The Distribution of the Enzyme Arginase in the Tissues of Selected Cichlidae Species: *Tilapia zillii, Sarotherodon galilaeus* and *Oreochromis niloticus*

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Abstract

The paper reports the tissue distribution of the enzyme arginase in three different Cichlids: *Tilapia zilli, Sarotherodon galilaeus* and *Oreochromis niloticus*, from the Aiba and Osinmo reservoirs, located in the south-western Nigeria. The tissues of *S. galilaeus* showed very high activity of arginase as compared with the other two species. The liver of *O. niloticus* and the gut of *T. zillii* showed very high activity of arginase in the Osinmo reservoir. The high arginase activity observed in the tissues of these organisms is attributed to ureotelism and is similar to the result obtained for tilapia, *Alcolapia grahami*, from lake Magadi, Kenya.

Introduction

Arginase is a hydrolytic enzyme responsible for the conversion of L-arginine to L-ornithine and urea (Jenkinson *et al.*, 1996). Arginases have been reported to subserve a number of different roles in different tissues and in different organisms. These roles include polyamine metabolism (Russell & McVicker, 1972), proline and glutamate synthesis (Verma & Boutwell, 1981), g-aminobutyric acid (GABA) formation (Johnson & Roberts, 1984), immune system function and nitric oxide synthesis (Cook *et al.,* 1994, Jenkinson *et al.*, 1996). The function of arginase in tissues lacking complete urea cycle is thought to be the production of L-ornithine, a biosynthetic precursor of L-proline (Yip & Knox, 1972). It has played a valuable role in the elucidation of key metabolic pathways and concepts which include urea cycle, Kreb Cycle, amino acid biosynthesis (transamination) and ammonium metabolism (Jenkinson *et al*., 1996; Ash, 2004).

In uricotelic organisms, that include bacteria, fungi, invertebrates, reptiles and birds (Bellairs, 1969; Jenkinson *et al.,* 1996) and ammonotelic organisms such as fish and amphibians (at early stage of development) (Mora *et al*., 1965ab; Bellairs, 1969; Jenkinson *et al.,* 1996), complete urea cycle enzymes are lacking. Thus, the function of arginase in these organisms is the production of ornithine which is channelled into metabolic pathways associated with proline and glutamate production (Jenkinson *et al*, 1996). Aquatic animals, including invertebrates, fish and larval amphibians, excrete mostly ammonia (Wood, 1993; Wright & Land, 1998). Ammonia is highly soluble in water and permeates cell membranes relatively easily.

Tilapia fishes are mainly freshwater fishes and are members of Teleost (bony) fishes (Wood *et al*., 1989). Ammonia is the main nitrogenous excretion product of freshwater Teleost fishes while urea excretion in teleost make up the secondary but significant

component of total nitrogen excretion (Campbell & Anderson, 1991; Wood, 1993). Its waste basically occurs through the gills, and the ammonium ion (NH_4^+) prevails over the non ionic ammonia (NH_3), the practically impermeable form.

Environmental conditions have been reported by Campbell & Anderson (1991) to be the common stimulus for urea synthesis in teleost fishes. For instance, Lake Magadi in Kenya *Oreochromis alcalicus grabami* live in very high alkaline water (*p*H 10) that is unfavourable for ammonia diffusion across the gills (Mommsen & Walsh, 1992; Wood, 1993). However, the activities around two reservoirs (Aiba & Osinmo reservoirs in south-western Nigeria) have been observed as those that enhance increase in environmental pollution, especially the use of fertilizers and other chemicals by farmers, and poor sewage disposal by the locals (Atobatale, 2008; Komolafe & Arawomo, 2008). These pose difficult environmental conditions such as alkaline waters and seasonal drought. The main objective of the present study, therefore, was to measure the activities of the enzyme arginase in different tissues of some *Tilapia* species (*Tilapia zillii, Sarotherodon galileus* and *Oreoch-romis niloticus*) in these reservoirs.

