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BIOMPHALARIA GLABRATA, A CONTINUING ENVIRONMENTAL BIODETERIOGEN: ALTERATIONS IN THE LEVELS OF SOME HYDROLASES FOLLOWING SCHISTOSOMA MANSONI INFECTION. 1. PHOSPHATASES AND N-ACETYL-BETA-D-GLUCOSAMINIDASE

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INTRODUCTION

The snail intermediate host of Schistosoma mansoni, has been considered the weakest link in the life cycle of this parasite. Chemical molluscicides are not specific for fresh water snails, and as a result all aquatic biota suffer (Duncan, 1980) and limited progress has been made in biological control research (Mone et al., 1986). The ideal of attaining a more effective and specific molluscicide has not yet materialized because of our limited knowledge of the physiological and biochemical characteristics of the Schistosoma-Biomphalaria relationship (for review see, Jordan, 1985). As part of our efforts to understand the physiology of this host-parasite relationship we investigated the kinetic changes in the activities of phosphatase and n-acetyl ß-D-glusosaminadase in B. glabrata following S. mansoni infection.

MATERIALS AND METHODS

The specimens of B. glabrata were either raised in the laboratory at the Centers for Disease Control, Chamblee, GA or obtained from the University of Lowell Research Foundation, Lowell, MA. The snails were infected with a Puerto Rican strain of S. mansoni.

The tissue extracts were prepared by homogenizing the head-foot region and the digestive tract of the snails in an ice-cold 0.25 M solution of sucrose and centrifuged at 300g for 20 minutes. The supernatants were either stored at -80°C or used immediately for enzyme assays.

Acid phosphatase (ACP; EC 3.1.3.2.) and alkaline phosphatase (ALP; EC 3.1.3.1.) activities were determined as described by Siddiqui and Nizami (1982) with some modifications. The assay mixture (total volume 2.0 ml) contained protein samples (100-150 µg protein), 0.05 M buffer of appropriate pH and 0.5% Triton X-100. The reaction was started after 10 min equilibration at 37°C in a shaking water bath, by the addition of 2.5 mM p-nitrophenyl phosphate. The reaction was stopped after 30 min by the addition of 1.0 M NaOH. The liberated p-nitrophenol was measured at 410 nm.

N-acetyl B-D-glucosaminase (NAG; EC 3.2.1.30.) activity was determined by the modified method of Siddiqui (1986). The reaction mixture (total volume 2.0 ml) contained 0.05 M acetate buffer, pH 5.5, protein sample (100-150 mg protein) and 0.5% Triton X-100. After 10 min equilibration at 37°C in a shaking water bath, the reaction was started by the addition of 2.5 mM p-nitrophenyl n-acetyl B-D-glycosaminide. The reaction was stopped after 30 min by the addition of 1.0 M NaOH and the absorbance was recorded at 410 nm.

The effect of pH on activities of the above enzymes was observed by using the following buffers of 0.05 M strength; pH 2-4 (Sodium acetate-HCl), pH 4.5-6.5 (acetate), pH 7-9 (Tris-HCl), pH 9.5-11 (glycine-NaOH) and pH 11.5-12 (KCl-NaOH). All other conditions were as described above. Calculations of Michaelis' constants (K_m) were made from the reciprocal plots of data (Siddiqui and Nizami, 1982). The effects of certain activators and inhibitors on enzyme activities were examined in different concentrations as shown in Tables 2 and 3. The protein concentrations were determined by the dye binding method of Bradford (1976) as modified by Spector (1978).

RESULTS

Homogenates of uninfected specimens of B. glabrata showed four distinct peaks of activity for the hydrolysis of p-nitrophenyl phosphate at pH 5.0, 7.5, 9.5 and 11.5 (Figure 1A). The same pattern was also observed for the infected specimens of B. glabrata, however, an increase of 60%, 25%, 40% and 70% was noted in the levels of enzymes at pH 5.0, 7.5, 9.5 and 11.5, respectively (Figure 1A). Furthermore, using tissue extracts of uninfected snails, only one peak of enzyme activity was detected for the hydrolysis of p-nitrophenyl-n-acetyl B-D-glucosaminide at pH 5.5 (Figure 1B). When compared with uninfected snail specimens, the enzyme activity for NAG was 50% higher in infected B. glabrata (Figure 1B).

