Rhodanese and Mercaptopyruvate Sulphur Transferase Enzymes in the Tissues of Chichilidae (Tilapia Zillii, Sarotherodon Galilaeus) and Hepsetidae (Hepsetus Odoe) in Igun River, Ilesa, Southwestern Nigeria

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Abstract

The cyanide detoxifying enzymes (rhodanese and mercaptopyruvate sulphurtransferase [MST]) were estimated in different tissues of three fishes (Tilapia zillii, Hepstus odeo and Sarotherodon galileaus) from Igun River in Ilesa, South-Western region of Nigeria. The enzyme activities were carried out by measuring the amount of thiocyanate produced by the two enzymes using specific substrates in each case. The results also showed that both enzymes have a statistically high specific activity in Hepstus odeo in all the tissues, followed by Sarotherodon galileaus and Tilapia zillii. Moreover, the tissue with the highest protein concentration is the gut, then the gills and the flesh, although the differences in their protein concentrations were statistically insignificant. The gut also showed the highest MST specific activity statistically, afterwards the gills and the flesh. Conversely, the rhodanese specific activity was however revealed to be high in the flesh than other tissues, but not statistically significantly different from the other tissues. The study showed the activities of two cyanide detoxifying enzymes (rhodanese and mercaptopyruvate sulphurtransferase) in the different fishes indicating the existence of a strong cyanide detoxifying mechanisms.

INTRODUCTION

From the distant past, cyanide has been known as a highly toxic compound that is readily absorbed and cause death by preventing the use of oxygen by tissues [1,2]. This toxicant is widespread in the environment. Many naturally occurring substances as well as industrial products contain cyanide [1]. More than 2,000 species of plants are known to contain cyanogenic glycosides [3]. It has been reported that ingestion of cyanogenic glycosides in forage crops can result in the death of grazing animals [4]. Many studies report the death of birds from cyanide poisoning through several routes, including exposure to cyanide salts or ingestion of cyanogenic plants [5]. Numerous accidental spills of sodium cyanide or potassium cyanide into rivers and streams have resulted in massive kills of fishes, amphibians, aquatic insects, and aquatic vegetation [6]. Adverse effects of cyanide on fish include delayed mortality, pathology, impaired swimming ability and relative performance, susceptibility to predation, disrupted respiration, osmoregulatory disturbances, and altered growth patterns [2]. Cyanide acts rapidly in aquatic environments, does not persist for extended periods, and is highly species selective; organisms usually recover quickly on removal to clean water. The critical sites for cyanide toxicity in freshwater organisms include the gills, egg capsules and other sites where gaseous exchange and osmoregulatory processes occur [7].

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Two major enzymes (rhodanese: EC. 2.8.1.1, thiosulphate: cyanide sulphurtransferase and mercaptoptyruvate sulphurtransferase: EC. 2.8.1.2) are involved in cyanide metabolism. Rhodanese is a ubiquitous enzyme that is known to be responsible for the biotransformation of Cyanide to thiocyanate using thiosulphate as the donor substrate [8,9]. The enzyme rhodanese is well characterized among the cyanide detoxifying enzymes. The liver has always been considered to be the major source of rhodanese and is believed to be the major site of cyanide detoxification [10].

Unlike rhodanese, MST has not been fully characterized. The enzyme is also widely distributed in prokaryotes and eukaryotes and it is located mainly in the cytosol compared to rhodanese which is a mitochondrial enzyme [11]; [8,11-12]. Recent works have shown that different genes codes for the two cyanide detoxifying enzymes with speculations that they have the same origin [12,13].

Reports have also shown that the pattern of distribution of these two enzymes might affect the physiological fate of cyanide [14]. Also, the activity of the enzymes in a particular tissue/organ may reflect the ability of that tissue/organ to detoxify cyanide [14].

We report here the pattern of distribution of rhodanese and 3-MST in different tissues from three types fishes captured from Igun River. The results might indicate the parts of the fish (tissues) that are extensively exposed to, and involved in cyanide metabolism.

MATERIALS AND METHODS

Study area and collection of fish samples

Cast-net was used to collect fish samples in Igun River. The fish samples were stored in an ice-chest covered with ice before transporting to the laboratory where they were stored at temperature below 0°C until ready for use. The fish species, *Tilapia zillii*, *Hepstus odea* and *Sarothodelon galileaus* were identified using the keys by [15,16,17]. All reagents used were of analytical grades.

Preparation of tissue extract

The *Tilapia zillii*, *Hepstus odea* and *Sarothodelon galileaus* fish species were slit open and the various tissues of interest (gill, flesh and gut) were removed and stored at 4°C until required. Tissue extracts were prepared by homogenising 10 g (w/v) of each tissue in 30 ml of homogenisation buffer (phosphate buffer, pH 7.2). The suspensions were centrifuged for 20 min at 4,000 rpm in a Microfield Centrifuge Model 800 D. The supernatants were used as the source of enzyme.