Materials and methods

Tris-HCl, tris-base, manganese chloride, and arginine were all purchased from Sigma Aldrich Chemicals, USA. All other reagents used were of analytical grades.

Collection of samples

Cast-net was used once a month to collect fish samples between April and August 2009 in Aiba and Osinmo reservoirs, both in south-western region of Nigeria. The fish samples were stored in an ice-chest before transporting to the laboratory where they were stored at temperature below 0 °C until ready for use. The fish species *viz*. *T. zillii, S. galilaeus* and *O. niloticus* were identified using the keys by Holden & Read (1978) and Adesulu & Sydenham (2008) at the Fish Culture Laboratory, Department of Zoology, Obafemi Awolowo University, Ile-Ife, Nigeria.

Preparation of tissue extract

Prior to extraction, the Cichlid species were slit open and the various tissues of interest (liver, guts and gills) were removed and stored at 4 °C until required. Tissue extracts were prepared by homogenising 10 g (w/v) of each tissue in three volumes of homogenisation buffer (phosphate buffer, *p*H 7.2). The suspensions were centrifuged for 20 min at 4,000 r.p.m. in a Microfield Centrifuge Model 800 D. The supernatants were used as the source of enzyme.

Protein and enzyme assays

Arginase activity was determined by the measurement of urea produced by the reaction of Ehrlich's reagent according to the modified method of Kaysen & Strecker (1973). The reaction mixture contained in final concentration 1.0 m*M* Tris-HCl buffer, *p*H 9.5 containing 1.0 m*M* $MnCl_2$ 0.1 *M* arginine solution and 50 mml of the enzyme preparation in a final volume of 1.0 m. The mixture was incubated for 10 min at 37 °C. The reaction was terminated by the addition of 2.5 ml Erhlich reagent (2.0 g of p-dimethylaminobenzaldelyde 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 min at 450 nm. The urea produced was estimated from the urea curve (graph of optical density against urea concen-tration). The unit of activity of arginase is defined as the amount of enzyme that will produce one mmol of urea per min at 37 °C. Bradford method (1976) 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 by using SAS/PC software 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 activity of arginase in the three Cichlid species is presented in Table 1. In Aiba reservoir, activity of arginase was found to be as high as 0.40 ± 0.00 and 0.49 ± 0.01 in the liver and gut of *S. galilaeus,* respectively. A slight variation was observed in Osinmo reservoir where arginase activity was highest in the liver (0.53±0.04) of *O. niloticus,* followed by the gut (0.48±0.01) and gills (0.29±0.02) of *T. zillii* (Table 1).

Discussion

Ureogenesis has been considered less important for most Teleosts (Wright, 1995). It was assumed for many years that genes of urea cycle (UC) enzymes had been lost from the Teleost genome (Wright, 1995; Jenkinson *et al*., 1996; Terjesen *et al*., 2001). However, the presence of UC enzymes in early life stages is being reported in some species, and proposed as a fact for most (Jenkinson *et al*., 1996), though it was not very clear whether ammonia excretion prevails over the urea. In spite of a few UC enzymes with different specific activities reported in many species (Nener, 1988; Mommsen & Walsh, 1992), the full set is uncommon.

It was reported that in difficult environmental conditions such as alkaline waters and seasonal drought, urea cycle enzymes remain active in a few adult Teleost species (Randall *et al.*, 1989). Arginine conversion to ornithine is catalyzed by arginase. This enzyme is widely distributed and its activity in fishes is supposed to change with the ingested protein level (Berge *et al.*, 1997). Usually, ammoniotelism

TABLE 1

Mean concentration of arginase activity (µmol/ml/min) from Aiba and Osinmo reservoirs