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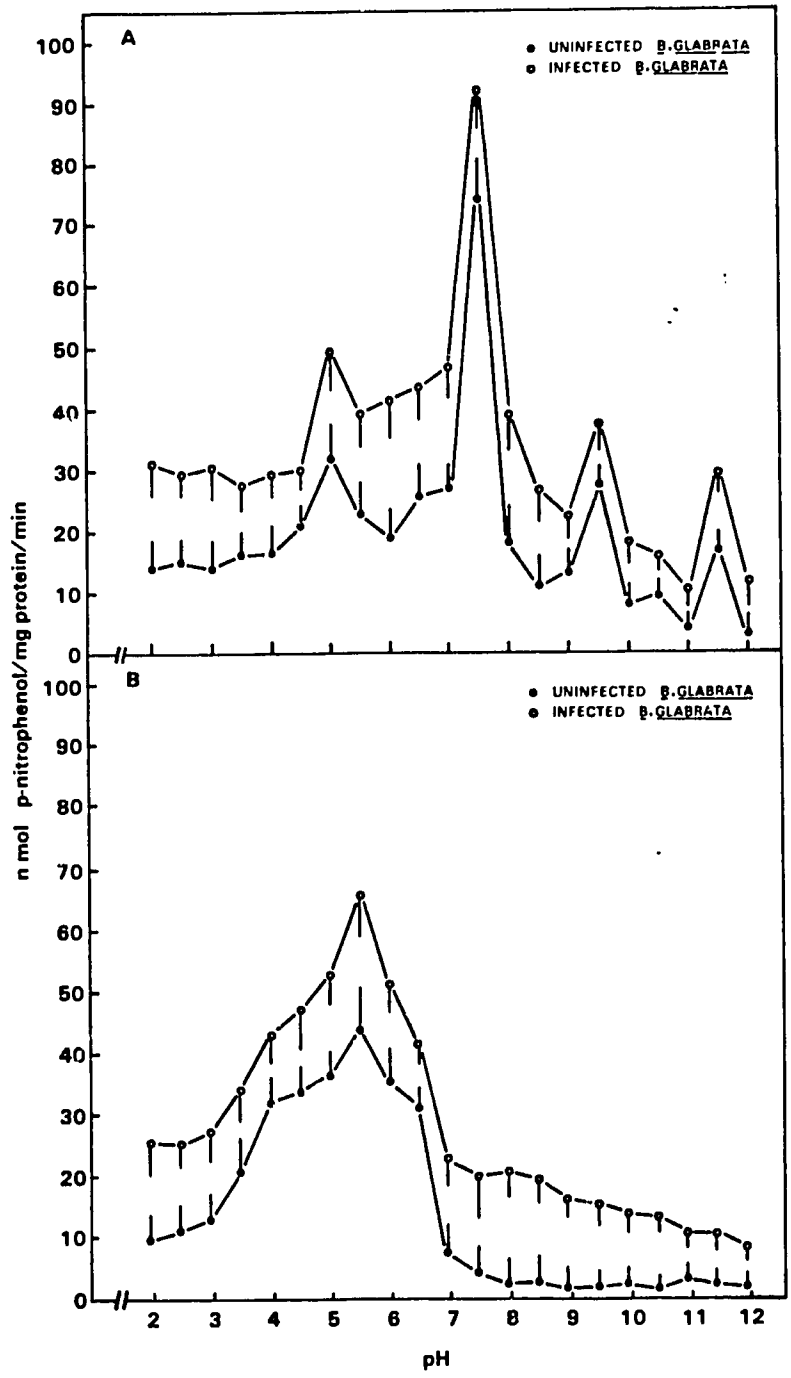

Figures 1A and 1B. Activities of Some Hydrolases of Uninfected and Infected Biomphalaria glabrata at Different pH Units, (A) Acid and Alkaline Phosphatases; (B) n-acetyl-B-D-glucosaminidase.

The Michaelis' constants (K_m) and maximum specific activities (V_{max}) obtained for ACP, ALP and NAG of uninfected and infected B. glabrata are shown in Table 1. The results of the effects of certain inhibitors and stimulators of enzyme activities of ACP and ALP are summarized in Table 2 and for NAG in Table 3, respectively.