Arginase assays

Arginase activity was determined by the measurement of urea produced by the reaction of Ehrlich’s reagent according to the modified method of [18]. The reaction mixture contained, in final concentration, 1.0 mM Tris-HCl buffer, and pH 9.5 containing 1.0 mM MnCl₂, 0.1 M arginine solution and 50 µl of the enzyme preparation in a final volume of 1.0 ml. The mixture was incubated for 10 min at 37 °C. The reaction was terminated by the addition of 2.5 ml Erlich reagent (2.0 g of p-dimethylaminobenzaldehyde in 20.0 ml of concentrated hydrochloric acid and made up to 100 ml with distilled water). The optical density reading was taken after 20 minutes at 450 nm. The urea produced was estimated from the urea curve (graph of optical density against urea concentration). The unit of activity of arginase is defined as the amount of enzyme that will produce one μmol of urea per min at 37°C.

Rhodanese and protein assay

Rhodanese activity was measured during purification and routinely according to the method employed by [9] using KCN and Na₅SO₃, as substrates. The activity of the enzyme is expressed in Rhodanese unit (RU). One Rhodanese unit is taken as the amount of enzyme which under the given conditions will produce an optical density reading of 1.08 at 460 nm [19]. Method was used to measure the protein concentration of the enzyme using Bovine Serum Albumin (BSA) as standard.

Statistical analysis

The results are presented as means ± SD. Data were analyzed by one-way ANOVA using SAS/PC soft ware. Duncan multiple range tests were used for paired comparisons. A p-value < 0.05 was considered statistically significant.

RESULTS

The activities of the two enzymes are presented in (Tables 1-3). The protein concentrations of *Tilapia zillii*, *Hepstus odea* and *Sarothodelon galileaus* gills were estimated to be 5244.37 ± 391.09, 1490.08 ± 105.58 and 1166.59 ± 368.07 respectively; while in their flesh, it was estimated to be 4431.94 ± 56.82, 1606.89 ± 142.94 and 799.47 ± 1.81 respectively. Also, the protein concentration in the gut was found to be 4084.26 ± 1285.08, 1530.19 ± 214.32 respectively. From these results, *Tilapia zillii* has the highest protein concentration, followed by *Sarothodelon galileaus* and *Hepstus odea*.

The results also showed that both enzymes have a statistically high specific activity in *Hepstus odea* in all the tissues, followed by *Sarothodelon galileaus* and *Tilapia zillii*. Moreover from table 3, the tissue with the highest protein concentration is the gill, then the flesh and the gut, although the differences in their protein concentrations were statistically insignificant. The gill also showed the highest MST specific activity statistically, afterwards the flesh and the gut, and then rhodanese specific activity was however revealed to be high in the gut than other tissues, but not statistically significantly different from the other tissues.

DISCUSSION

Fish and aquatic invertebrates are particularly sensitive to cyanide exposure. Free cyanide was reported to be the primary toxic agent in the aquatic environment. Environmentally relevant exposures to cyanide ions can cause stress, increase in mortality and place an appreciable metabolic load on fishes and other aquatic organisms [7]. Sulphurtransferases are widely distributed enzymes of prokaryotes and eukaryotes [20]. The enzymes catalyze the transfer of sulphane sulphur from a donor molecule, such as thiosulphate or 3-mercaptopyruvate, to a nucleophilic acceptor, such as cyanide or mercaptoethanol.
Table 1: Protein concentration and enzyme activity assays in different tissues of the three fishes.

<table>
<thead>
<tr>
<th>TISSUE</th>
<th>Protein Concentration</th>
<th>Rhodanese Specific Activity</th>
<th>MST Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TZ GILLS</td>
<td>5244.37 ± 391.09</td>
<td>0.09 ± 0.00</td>
<td>0.07 ± 0.00</td>
</tr>
<tr>
<td>HO GILLS</td>
<td>1490.08 ± 105.58</td>
<td>0.19 ± 0.07</td>
<td>0.08 ± 0.02</td>
</tr>
<tr>
<td>SG GILLS</td>
<td>1166.59 ± 368.07</td>
<td>0.17 ± 0.02</td>
<td>0.09 ± 0.01</td>
</tr>
<tr>
<td>TZ FLESH</td>
<td>4431.94 ± 56.82</td>
<td>0.13 ± 0.03</td>
<td>0.08 ± 0.01</td>
</tr>
<tr>
<td>HO FLESH</td>
<td>1606.89 ± 142.94</td>
<td>0.15 ± 0.01</td>
<td>0.14 ± 0.03</td>
</tr>
<tr>
<td>SG FLESH</td>
<td>799.47 ± 1.81</td>
<td>0.16 ± 0.03</td>
<td>0.09 ± 0.02</td>
</tr>
<tr>
<td>TZ GUT</td>
<td>4084.26 ± 1285.08</td>
<td>0.17 ± 0.02</td>
<td>0.13 ± 0.03</td>
</tr>
<tr>
<td>HO GUT</td>
<td>456.49 ± 67.63</td>
<td>0.24 ± 0.02</td>
<td>0.15 ± 0.01</td>
</tr>
<tr>
<td>SG GUT</td>
<td>1530.19 ± 214.32</td>
<td>0.10 ± 0.01</td>
<td>0.12 ± 0.02</td>
</tr>
<tr>
<td>Overall Mean</td>
<td>2312.25</td>
<td>0.18</td>
<td>0.104</td>
</tr>
<tr>
<td>p Value</td>
<td>&lt;.0001</td>
<td>0.3678</td>
<td>0.08</td>
</tr>
</tbody>
</table>

TZ = *Tilapia zillii*; HO = *Hepstus odea*; SG = *Sarothodelon galileaus*
The values in table represent the mean ± SD (n = 5), all values in the same group are significantly different at (p < 0.05). Different alphabet superscript shows that the values are statistically different from other groups.

