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Full Length Research Paper

The distribution of cyanide detoxifying enzymes (rhodanese and 3- mercaptopyruvate sulphurtransferase) in different species of the family Cichlidae (*Tilapia zillii, Sarotherodon galilaeus* and *Oreochromis niloticus*)

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The cyanide metabolising enzymes (rhodanese and mercaptopyruvate sulphurtransferase) were estimated in different tissues of three species of tilapia (*Tilapia zilli, Sarotherodon galileus,* and *Oreochromis niloticus*) from two locations (Aiba and Osinmo reservoirs, both in the 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. There was no significant difference in the activity of rhodanese in the tissues of the tilapia species from Aiba River. Mercaptopyruvate sulphurtransferase (3-MST) presented a different pattern of distribution with significant difference in the gut of the tilapia species. The study showed the activities of two cyanide detoxifying enzymes (rhodanese and mercaptopyruvate sulphurtransferase) in the different tilapia species indicating the presence of strong cyanide detoxifying mechanisms.

Key words: Rhodanese, mercaptopyruvate sulphurtransferase, cyanide, tilapia, detoxification.

INTRODUCTION

Cyanide is a highly toxic compound that is readily absorbed and causes death by preventing the use of oxygen by tissues (Egekeza and Oehme, 1980). This toxicant is widespread in the environment. Many naturally occurring substances as well as industrial products contain cyanide (Egekeza and Oehme, 1980). More than 2,000 species of plants are known to contain cyanogenic glycosides (Vennesland et al., 1982). It has been reported that ingestion of cyanogenic glycosides in forage crops can result in the death of grazing animals (Keeler et al., 1978). Many studies report the death of birds from cyanide poisoning through several routes, including exposure to cyanide salts or ingestion of cyanogenic plants (Wiemeyer et al., 1986). 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 (Leduc, 1984).

Two major enzymes (rhodanese: EC. 2.8.1.1., thiosulphate: cyanide sulphurtransferase and mercaptopyruvate 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 (Westley, 1981). The enzyme is well characterised 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 (Marrs and Ballantyne, 1987). However, Aminlari and Gilanpour (1991) and Aminlari and Shahbazi (1994) have shown that different parts of the

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stomach in sheep and cattle and the proventriculus in chickens contain greater rhodanese activity than liver.

Unlike rhodanese, MST has not being fully characterised. 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 (Jarabak and Westley, 1974; Jarabak and Westley, 1978; Westley, 1981; Nagahara et al., 1995). In the case of MST, mercaptopyruvate was found to be the donor substrate (Westley, 1980). Recent works have shown that different genes codes for the two cyanide detoxifying enzymes with speculations that they have the same origin (Nagahara et al., 1995; Nagahara et al., 1996). Reports have also shown that the pattern of distribution of these two enzymes might affect the physiological fate of cyanide (Ali et al., 2000). Also, the activity of the enzymes in a particular tissue/organ may reflect the ability of that tissue/organ to detoxify cyanide (Ali et al., 2000).

We report here the pattern of distribution of rhodanese and 3-MST in different internal sections of the tilapia species obtained at different locations. The results might indicate the parts of the tilapia species that are significantly involved in cyanide metabolism.

MATERIALS AND METHODS

Materials

Potassium cyanide, sodium thiosulphate, boric acid, sodium borate and mercaptoethanol were all purchased from Sigma Aldrich Chemicals, USA. All reagents used were of analytical grades.

Methods

Collection of samples

Different tilapia species were collected between April and August 2009, during a fishing tour of two reservoirs (Aiba and Osinmo reservoirs, both in the South-Western region of Nigeria). The fish species were collected alive from their normal environment and stored in an ice container before transporting to the laboratory where they were stored at temperature below 0°C until ready for use.

The fish species were *Tilapia zilli, Sarotherodon galileus* and *Oreochromis niloticus*. They were identified at the Fish Culture Laboratory, Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of tissue extract

Prior to extraction, the tilapia species were dissected and the various tissues of interest (liver, guts and gills) were removed. These were later kept in the refrigerator until required. Tissue extracts were prepared by homogenising 10 g (w/v) of each tissue in 3 volume 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.

Protein and enzyme assays

Bradford method (1976) was used to measure the protein concentration of the enzyme using bovine serum albumin (BSA) as standard. Rhodanese was assayed by the method of Agboola and Okonji (2004). The reaction mixture contained 50 mM sodium thiosulphate, 50 mM potassium cyanide, 0.25 mM borate buffer, pH 9.4 and 10 µl of enzyme solution in a final volume of 1.0 ml. The reaction was carried out for 1 min at 37°C and stopped by adding 0.5ml 15% formaldehyde and 1.5 ml of Sorbo reagent (which is made up of ferric nitrate solution containing 0.025 g $Fe(NO_3)_3$,9 H_2O in 0.74 ml water and 0.26 ml concentrated nitric acid). Absorbance was measured at 460 nm. The unit of enzyme activity was defined as micromoles thiocyanate formed per minute at 37°C and pH 9.2.

Mercaptopyruvate sulphurtransferase activity was measured according to the modified method of Taniguchi and Kimura (1974). The reaction mixture in a final volume of 1 ml contained 0.38 M of Tris HCl buffer, pH 7.8, 0.5 M potassium cyanide, 0.3 M mercaptoethanol and 30 µl of enzyme solution. Absorbance was also measured at 460 nm. The unit of enzyme activity was defined as micromoles mercaptocyanate formed per minute at 37°C and pH 7.8

Statistical analysis

The results are presented as means ± SD. Data were analyzed by one-way ANOVA by using SAS/PC soft ware to examine whether there was any statistical difference among groups. Duncan multiple range test was used for paired comparisons. A *P* value less than 0.05 was considered statistically significant.