DISCUSSION

Data presented here on ACP, ALP and NAG activities are in agreement with those reported for a number of invertebrate tissues. For example, pH optima recorded for the three under-study enzymes are in the same range as reported for many molluscs (Principato et al., 1982), mosquitoes (Hauk and Hardy, 1984), Platyhelminths (Nizami et al., 1975; Siddiqui et al., 1985; 1986) and protozoans (Glew et al., 1982).

On the basis of K_m values, the enzymes studied by us showed similar enzyme-substrate affinities, comparable to those observed in ACP, ALP and NAG from invertebrate at a lower evolutionary level (Annelida; Giovanni et al., 1978), (Platyhelminths; Siddiqui et al., 1985) or in Escherichia coli. Such affinities, on the contrary, are lower in enzymes obtained from more advanced invertebrates (Echinodermata; Principato et al., 1982) or mammals (Brenna et al., 1975).

As shown in Tables 2 and 3, various inhibitors and stimulators have different effects on ACP, ALP and NAG. Stimulation or inhibition appears to be concentration dependent. The most potent inhibitors for ACP were sodium fluoride and sodium arsenate whereas ALP was completely inhibited only by sodium arsenate. These results are in agreement with those reported for many invertebrates (Siddiqui and Nizami, 1982; Hauk and Hardy, 1984). Sodium arsenate was also found to be a good inhibitor of NAG (Table 3). In the presence of Mg^{++}, Mn^{++} and Ca^{++}, ACP, ALP and NAG were activated, although the degree of stimulation was very different. Mn^{++} was found to be the best stimulator for ACP and ALP, and Ca^{++} for NAG. These results are basically in agreement with those reported for many vertebrates and invertebrates (Glew et al., 1982).

At the present stage of research it is difficult to state the exact biological significance of the increased levels of ACP, ALP and NAG in B. glabrata following the infection of S. mansoni, since little is known about the metabolic rate(s) of these enzymes in invertebrates (Principato et al., 1982). However, it is established that in incompatible snail hosts, the larval schistosomes are destroyed by encapsulation (Cheng and Garrabrant, 1977), and this host response is accompanied with increase in the levels of some enzymes (Granath and Yoshio, 1983 a, b). Previous workers have shown

Table 1. A Summary of the Kinetic Parameters for Acid Phosphatase, Alkaline Phosphatase and n-acetyl Beta-D-Glucosamnidase Activities Associated with uninfected and Infected Specimens of *Biomphalaria glabrata*. Maximum specific activities (V_{max} = nmol p-nitrophenol mg protein/min); Michaelis' constants (k_m = mM substrate).

Enzymatic Activities and Parameters	K_m	V_{max}
	Uninfected *B. glabrata*	
ACP[a] (pH 5.0)	0.36 ± 0.03	30.53 ± 2.74
ALP[b] (pH 7.5)	0.14 ± 0.01	75.51 ± 7.64
ALP (pH 9.5)	0.40 ± 0.03	25.14 ± 1.10
ALP (pH 11.5)	0.46 ± 0.04	14.53 ± 1.16
NAG[c] (pH 5.5)	0.83 ± 0.07	47.07 ± 2.82
	Infected *B. glabrata*	
ACP[a] (pH 5.0)	0.24 ± 0.01	50.53 ± 4.46
ALP[b] (pH 7.5)	0.12 ± 0.007	93.57 ± 7.48
ALP (pH 9.5)	0.32 ± 0.02	37.54 ± 3.37
ALP (pH 11.5)	0.38 ± 0.03	25.59 ± 1.15
NAG[c] (pH 5.5)	0.64 ± 0.04	68.37 ± 6.01

[a]ACP = Acid Phosphatase

[b]ALP = Alkaline Phosphatase

[c]NAG = N-acetyl Beta-D-Glucosaminidase

Table 2. Effect of Various Inhibitors and Stimulators on Acid and Alkaline Phosphatase of Uninfected and Infected Specimens of *Biomphalaria glabrata* (mean inhibition/stimlation ± standard error).

