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Short Communication

Studies on the pH tolerance of *Biomphalaria glabrata* snails infected with *Schistosoma mansoni*

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ABSTRACT

Based on the results of our earlier study on pH tolerance of uninfected snails, we conducted further studies on pH tolerance of *Biomphalaria glabrata* snails infected with *Schistosoma mansoni*. The metabolic processes of the snails patently infected with *S. mansoni* are presumably quite different than those of non-infected snails. In methods similar to those used in the previous study, we examined the effects of pH on the lipids of infected snails. Using high performance thin layer chromatography, we found that infected snails, unlike the uninfected ones, showed significant lipid changes within the pH ranges examined. Free fatty acid amounts of the infected snails were significantly greater in deionized (DI) water at pH 10 than those of cohorts maintained in DI water, artificial spring water, and DI water at pH 4.0. We also found that the amounts of triacylglycerols in the infected snails were significantly greater in snails maintained in DI water than in solutions at other pH values.

KEYWORDS: *Biomphalaria glabrata*, thin layer chromatography, *Schistosoma mansoni*, lipids, acid-base tolerances

INTRODUCTION

The study of *Biomphalaria glabrata* is important because of the unique characteristics of this snail. It is an intermediate host of the trematode *Schistosoma mansoni*, an important parasite that causes the disease schistosomiasis in millions of people around the world. Understanding more about the environment in which the infected snail lives will provide valuable information as our climate continues to change. The water pH is an important abiotic factor that influences the growth of many aquatic species but has not been extensively studied as a variable in the laboratory. Our previous paper [1] reviewed the pH tolerance literature on infected and non-infected snails. In our present study, we present our biochemical findings on the pH tolerance of *B. glabrata* snails patently infected with *S. mansoni* in the laboratory.

EXPERIMENTAL

For this experiment, we used similar methods to those described in O'Sullivan *et al.* [1] with the exception of our studied pH values. For this experiment, we looked at pH values of 4, 10, and 11 and for our controls we used both deionized (DI) water and artificial spring water (ASW). ASW is a commonly used solution for freshwater aquatic snails as it contains ions that are found in natural water conditions. We studied both DI and ASW as controls to see if there were any differences between the two solutions. We used the pH values of 4, 10, and 11 because they were the pH values at which the snails survived reasonably well. We eliminated the pH values of 6 and 8 used in our earlier study [1] because those values were very similar to the control pH values in our experiment. We used only one time endpoint at 72 hr instead of both 48 and 72 hr as used in our earlier study [1]. The lack of differences in results between the different endpoints lead us to believe it was not necessary to maintain two different experimental times.

Samples were prepared first by removing the snail bodies from their shells by lightly tapping the shell with a hammer and removing the shell with forceps. With a pair of dissecting scissors, the digestive gland gonad complex (DGG) was separated from the rest of the body and used for analysis. Lipids were then extracted from the sample in a 2:1 chloroform-methanol solution in a 7 mL capacity Wheaton (Millville, NJ, USA) glass homogenizer in accord with the Folch *et al.* [2] method. The nonlipophilic material was separated and removed by adding 1 part Folch *et al.* wash (0.88% KCl, w/v, in DI water) for every 4 parts of chloroform-methanol (2:1). The aqueous top layer was removed and discarded. The samples were then placed in a warm water bath and taken to dryness by a stream of nitrogen gas. The dried samples were reconstituted in known volumes of chloroform-methanol (2:1) to produce sample zone scan areas that fell within the HPTLC-densitometry calibration range.

HPTLC-densitometry was carried out as described in detail by Counihan *et al.* [3]. In short, neutral lipid standard solutions and reconstituted sample solutions were applied to Analtech Inc., (Newark, DE, USA) 10 x 20 cm HPTLC silica gel preadsorbent channeled glass plates (Catalog No. 61927) using a Drummond (Broomall, PA, USA) 10 µL digital microdispenser. Plates were developed in a CAMAG (Wilmington, NC, USA) HPTLC twin trough chamber using the mobile phase petroleum ether-diethyl ether-glacial acetic acid (80:20:1). Lipids were visualized as dark blue band shaped zones on a yellow background by spraying with 5% ethanoilc phosphomolybdic acid reagent and heating on a CAMAG plate heater at 110°C for 10 min. The lipids were then quantified using a CAMAG Scanner 3 in the absorbance-reflectance mode with slit dimensions of 4.00 mm x 0.45 mm Micro and a scanning rate of 20.0 mm/second. The tungsten light source was set at 610 nm. The winCATS software automatically created polynomial calibration curves relating standard zone weights to their peak areas, and unknown sample zone weights were interpolated from the curve based on their bracketed peak areas. The percent of each lipid in a sample was calculated using the following equation:

$$\text{Percent lipid} = [w \times R \times 100]/[\text{Initial DGG sample mass } (\mu g)]$$

where w = lipid mass (µg) of sample interpolated from calibration curve, R = reconstituted volume (µL)/spotted volume (µL). Statistical analysis of these lipid samples was conducted using Microsoft Excel's Student's *t*-test function to determine statistical significance of the quantitative data.

RESULTS

After biochemical analysis, we found that there were several significant differences among the various lipid fractions of infected snails at different pH values. These data are summarized in Table 1.

As can be seen in Table 1, free fatty acid content of the infected snail DGGs was significantly

Table 1. Weight percentage (Mean ± standard error) of neutral lipids in the DGG of snails.

Sample	Free sterols	Free fatty acids	Triacylglyercols	Methyl esters	Steryl esters
DI	0.13 ± 0.09	0.3 ± 0.1	1.1 ± 0.5[b]	N/A	0.09 ± 0.03
ASW	0.15 ± 0.09	0.51 ± 0.07	N/A	0.074 ± 0.009	0.06 ± 0.03
pH 4	0.13 ± 0.09	0.5 ± 0.2	0.17 ± 0.09	0.11 ± 0.05	0.13 ± 0.05
pH 10	0.20 ± 0.06	1.1 ± 0.1[a]	0.9 ± 0.6	0.06 ± 0.03	0.10 ± 0.03
pH 11	0.10 ± 0.02	0.8 ± 0.2	0.3 ± 0.2	0.07 ± 0.02	0.13 ± 0.09

[a]statistically greater than DI, ASW and pH 4, $P<0.05$.
[b]statistically greater than pH 11, $P<0.05$.

greater in DI water at pH 10 than in DI water at pH 4, ASW, and DI water. The triacylglycerol content was also significantly greater in DI water than DI water at pH 4 and pH 11.

CONCLUDING REMARKS

In our previous biochemical study [1] on the effects of subjecting uninfected *B. glabrata* to a range of pH values, such changes did not alter the neutral lipid or phospholipid content of the snails. Interestingly, in the current study using *B. glabrata* infected with *S. mansoni*, altered pH of the aquarium water affected the lipid concentrations, particularly the free fatty acids and triaclglycerols of the snails. The significance of these findings awaits further biochemical studies.

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