Neurochemical correlates of aestivation: Aminotransferase heterogeneity in the cerebral ganglion of the amphibious snail *Pila globosa*

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**ABSTRACT**

The activity levels of Aspartate aminotransferase (AAT, E.C. 2.6.1.1) and alanine aminotransferase (AIAT, E.C. 2.6.1.2) decreased in the cerebral ganglia of aestivating snail, *Pila globosa*. Heterogeneity of aminotransferases in the cerebral ganglia of normal and aestivated snails (studied by agar gel electrophoresis) revealed a decrease and also loss of isozymes during aestivation.

1. **INTRODUCTION**

Considerable information is available on enzymatic activities in the Indian apple snail, *Pila globosa*, during aestivation. A reduction in the activity levels of hepatic dehydrogenases, an inhibition of respiratory enzymes in the different tissues, and a deceleration in the activity level of urease in the kidney and mantle of *Pila globosa* during aestivation has been reported. Murali Mohan detected significant inhibition in the activity levels of aminotransferases in the entire nervous system of *Pila* during aestivation. However, very little information is available on the multiple forms of enzymes during such aestivation, and hence the present studies were undertaken.

2. **MATERIALS AND METHODS**

*Pila globosa* were collected from the local fresh water ponds and were maintained in glass aquaria at 24 ± 2°C, for a week. They were fed on Hydrilla plants. Actively feeding snails were aestivated by embedding them in dry sand for eight months.

The animal was dissected on a wax plate kept on ice blocks at 0°C. Cerebral ganglia were isolated and kept in a cavity glass at 0°C.
pooled ganglia (from 5–8 animals) were weighed in ice cold Pila Ringer and immediately used for experimentation.

10% (W/V) homogenates of cerebral ganglia were prepared in 0.1 M potassium phosphate buffer using pestle and mortar and centrifuged at 3,000 g for 10 minutes to remove the cell debris. The supernatants were used for the enzyme assay. The AAT and AIAT activities were determined following the colorimetric procedure of Reitman and Frankel as described by Bergmeyer.9

The electrophoretic mobilities of AAT and AIAT of the supernatants of cerebral ganglia (pooled from 15–18 snails) were studied on agar gels in a horizontal chamber. The tissue content of all the samples was equalized by appropriate dilution. 10 microlitres of each sample was spotted on the gel layer well. Veronal-acetate buffer (pH 8.6) was used as the separating medium. A voltage gradient of 220 volts D.C. was applied across a 12 inch chamber for 16 hours at 10°C. After the run, the individual gels were cut into 5 mm wide serial sections, and were eluted in separate test tubes with 1 ml of phosphate buffer by vigorous stirring. The contents of each tube were centrifuged and an aliquot of 0.1 ml of the supernatant from the elution was used for aminotransferase determination.

3. RESULTS AND DISCUSSION

The data after statistical analysis are summarized in table 1 and figures 1–2.

The activity levels of AAT and AIAT in general showed a decrease in the cerebral ganglia during aestivation (table 1). The AAT and AIAT ratio did not show significant change during aestivation (table 1). In general the activity level of AAT was more than that of AIAT in (both) the normal and aestivated cerebral ganglia (table 1). The general decrease in the activities of these two aminotransferases clearly indicates that the contribution of the glucogenic amino acids to carbohydrate metabolism is less during aestivation.5

**Isozymic Pattern of Aminotransferases:** It is evident from the isozymic pattern of AAT and AIAT (figures 1–2) that there is both decrease and loss of isozymes during aestivation.