Inhibitors/ Stimulators	Conc. (mM)	Uninfected *B. glabrata*			
		pH			
		5.0	7.5	9.5	11.5
Magnesium Chloride (n=3)	2.5	+ 6.16 ± 0.4	+ 13.84 ± 2.6	+ 18.14 ± 1.1	+ 8.34 ± 0.2
	5.0	+ 19.28 ± 1.7	+ 34.17 ± 5.4	+ 23.12 ± 2.1	+ 30.32 ± 1.5
	10.0	+ 31.94 ± 2.2	+ 47.12 ± 7.5	+ 36.71 ± 2.7	+ 47.13 ± 3.4
	15.0	+ 46.17 ± 4.3	+ 51.31 ± 7.1	+ 57.12 ± 4.8	+ 48.74 ± 2.6
Manganese Chloride (n=3)	2.5	+ 36.14 ± 3.2	+ 29.34 ± 3.6	+ 21.14 ± 1.8	+ 38.12 ± 2.6
	5.0	+ 67.54 ± 4.6	+ 57.82 ± 8.1	+ 48.16 ± 1.5	+ 51.11 ± 4.2
	10.0	+ 70.84 ± 6.3	+ 61.84 ± 8.5	+ 67.27 ± 4.2	+ 57.84 ± 6.3
	15.0	+ 94.64 ± 7.5	+101.11 ± 9.9	+ 86.84 ± 7.9	+ 91.84 ± 7.9
Calcium Chloride (n=3)	2.5	+ 3.78 ± 0.3	+ 16.23 ± 1.2	+ 4.54 ± 0.2	+ 7.84 ± 0.7
	5.0	+ 11.24 ± 0.8	+ 18.76 ± 1.6	+ 8.93 ± 1.1	+ 9.83 ± 0.1
	10.0	+ 17.84 ± 1.3	+ 19.18 ± 2.7	+ 9.13 ± 0.6	+ 10.28 ± 1.7
	15.0	+ 18.14 ± 1.8	+ 19.76 ± 3.1	+ 10.74 ± 1.2	+ 14.87 ± 1.2
Sodium Fluoride (n=3)	2.5	- 38.94 ± 2.7	- 9.46 ± 0.8	---	---
	5.0	- 89.76 ± 8.0	- 13.12 ± 0.9	---	---
	10.0	-100.00	- 17.30 ± 1.6	- 8.78 ± 0.8	- 9.86 ± 0.6
	15.0	-100.00	- 23.17 ± 1.9	- 17.84 ± 1.6	- 19.64 ± 1.1
Copper Sulfate (n=3)	2.5	- 16.11 ± 1.2	- 23.43 ± 7.2	- 17.54 ± 1.3	- 20.23 ± 1.2
	5.0	- 38.36 ± 3.4	- 31.83 ± 13.2	- 26.84 ± 2.1	- 41.34 ± 3.6
	10.0	- 56.13 ± 6.1	- 47.77 ± 23.1	- 59.17 ± 1.7	- 60.37 ± 3.7
	15.0	- 73.84 ± 8.8	- 63.26 ± 16.2	- 76.84 ± 4.7	- 83.46 ± 7.6
Sodium Arsenate (n=3)	2.5	- 38.24 ± 4.5	- 38.24 ± 4.5	- 31.11 ± 7.8	- 22.17 ± 2.8
	5.0	- 67.87 ± 13.4	- 73.84 ± 1.9	- 68.15 ± 9.7	- 56.86 ± 3.6
	10.0	- 96.81 ± 14.1	- 93.66 ± 11.6	- 83.78 ± 13.9	- 96.37 ± 17.3
	15.0	-100.00	-100.00	-100.00	-100.00
Potassium Cyanide (n=3)	2.5	---	---	---	---
	5.0	- 30.74 ± 2.4	- 16.17 ± 2.8	- 26.17 ± 3.8	- 38.96 ± 3.1
	10.0	- 53.63 ± 3.7	- 49.16 ± 7.4	- 51.06 ± 21.2	- 57.09 ± 5.6

6.

