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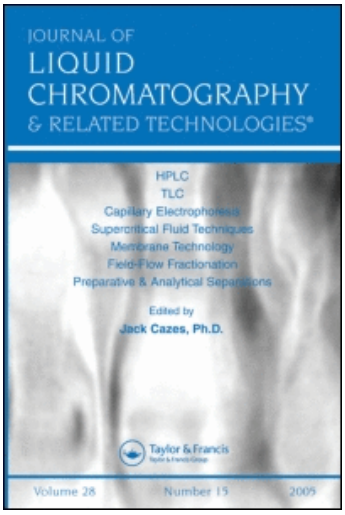

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HPTLC ANALYSIS OF AMINO ACIDS IN *BIOMPHALARIA GLABRATA* INFECTED WITH *SCHISTOSOMA MANSONI*

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HPTLC ANALYSIS OF AMINO ACIDS IN *BIOMPHALARIA GLABRATA* INFECTED WITH *SCHISTOSOMA MANSONI*

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ABSTRACT

HPTLC analysis was used to determine the amino acid content in the digestive gland-gonad complex (DGG) of *Biomphalaria glabrata* snails infected with larval *Schistosoma mansoni* for eight weeks. DGGs of infected and control snails were pooled, extracted in ethanol, and chromatographed using various sorbent-mobile phase combinations applicable to amino acid analysis. Zones were detected with ninhydrin and quantified by densitometry.

Qualitative analysis showed the presence of histidine, lysine, alanine, methionine, threonine, asparagine, proline, and leucine/isoleucine in both the infected and uninfected DGGs. Quantitative analysis was done on histidine, lysine, alanine, and methionine, but only lysine showed a significant reduction (Student's *t*-test, $P < 0.05$) in concentration in the DGGs of infected snails compared to the controls. Lysine, an essential amino acid

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for the developing schistosome larvae, is obtained in significant amounts from the snail DGG.

Key Words: HPTLC; Amino acids; *Biomphalaria glabrata*; Gastropoda; *Schistosoma mansoni*; Trematoda

INTRODUCTION

Numerous studies have been done on the amino acid content of *Biomphalaria glabrata* snails infected with the intramolluscan stages of *Schistosoma mansoni* (Trematoda). Most studies reported a reduction in free amino acid levels in the hemolymph and tissues of *B. glabrata* infected with larval *S. mansoni*. Earlier studies on this topic are those of Targett,[1] Senft,[2] and Gilbertson et al.[3] All of these works reported alterations in the amino acid content of tissues and hemolymph of *B. glabrata* infected with *S. mansoni*. Targett[1] found that infected snails had an overall reduction in their free amino acid content, and that certain amino acids, e.g., methionine, are quantitatively reduced more than others; Targett suggested that the schistosome larvae obtained part of its free amino acid requirements from the snail host. Gilbertson et al.[3] reported a decrease in the free hemolymph levels of amino acids by 10 days post-infection (PI) in *B. glabrata* infected with *S. mansoni* and that the level of total free amino acids was decreased to about one-half that of the uninfected snails at 32 days. These findings were later confirmed by Stanislawski et al.[4] and Schnell et al.[5]

Most of the aforementioned studies used analytical techniques such as automated amino acid analysis, thin layer chromatography (TLC), and paper chromatography (PC). There are no studies that examined the effects of *S. mansoni* infection on *B. glabrata* snails using high performance thin layer chromatography (HPTLC). The purpose of this paper is to report our results using HPTLC on the effects of *S. mansoni* infection on the amino acid content in the digestive gland-gonad complex (DGG) of the *B. glabrata* snail at eight weeks PI.

EXPERIMENTAL

Maintenance of Snails and Sample Preparation

Snails were exposed to *S. mansoni* miracidia and maintained in aquaria, as described in Fried et al.[6] and fed ad libitum on the leafy portion of Romaine lettuce. The infected snails were extracted and analyzed by HPTLC at eight weeks PI along with matched uninfected controls. DGG samples from a pool of three to eight snails (0.06–0.09 g) were collected and amino acids extracted in 70% ethanol as described in Pachuski et al.[7] After evaporation under a stream of air at

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45°C, the DGG samples were reconstituted in 250 μL of 70% ethanol prior to TLC analysis.