**AAT:** Specific pattern of AAT multiplicity characteristic of the cerebral ganglia of normal and aestivated Pila were distinguishable (figure 1). In the aestivated snails three distinct and characteristic fast moving forms
Table 1. Changes in weights and activity levels of Aminotransferases in the cerebral ganglion of control (normally active) and aestivated (for 8 months) *Pila globosa*

<table>
<thead>
<tr>
<th>Constituent measured</th>
<th>Control (Normally active)</th>
<th>Aestivated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (wet)</td>
<td>3.2±0.02</td>
<td>1.9±0.16</td>
</tr>
<tr>
<td>Mg/g</td>
<td>-40.6* 1.33≠</td>
<td>P&gt;0.5</td>
</tr>
<tr>
<td>+AAT</td>
<td>0.285±0.016</td>
<td>0.237±0.021</td>
</tr>
<tr>
<td>μ moles of Keto acid/g/hr</td>
<td>-16.8* 3.2≠</td>
<td>P&gt;0.01</td>
</tr>
<tr>
<td>+A1AT</td>
<td>0.232±0.018</td>
<td>0.186±0.015</td>
</tr>
<tr>
<td>μ moles of Keto acid/g/hr</td>
<td>-19.8* 9.5+</td>
<td>P&gt;0.001</td>
</tr>
</tbody>
</table>

Values are Mean of 4 observations ± S.D. For each observation ganglia from 5 animals were pooled.
≠ = t value.
*Percentage change = Sign (−) indicating decrease over controls.
+= AAT/A1AT = in normal (control) snails = 1.23.
+=AAT/A1AT = in aestivated snails = 1.28.

Figure 1. Profiles of AAT isozymes in the cerebral ganglion of the Indian apple snail, *Pila globosa*, as a function of aestivation.

cationic in nature and anodal in their migration could be resolved. All the three forms exhibited higher activity over controls. Besides these, two slow moving anodal and a cathodal isozyme (anionic in nature) with comparatively low activity were found (figure 1). The control animals exhibited an isozymic pattern with one cathodal (anionic in nature) and six distinct cationic isozymes. The cathodal isozymes of control animals exhibited higher activity compared to the cathodal form of aestivated snails. Of the
Figure 2. Profiles of AIAT isozymes in the cerebral ganglion of the Indian apple snail, *Pila globosa*, as a function of aestivation.

C, cathodal migration; A, anodal migration; Spt., point of application of the sample.

- - - - - represents control; - - - represents experimental.

Each point in figures is an average of 3 observations. For each observation ganglia from 15-18 animals were pooled.

1-28 in 'X' axis indicate the number of sections analyzed in the serial order from cathode to anode for the enzyme activity.

six anodal forms from controls, the first three were comparatively slow moving and exhibited characteristically higher activity levels compared to experimental forms. Of the three fast moving forms the isozyme with the highest electromobility showed comparatively lower activity and in general these three isozymes exhibited lower activity levels compared to three fast moving isozymes from the cerebral ganglia of aestivated animals (figure 1 and table 2).

**AIAT**: Characteristically a similar isozyme pattern as that of AAT was detected in the cerebral ganglia of control (normally active) and to a great extent in experimental (aestivated) animals. For instance, like AAT, one cathodal and six distinct forms with anodal migration could be resolved in control snails. But this pattern was slightly altered in the experimental individuals. For instance the form corresponding to the isozyme with anodal migration exhibiting slowest mobility (of controls) has completely lost its expression (figure 2). But one cathodal isozyme and five components (isozymes) with anodal migration corresponding to the isozymes of controls could be detected. Of these isozymes (cationic in nature) three were prominent. The anodal form with relatively lesser mobility compared to the fastest group was not defined.
Thus the isozymic pattern of AAT in control and aestivated *Pila globosa* followed a similar pattern as was exhibited by AlAT. It is therefore possible that the components of AAT and AlAT had similar electromobility suggesting that both the enzymes are associated with a single protein based particle. The aestivated state induced a general deceleration in the activities of aminotransferases but did not affect the basic isozyme pattern of either aminotransferase in the cerebral ganglia. However the difference in the enzyme activity between the components was more pronounced for AAT than for AlAT (figures 1, 2).

**REFERENCES**