Table 2 continued

Inhibitors/ Stimulators	Conc. (mM)	Infected *B. glabrata* pH 5.0	pH 7.5	pH 9.5	pH 11.5
Magnesium Chloride (n=3)	2.5	+ 8.24 ± 0.8	+ 18.91 ± 1.3	+ 12.14 ± 0.6	+ 7.14 ± 0.1
	5.0	+ 22.16 ± 1.2	+ 26.84 ± 1.6	+ 25.82 ± 2.6	+ 29.26 ± 1.2
	10.0	+ 37.84 ± 2.9	+ 38.12 ± 4.2	+ 33.16 ± 4.7	+ 43.74 ± 3.4
	15.0	+ 43.52 ± 6.5	+ 47.81 ± 3.7	+ 56.64 ± 6.7	+ 53.64 ± 6.2
Manganese Chloride (n=3)	2.5	+ 26.12 ± 2.1	+ 37.56 ± 2.5	+ 37.24 ± 2.6	+ 27.86 ± 0.9
	5.0	+ 63.42 ± 4.2	+ 56.12 ± 3.7	+ 83.12 ± 3.7	+ 61.17 ± 7.3
	10.0	+ 77.64 ± 6.7	+ 73.84 ± 6.1	+ 82.14 ± 4.2	+ 78.94 ± 9.3
	15.0	+ 83.84 ± 7.6	+100.00 ± 9.6	+ 86.11 ± 7.3	+ 93.74 ± 10.8
Calcium Chloride (n=3)	2.5	+ 14.84 ± 0.7	+ 5.64 ± 1.4	+ 5.99 ± 1.1	+ 5.88 ± 1.1
	5.0	+ 19.26 ± 1.2	+ 14.92 ± 1.1	+ 10.84 ± 0.7	+ 11.84 ± 1.7
	10.0	+ 21.11 ± 1.7	+ 16.34 ± 1.6	+ 14.64 ± 0.9	+ 14.63 ± 2.4
	15.0	+ 23.61 ± 2.4	+ 20.11 ± 0.8	+ 16.14 ± 1.3	+ 17.21 ± 6.2
Sodium Fluoride (n=3)	2.5	- 46.12 ± 4.2	- 16.81 ± 1.2	---	---
	5.0	- 82.11 ± 4.6	- 21.11 ± 1.8	---	---
	10.0	-100.00	- 28.12 ± 1.9	- 10.11 ± 2.7	- 13.72 ± 1.6
	15.0	-100.00	- 32.50 ± 2.1	- 20.01 ± 1.6	- 27.92 ± 2.6
Copper Sulfate (n=3)	2.5	- 13.27 ± 0.2	- 19.73 ± 1.6	- 27.84 ± 3.6	- 13.13 ± 2.6
	5.0	- 22.76 ± 1.1	- 38.84 ± 2.6	- 36.11 ± 4.1	- 39.74 ± 5.2
	10.0	- 52.16 ± 5.1	- 56.72 ± 5.1	- 51.32 ± 4.1	- 55.56 ± 9.6
	15.0	- 69.18 ± 4.2	- 76.17 ± 7.6	- 68.96 ± 7.3	- 87.76 ± 14.6
Sodium Arsenate (n=3)	2.5	- 30.91 ± 3.8	- 46.11 ± 3.2	- 29.27 ± 3.9	- 35.82 ± 1.7
	5.0	- 51.64 ± 7.8	- 69.96 ± 8.7	- 59.76 ± 7.6	- 60.27 ± 3.2
	10.0	- 98.27 ± 11.8	- 79.79 ± 10.0	- 77.71 ± 14.6	- 87.73 ± 9.7
	15.0	-100.00	-100.00	-100.00	-100.00
Potassium Cyanide (n=3)	2.5	---	---	---	---
	5.0	- 22.54 ± 2.6	- 20.42 ± 3.8	- 23.74 ± 2.8	- 37.07 ± 8.7
	10.0	- 51.79 ± 10.2	- 43.21 ± 7.2	- 69.87 ± 3.8	- 61.18 ± 9.7

Table 3. Effect of Various Inhibitors and Stimulators on n-acetyl Beta-D-glucosaminidase of Uninfected and Infected Specimens of *Biomphalaria glabrata* (mean inhibition/ stimulation ± standard error).