Fish species	*Liver (10 g: w/v)*		*Gut (10 g: w/v)*		*Gills (10 g: w/v)*	
	A	*O*	*A*	*O*	*A*	*O*
Oreochromis niloticus	0.36 ± 0.01	0.53 ± 0.04	0.09 ± 0.02	0.23± 0.04	0.25 ± 0.04	0.12 ± 0.01
Sarotherodon galilaeus	0.40 ± 0.00	0.23 ± 0.07	0.49 ± 0.01	0.13± 0.02	0.21 ± 0.02	0.27 ± 0.02
Tilapia zillii	0.20 ± 0.01		0.33 ± 0.03	0.07 ± 0.02	0.48± 0.01	0 . 2 3 ±
0.01	0.29 ± 0.02					

Values are means ± SD (n = 20); Results are mean of five determinations. A = Aiba; O = Osinmo; w/v = weight per volume.

predominates among the teleosts, and the transition to ureotelism is not clear. Alkaline environments frequently impair ammonia excretion by inhibiting NH_3 conversion to NH_4^+. This avoids diffusion through the gills (Timothy & Iwama, 1992; Wilkie *et al.*, 1993) and increases the plasma NH_3 (McGeer & Eddy, 1998).

In the present work, the distribution of arginase was found to be highest in the *S. galilaeus*. The three tissues (liver, gut and gill) of *S. galilaeus* from the Aiba reservoir showed relatively high activity of arginase (Table 1). The arginase activity was found to be high (between 0.23 ± 0.07 and 0.53 ± 0.04) in the liver tissues of *Tilapia* species from Osinmo reservoir, characterised with high alkalinity of 84.7 mg/l and 88.5 mg/l in rainy and dry season, respectively (Komolafe & Arawomo, 2008). However, the activities around the two reservoirs (Aiba and Osinmo rivers) have been observed as those that enhance increase in environmental pollution, especially the use of fertilizers and other chemicals by farmers, and poor sewage disposal by the locals (Atobatale, 2008; Komolafe & Arawomo, 2008). These pose difficult environmental conditions such as alkaline waters and seasonal drought. As consequence, urea cycle enzymes remain active in Teleost species.

Similar result was obtained by Cvancara (1969), whose findings on arginase activity from the liver tissue homogenates of 19 species of freshwater teleosts reveal that arginase levels were highest in liver tissue, followed by kidney and heart tissue. He went further to suggest that diet may play an important role in the level of liver arginase activity and that degradation of dietary arginine may contribute substantially to urea production in teleosts. Also Ozan *et al.* (1999), working on Rainbow trout (*Oncorhynchus mykiss*), found that arginase level in the liver was highest followed by kidney, skin, gills, muscle and eyes. The high level of arginase in the liver of these *Tilapia* species has been found to be caused by increase in the environmental *p*H which decreases ammonia transport through the gills, and then impairing nitrogenous waste (Campbell & Anderson, 1991; Moraes & Polez, 2003). The consequent toxicity is usually drastic to most fishes. A few species are able to synthesize urea as a way to detoxify plasma ammonia.

Moraes & Polez (2003) in their study of three teleost family of Erythrinidae (*Hoplias lacerdae*, *Hoplerithrynus unitaeniatus* and *Hoplias malabaricus*), living in distinct environments with distinct biochemical behaviours, were subjected to alkaline water and the urea excretion rate was determined. The studied species showed ureogenicity. Urea excretion of *H. malabaricus* was high in alkaline water, and the transition to ureotelism was proposed (Moraes & Polez, 2003). Hartenstein (1971) reported the distribution of crayfish arginase which occurs in soluble and particulate fractions of the hepatopancreas, abdominal muscle and thoracic wall of the crayfish. Singh & Singh (1988) also described the presence of arginase in the tissues (brain, liver, heart, kidney, spleen, gills, ovary and testis) of freshwater teleostean fish, *Clarias batrachus*. Of these tissues, liver, kidney and heart were found to contain the highest activities in that order, representing the tissues of high metabolic activities.

Biochemical functions and the importance of arginase in the liver, gill and other tissues of *Tilapia* species are not well understood. However, the high arginase activity observed in the tissues of these organisms could be an indication of ureotelism in alkaline water, as observed by other workers (Mommsen & Walsh, 1992; Wood, 1993; Wright, 1995; Moraes & Polez, 2003).

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