RESULTS

The activities of the two enzymes are presented in Tables 1-4. In most of the fishes, there were no significant differences in the values, though, the highest enzyme activities (rhodanese and 3-MST) were found in the livers of the three species of tilapia fish. Except for the livers of *S. galileus and T. zilli* where the 3-MST activity were lower compared to the activities found in the guts and gills of the fishes.

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 (Eisler, 1991). Sulphurtransferases are widely distributed enzymes of prokaryotes and eukaryotes (Nakamura et al., 2000; Bordo and Bork, 2002). 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. Sulphurtransferases may also play a part in the management of the cytotoxicity of reactive oxygen species in aerobic tissues (Nandi et al., 2000). However the natural

Table 1. Mean rhodanese activity of tilapia species from Aiba reservoir.

Fish species	Liver	Gut	Gills
Oreochromis niloticus	0.132 ± 0.01 A	0.117 ± 0.04 A	0.113 ± 0.02 A
Sarotherodon galileus	0.298 ± 0.07 A	0.228 ± 0.05 A	0.118 ± 0.04 A
Tilapia zilli	0.146 ± 0.05 A	0.151 ± 0.01 A	O.127 ± 0.03 A
P- value	< 0.165	< 0.431	< 0.411

Values are means ±SD (n=15); Results are mean of five determinations.

Table 2. Mean 3-MST activity of tilapia species from Aiba reservoir.

Fish species	Liver	Gut	Gills
Oreochromis niloticus	0.157 ± 0.02 A	0.135 ± 0.02 A	0.140 ± 0.00 A
Sarotherodon galileus	0.100 ± 0.01 A	0.082 ± 0.01 B	0.075 ± 0.01 B
Tilapia zilli	0.196 ± 0.02 A	0.127 ± 0.01 A	0.144 ± 0.01 A
P-value	< 0.083	< 0.144	< 0.251

Values are means ±SD (n=15); Results are mean of five determinations

Table 3. Mean rhodanese activity of tilapia species from Osinmo reservoir.

Fish species	Liver	Gut	Gills
Oreochromis niloticus	0.199 ± 0.03 A	0.168 ±0.05 A	0.770 ± 0.06 A
Sarotherodon galileaus	0.326 ±0.03 A	0.222 ± 0.02 A	0.172 ± 0.08 A
Tilapia zilli	0.161 ± 0.04 A	0.503 ± 0.04 A	0.134 ± 0.07 A
P- value	< 0.112	< 0.218	< 0.156

Values are means ± SD (n = 15); Results are mean of five determinations.

Table 4. Mean 3-MST activity of tilapia species from Osinmo reservoir.

Fish species	Liver	Gut	Gills
Oreochromis niloticus	0.311 ± 0.00 A	0.145 ± 0.00 A	0.132 ± 0.02 A
Sarotherodon galileus	0.087 ± 0.01 B	0.136 ± 0.00 A	0.087 ± 0.01 B
Tilapia zilli	0.080 ± 0.01 B	0.139 ± 0.01 A	0.154 ± 0.03 A
P-value	< 0.491	< 0.622	<0.225

Values are means ±SD (n=15); Results are mean of five determinations.

sulphane donors and acceptors and the physiological functions of most Sulphurtransferases remain uncertain. The activities around the two rivers (Aiba and Osinmo reservoirs) have been described as those that enhance increase in environmental pollution especially the use of fertilizers and other chemicals by farmers, effluent from industries and poor sewage disposal by the locals. Tilapia fish (a 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.

In this work, cyanide detoxifying enzymes (rhodanese and 3-MST) were estimated in some tissues of different tilapia species. There was no significant difference in the activity of rhodanese in the tissues of the three species of tilapia from Aiba reservoirs (Table1). In Table 2, there were slight variations in the distribution of 3-MST in the tilapia species from Aiba reservoirs. However there was significant correlation in the activity of 3-MST in the gut and gills of *S. galileus* as compared to the other two species.

The distribution pattern of rhodanese in the different species of tilapia in Osinmo reservoirs was similar to that of Aiba reservoirs (Table 3 and 4). Though no specific study of the subcellular distribution has been made. This

distribution pattern may be attributed to the function of rhodanese in the detoxification of cyanide as this could be the primary function of rhodanese in these species. The activity of rhodanese in the gut of *T. zillii* was found to be very high with no significant difference as compared with the other two species. The activity of rhodanese in the gills of *Oreochromis* was highest compared to the other two species (Table 3). Kenneth (1991) had shown that gills are multifunctional organs responsible for respiration, osmoregulation and acid base balance. Therefore, the high activity of rhodanese in the gill of the tilapia species 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 liver rhodanese activity of the three species. Although the rhodanese activity was highest in the liver of *S. galileus*, the distribution of MST among the species showed similar activity in the gut of the tilapia species while the activity of the enzyme in the gill was not significantly different in the *O. niloticus* and *T. zilli*. In the liver, MST in *O. niloticus* was significantly different from *T. zilli* and *S. galileus.* The presence of these sulphurtranferases (rhodanese and 3-MST) in the tissues of these tilapia species is an indication of high cyanide detoxifying mechanism, a protective and possible physiological mechanism for the survival of these organisms in their environment.

In Nigeria, rapid urbanization as well as, high crop yield, severe pest problems, weeds, rodents, locusts and grain eating birds have increased reliance on the use of pesticides, herbicides and fertilizers. Most of which are a cheap sources of cyanides and are widely used. Also co-disposal of industrial, domestic and medical waste in open dumps has contributed immensely to pollution menace in the region. The public awareness on health hazards and risks associated with pesticides and herbicides are also relatively low in Africa, hence the need for continuous monitoring of pollutants such as cyanides in fresh and marine waters and also biota such as fish.

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