.3.1., orthophosphoric monoester phosphohydrolase) respectively. β-glucuronidase (EC 3.2.3.1., β-D-glucuronide glucuronohydrolase) was determined by the procedures of Fishman et al (1948). The activities of GOT (EC 2.3.1.1., L-aspartate: 2-oxoglutarate aminotransferase) and GPT (EC 2.6.1.2., alanine-2-oxoglutarate aminotransferase) were determined spectrophotometrically at 505nm by the method of Reitman & Frankel (1957).

RESULTS

The specific activities of acid phosphatase, alkaline phosphatase, β-glucuronidase, lysozyme, GPT and GOT for the homogenates of the head-foot and digestive gland tissues from *B. glabrata* (Touros), *B. tenagophila* (Caçapava) and *B. straminea* (Monsenhor Gil) are presented in Table I.

High specific activities were found for glutamic-pyruvate and glutamicoxalacetate transaminases in the head-foot and digestive gland extracts, for all three species of *Biomphalaria* when compared to the other four enzymes. However, when only the hydrolytic enzymes were compared, β-glucuronidase was found to be the most active. Lysozyme exhibited the lowest activity.

DISCUSSION

It is apparent that all six enzymes are associated with the head-foot and digestive gland tissues of *B. glabrata, B. tenagophila* and *B. straminea.* Moreover, the hydrolytic enzymes, lysozyme, β-glucuronidase, acid phosphatase and alkaline phosphatase, which are associated with lysosomes (Tappel, 1969) are present in the head-foot and digestive gland extracts.

High standard deviations in the specific activities of all enzymes studied were noted. This variation in activity is characteristic of many molluscs, especially pelecypods and gastropods which possess an open circulatory system (Cheng, 1975; Rodrick, 1979).

TABLE I

Specific activities on six enzymes associated with tissues of the head-foot and digestive gland of *Biomphalaria glabrata, B. tenagophila* and *B. straminea*

Enzymes	*Head-foot*			*Digestive gland*		
	B. glabrata	*B. tenagophila*	*B. straminea*	*B. glabrata*	*B. tenagophila*	*B. straminea*
Acid Phosphatase	12.41±1.40	13.12±2.11	8.11±1.41	22.81±1.91	24.81±2.81	17.41±3.11
(mu/mg protein)	(10)	(10)	(10)	(10)	(10)	(10)
Alkaline Phosphatase	8.31±1.78	7.48±1.81	6.84±1.12	25.41±3.11	21.22±2.12	19.71±2.41
(mu/mg protein)	(10)	(10)	(10)	(9)	(9)	(10
β-glucuronidase	56.79±19.46	46.61±12.81	39.18±12.32	60.41±1.60	45.18±4.11	39.99±3.19
(Sigma U/mg Protein)	(10)	(10)	(10)	(10)	(10)	(12)
Lysozyme	0.02±0.01	0.03±0.01	0.02±0.01	0.06±0.03	0.05±0.02	0.04±0.02
(DOD/mg protein)	(12)	(12)	(12)	(9)	(9)	(10)
GPT	74.19±7.71	71.68±6.47	64.71±5.44	81.09±4.19	73.47±6.16	74.91±10.11
(Sigma Frankel U/mg protein)	(9)	(10)	(10)	(8)	(9)	(8)
GOT	71.98±9.91	62.99±8.62	60.95±7.89	89.76±6.99	71.92±2.11	76.65±9.08
(Sigma Frankel U/mg protein)	(9)	(10)		(8)	(9)	(10)

Because all gastropods possess an open circulatory system, it is conceivable that hydrolases originating in tissues other than hemocytes, specifically the head-foot and digestive gland, could contribute to the enzyme pool in serum. These enzymes, if occurring at sufficiently high levels, may serve as a defense function against invading biotic and abiotic materials (Cheng, 1980).

Biomphalaria glabrata, B. tenagophila and *B. straminea* are of major biomedical importance in Brazil, as vectors of *Schistosoma mansoni.* Various factors, both extrinsic and intrinsic, are known to influence the establishment of such parasites in many gastropods (Cheng, 1973). Clearly, one important factor which merits careful attention is the action of intracellular hydrolytic enzymes within various molluscan tissues.

RESUMO

Foram determinadas, na massa cefalopedal e na glândula digestiva de *Biomphalaria glabrata* de Touros (Rio Grande do Norte), *B. tenagophila* de Caçapava (São Paulo) e *B. straminea* de Monsenhor Gil (Piauí), as atividades específicas das seguintes enzimas: fosfatase ácida, fosfatase alcalina, β-glucuronidase, lisozima, transaminase glutâmico-oxalacética e transaminase glutâmico-pirúvica.

As seis enzimas referidas foram detectadas no sobrenadante a 3000g. Ambas as enzimas citoplasmáticas – transaminases glutâmico-oxalacética e glutâmico-pirúvica – mostraram as atividades específicas mais altas. No caso das quatro enzimas hidrolíticas, a β-glucuronidase revelou a mais alta atividade específica, enquanto a lisozima revelou a mais baixa.

É admitido que todas as seis enzimas são produzidas por células presentes na massa cefalopedal e na glândula digestiva das três espécies de moluscos examinadas.

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