HPTLC Analysis of Amino Acids

Standards of 19 amino acids were purchased from Sigma (St. Louis, MO, USA) and were prepared as 0.100 μg mL^{-1} solutions in 70% ethanol. Qualitative and quantitative analyses of amino acids were performed on four different systems: 20 cm × 10 cm silica gel HPTLC plates with a preadsorbent sample application zone and 19 channels (LHPKDF #4806-711; Whatman, Clifton, NJ, USA); 20 cm × 20 cm strong acid cation-exchange sheets (POLYGRAM® IONEX-25 SA-Na; Macherey-Nagel, Easton, PA, USA); 20 cm × 20 cm reversed phase plates with preadsorbent zone (LKC18F #4800-820; Whatman, Clifton, NJ, USA); and 20 cm × 10 cm HPTLC cellulose F plates (Art. 15036; EM Science, Gibbstown, NJ, USA, an affiliate of Merck KGaA, Darmstadt, Germany). All layers were pre-cleaned by development with dichloromethane–methanol (1 : 1) and dried before use.

Samples and standards were applied with a 10 μL digital microdispenser (Drummond, Broomall, PA, USA). Qualitative standards for identification of sample zones were applied in volume aliquots of 10.0 μL. Quantitative standards were applied in various increments ranging from 1.00 to 30.0 μL to bracket the amounts of amino acids in the 10.0 to 40.0 μL aliquots of reconstituted samples that were applied. Application of the larger volumes in small initial zones was aided by directing a stream of air onto the origin and applying repeated, small volume increments.

For both qualitative and quantitative amino acid analyses, initial zones were allowed to air dry, and plates were developed in a twin-trough TLC chamber (Camag, Wilmington, NC, USA) containing a saturation pad (Analtech, Newark, DE, USA) using pH 3.3 citrate buffer as the mobile phase for the ion exchange sheet, *n*-propanol-0.5 M NaCl (4 : 6) for the reversed phase layer, and *n*-butanol–acetic acid–water (3 : 1 : 1) for the silica gel and cellulose layers.[8] The ion-exchange sheet was supported in the tank by a 20 cm × 20 cm glass plate to prevent it from bending as the mobile phase advanced. Development on the four layers required 1.5 to 4 hr. Detection of the zones was achieved by spraying the developed and air-dried plates with ninhydrin reagent, as described by Steiner et al.[8] air drying for 30 min, and heating for 10 min at 110°C on a Camag plate heater to produce purple zones for all amino acids, except for proline, which had a yellow color.

In the quantitative amino acid analyses, the areas of the bands were measured using a Camag TLC Scanner II with the tungsten light source set at 495 nm for histidine and 610 nm for all other acids, slit width 3, slit length 7, and

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scan speed 4 mm s^{-1}. The CATS software automatically produced a calibration curve relating the scan areas of the standard zones to their respective weights (0.100–3.00 μg) by polynomial regression and interpolated the weights of sample zones from the curve based on their areas.

The interpolated sample weight of amino acid was then multiplied by the ratio of the reconstituted volume to the spotted volume. This value, multiplied by 100%, was then divided by the total wet weight of the DGG sample, in μg, to give the reported weight % (wt%). The uninfected and infected samples were compared using Student's *t*-test to determine if the values were different with $P < 0.05$ being considered significant.

RESULTS AND DISCUSSION

By comparison with the migration of standards, histidine, lysine, alanine, methionine, threonine, asparagine, and proline, were identified as amino acids found in both the infected and uninfected DGG. An additional zone was identified as either leucine or isoleucine. Other zones were also found, but could not be positively identified. All four layers were required for the identification of the amino acids.

Table 1 lists the quantitative data for histidine, lysine, alanine, and methionine in the DGG of infected and uninfected snails, as found from quantification on either the silica gel or cellulose plates. There was a significant decrease in the wt% of lysine in the infected snails. Irrespective of infection, differences in the concentrations of histidine, alanine, and methionine were not significant.

Lysine is probably an essential amino acid for the developing intramolluscan stages, i.e., sporocysts and cercariae, of the *S. mansoni* parasite. Since this amino acid cannot be synthesized de novo, it must be provided by the *B. glabrata*

Table 1. Quantitative Data for Amino Acid Analysis in the DGG of *S. mansoni* Infected (I) and Control (C) Snails at 8 wk PI

	Histidine Mean wt% (μg) ($n = 3$)	Lysine* Mean wt% (μg) ($n = 3$)	Alanine Mean wt% (μg) ($n = 3$)	Methionine Mean wt% (μg) ($n = 2$)
C	0.00687	0.00431	0.00921	0.139
I	0.00516	0.00147	0.00940	0.140

*The concentration of lysine was significantly reduced in the infected snails (Student's *t*-test, $P < 0.05$).

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snail host. The significant decline in lysine in the DGG of infected snails at 8-wk PI probably reflects the parasites' ability to obtain this amino acid from its host.

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