Table 2: Approximate protein concentration and enzyme activity assays in the three fishes.

<table>
<thead>
<tr>
<th>Fish Spp.</th>
<th>Protein Concentration</th>
<th>MST Specific Activity</th>
<th>Rhodanase Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>TZ</td>
<td>4586.9a</td>
<td>0.09b</td>
<td>0.13b</td>
</tr>
<tr>
<td>SG</td>
<td>1184.5b</td>
<td>0.10ab</td>
<td>0.14b</td>
</tr>
<tr>
<td>HO</td>
<td>1165.4b</td>
<td>0.12a</td>
<td>0.26</td>
</tr>
</tbody>
</table>

TZ = *Tilapia zillii*; HO = *Hepstus odea*; SG = *Sarothodelon galileaus*
The values in table represent the mean ± SD (n = 5), all values in the same group are significantly different at (p < 0.05). Different alphabet superscript shows that the values are statistically different from other groups.

Table 3: Protein concentration and enzyme activity assays in different tissues.

<table>
<thead>
<tr>
<th>Tissues</th>
<th>Protein Concentration</th>
<th>MST Specific Activity</th>
<th>Rhodanase Specific Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>GILLS</td>
<td>2633.7a</td>
<td>0.13a</td>
<td>0.17a</td>
</tr>
<tr>
<td>FLESH</td>
<td>2279.4a</td>
<td>0.10a</td>
<td>0.15a</td>
</tr>
<tr>
<td>GUT</td>
<td>2023.6a</td>
<td>0.08a</td>
<td>0.23a</td>
</tr>
</tbody>
</table>

TZ = *Tilapia zillii*; HO = *Hepstus odea*; SG = *Sarothodelon galileaus*
The values in table represent the mean ± SD (n = 5), all values in the same group are significantly different at (p < 0.05). Different alphabet superscript shows that the values are statistically different from other groups.

Sulphurtransferases may also play a part in the management of the cytotoxicity of reactive oxygen species in aerobic tissues [21].

Mining of gold and other mineral resources is very important in several developing countries like Nigeria. Active and abandoned primary and secondary goldmines have been observed to be major sources of metals into the environment. The presence in excess or above threshold level of heavy metals like Chromium (Cr), Copper (Cu), Cadmium (Cd), Zinc (Zn), Lead (Pb), Aluminum (Al) etc have been attributed to be major cause of several diseases in human body.

The unscrupulous gold mine activities in Igun town have been a major source of concern, as it exposes the rivers and waters in the environment to pollution through the leaching of cyanide and heavy metals to these rivers. Particularly, it is observed that the mining activities are not manned by any government regulations, therefore, the more it endangered the aquatic and human lives. Fishes (mainly herbivorous), ingest a wide variety of natural food organisms, including plankton, succulent green leaves, benthic organisms, aquatic invertebrates, larval fish, detritus and decomposing organic matter. The fish however, obtain substantial nutritional benefit from plant materials, some of which have been reported to be cyanogenic [2].

In this work, cyanide detoxifying enzymes (rhodanese and 3-MST) were estimated in some tissues of different fishes. From these results, it is clear that *Tilapia zillii* has the highest protein concentration, then *Sarothodelon galileaus* and *Hepstus odea*. The results also showed that both enzymes have a statistically high specific activity in *Hepstus odea* in all the tissues, followed by *Sarothodelon galileaus* and *Tilapia zillii*. Moreover from (Table 3), the tissue with the highest protein concentration is the gill, then the flesh and the gut, although the differences in their protein concentrations were statistically insignificant. The gill also showed the highest MST specific activity statistically, afterwards the flesh and the gut, and then rhodanese specific activity was however revealed to be high in the gut than other tissues, but not statistically significantly different from the other tissues [22]. Had shown that gills are multifunctional organs responsible for respiration, osmoregulation and acid base balance. Therefore, the high activity of MST in the gill of the fishes and gut could be...
physiological, since the organ (gill) is involved in respiration, a function that is prone to cyanide attack. There was no significant difference in the rhodanese activity of the three fishes. The presence of these sulphurtransferases (rhodanese and 3-MST) in the tissues of these fishes is an indication of high cyanide detoxifying mechanism and a possible way of coping with the heavy metal pollution in the water, a protective and possible physiological mechanism for the survival of these organisms in their environment.

In Nigeria especially Igun – Ijesa, gold mining activity is done under unregulated conditions. As a result, the potent pollution of ground water by cyanide and heavy metals which are toxic to aquatic lives and of those that feed on them. The public awareness of ground water by cyanide and heavy metals which are toxic to their environment.

REFERENCES