Inhibitors/ Stimulators	Conc. (mM)	Uninfected *B. glabrata* pH 5.5	Infected *B. glabrata* pH 5.5
Magnesium Chloride (n=3)	2.5	+ 3.42 ± 0.4	+ 6.39 ± 0.9
	5.0	+ 11.36 ± 1.3	+ 13.12 ± 2.8
	10.0	+ 13.14 ± 2.7	+ 14.28 ± 3.6
	15.0	+ 17.34 ± 2.1	+ 16.84 ± 2.4
Manganese Chloride (n=3)	2.5	+ 2.14 ± 0.1	+ 3.68 ± 0.2
	5.0	+ 8.43 ± 1.3	+ 7.68 ± 0.9
	10.0	+ 10.34 ± 2.6	+ 11.76 ± 1.6
	15.0	+ 13.81 ± 3.1	+ 12.67 ± 2.3
Calcium Chloride (n=3)	2.5	+ 16.84 ± 1.3	+ 19.11 ± 2.7
	5.0	+ 54.81 ± 2.9	+ 38.17 ± 11.2
	10.0	+ 87.13 ± 9.2	+ 78.17 ± 7.6
	15.0	+ 98.22 ± 3.8	+ 91.74 ± 11.7
Sodium Fluoride (n=3)	2.5	- 10.74 ± 2.8	- 16.84 ± 3.7
	5.0	- 40.34 ± 8.8	- 39.23 ± 9.1
	10.0	- 66.84 ± 11.6	- 68.17 ± 12.8
	15.0	- 79.24 ± 16.2	- 81.76 ± 14.2
Copper Sulfate (n=3)	2.5	---	---
	5.0	---	---
	10.0	- 3.94 ± 0.8	- 2.11 ± 0.6
	15.0	- 11.76 ± 1.3	- 9.24 ± 1.1
Sodium Arsenate (n=3)	2.5	- 18.74 ± 0.8	- 2.11 ± 0.6
	5.0	- 32.86 ± 12.6	- 39.36 ± 11.2
	10.0	- 87.87 ± 19.2	- 91.86 ± 12.0
	15.0	- 100.00	- 100.00
Potassium Cyanide (n=3)	2.5	- 20.40 ± 1.6	- 21.77 ± 9.1
	5.0	- 37.50 ± 7.6	- 41.82 ± 13.6
	10.0	- 73.81 ± 9.2	- 78.11 ± 16.7
	15.0	- 86.82 ± 13.3	- 92.86 ± 9.8

a 10-fold increase in some lysosomal hydrolases in snails which are resistant to S. mansoni whereas only three-fold increase in Schistosome-susceptible snails was observed (Kassim and Richards, 1978). Acid Phosphatase and NAG have been reported to be primarily localized in lysosomes, whereas, ALP in plasma membranes (Siddiqui and Podesta, 1985). Why the activities of these hydrolases are stimulated during the course of S. mansoni infection in susceptible snails is still open for speculation. Furthermore, it is not clear at this stage what the mechanisms are for determining survival of miracidia in an immunologically hostile environment, leading to an almost successful immune evasion by the parasite. Further studies are required to ascertain the exact role(s) of these enzymes in the snail host system as it relates to schistosome infections.

SUMMARY

This study was designed to compare B. glabrata with specimens infected with S. mansoni in order to determine if snails with prepatent infections can be identified through enzyme markers in snail populations. Activities of acid and alkaline phosphatases were determined using spectrophotometric methods. Enzymes of both infected and uninfected snails showed four peaks of activity at pH 5.0, 7.5, 9.5 and 11.5. The K_m for p-nitrophenyl phosphate (pNPP) was 0.36 mM (V_{max} 30.5 units), 0.14 mM (V_{max} 75.5 units), 0.40 mM (V_{max} 25.1 units) and 0.46 mM (V_{max} 14.5 units) at pH 5.0, 7.5, 9.5 and 11.5, respectively, for uninfected snails. The K_m values for pNPP were 0.24 mM (V_{max} 54.5 units), 0.12 mM (V_{max} 93.5 units), 0.32 mM (V_{max} 39.5 units) and 0.38 mM (V_{max} 32.5 units) for infected snails. The above reported enzyme marker(s) appears to be a useful tool for studying the infectivity potential and will help in our understanding of schistosome epidemiology.

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