

**CHARACTERIZATION OF ARGINASE FROM THE TROPICAL FRESHWATER
TELEOST FISH *HOPLERITHRINUS UNITAENIATUS* (JEJU)**

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Arginase (L-arginine amidinohydrolase) catalyzes the hydrolyses of L-arginine into ornithine and urea (Reczkonski & Ask, 1994). This enzyme is the terminal step of the ornithine-urea cycle and its role on ammonia detoxification is the most explored issue. However, its involvement with several other functions have been reported. Occurrence of arginase in the nuclei as well as in cytoplasm of mammalian liver cells was first described by Dounce and Beyer (1942) but it is generally considered to be a mitochondrial enzyme in fishes (Casey and Anderson 1985; Carvajal *et al* 1989; Cao *et al* 1991; Dkhar *et al* 1991).

Usually ammonia excretion is not a problem for aquatic animals. While terrestrial have to synthesize urea through ornithine urea cycle (OUC), those one are able to excrete ammonia to the environment. However, the role of (OUC) enzymes could not be strictly related to urea excretion as it happened with anaplerotic cycles. Urea synthesis can be relegated to some second alternative facing environmental disturbance (Wright, 1995).

The marine elasmobranchs (sharks, rays and skates) are placed among the well known urea producers (Griffith, 1991). Those fish are able to synthesize and retain urea in their tissue and body fluids at concentrations higher than 0.4 M. This synthesis has the purpose of osmoregulation in the sea water environment (Smith 1936; Wood *et al* 1995). The same phenomenon is not reported in freshwater elasmobranchs. Some marine and freshwater teleosts are also reported presenting some OUC enzymes. Nevertheless, the role of them remains under discussion (Campbell & Anderson, 1991).

The presence of one or other OUC enzyme in animal tissues is not some indicative of urea synthesis through this cycle. The urea detected in the plasma may comes from other source than synthesis from OUC enzymes. It may involve purine degradation (for review see Wood *et al* 1989). In fish, the presence of arginase came to be proposed by its role in arginine catabolism (Cvancara, 1969). The use of OUC as strategy against environmental factors was reported in *Oreochromis nilotica* from the Lake Maghali (Wood *et al* 1989). The presence of OUC in lung fish have been also reported.

Hoplerithrinus unitaeniatus (jeju) presents a very intensively vascularized swim bladder. This species is known presenting the common behavior of moving from pond to pond across dry lands. This fact let us to propose the ability of urea excretion in this fish under some kind of stress as well as the study of urea cycle enzymes.

The kinetic characteristics of arginase are quite similar in several species. The awareness of them permits inferences about urea excretion strategies and its regulation. However, the correlation of arginase and urea excretion may not be always assumed. Direct or indirect evaluation of the process as a whole have to be done for any conclusion about it.

Experiments with *H. unitaeniatus* have shown urea excretion to the environment at a rate of $0.17 \cdot 10^{-3} \mu\text{mols} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of fish. Animals injected with urea showed a significant rise in the excretion rate ($3.10 \cdot 10^{-3} \mu\text{mols} \cdot \text{min}^{-1} \cdot \text{gram}^{-1}$ of fish). The injection of arginine also leads to an increase in the urea excretion rate ($0.55 \cdot 10^{-3} \mu\text{mols} \cdot \text{min}^{-1} \cdot \text{gram}^{-1}$ of fish). However, ornithine administration resulted in a quite similar excretion rate compared to the control ($0.2 \cdot 10^{-3} \mu\text{mols}^{-1} \cdot \text{min}^{-1} \cdot \text{gram}^{-1}$ of fish).

The urea excretion was linear along the course of 120 minutes but this rate seems not to be the maximum when compared to values obtained in animals subjected to urea injections. Under that condition it was observed a rise of 18 times when compared to the control and the urea excretion, despite has been kept linear in the course of 20 minutes, presented an hyperbolic excretion profile along 120 minutes. All these results are presented in figure 1.

The urea excretion observed in jeju was 10 times lower than values observed in traira *Hoplias malabaricus* ($1.86 \cdot 10^{-3} \mu\text{mols} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of fish), another species belonging to Erythrinidae, and tilapia *Oreochromis nilotica* ($1.80 \cdot 10^{-3} \mu\text{mols} \cdot \text{min}^{-1} \cdot \text{gram}^{-1}$ of fish) (Moraes, unpublished results). This fact may reflect some kind of transport for urea in *H. unitaeniatus*. Future experiments with different injected concentrations of urea followed by determination of its plasma values could suggest the preferential type of transport involved in the excretion of this metabolite. However, different patterns of excretion, with and without administration of urea, tempt us to assume about some kind of transporter.

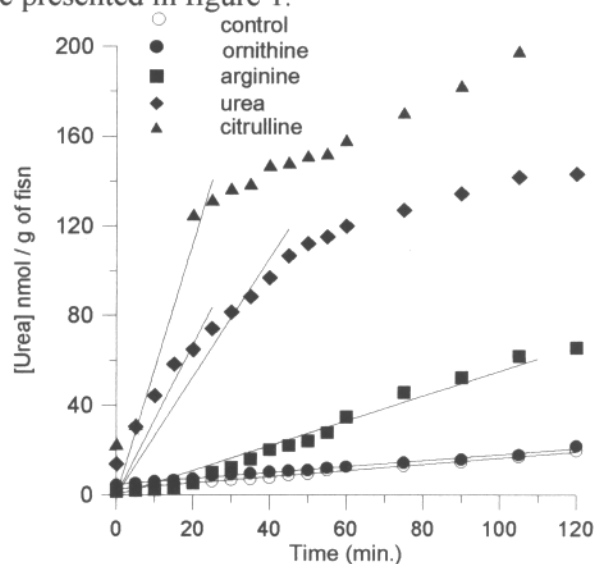


Fig. 1. Excretion rate of urea determined after urea, arginine, citrulline and ornithine injection against saline as a control

Previous administration of urea precursors as arginine citruline and ornithine, under the same conditions employed to urea excretion measurement, allow us to infer upon the steps of urea synthesis through OUC enzymes in *H. unitaeniatus*. Tilapia (*O. nilotica*), reported as ammoniotelic teleost, was employed as reference and, when injected with ornithine, resulted no increase in urea excretion rate. Although, administration of arginine increased 2.17 times the value determined for such species (Moraes, unpublished results). As well as in tilapia, only arginine has increased the urea excretion in jeju and the magnitude order observed was 3.2. Upon these results might be supposed the absence or non expression of all OUC enzymes in this fresh water teleost specially ornithine carbamoyl transferase. The way for denying such proposition was to study the urea synthesis step by step in this species.

Arginase was assayed and promptly detected in liver extracts of jeju. Arginase reaction conditions were optimized and the kinetic parameters were established for crude extract. The reaction kept linear along 40 minutes and 200 μg of wet tissue. The optimal pH observed was 9.3 and the optimal temperature was 45°C. The fit for arginine saturation curve is hyperbolic and the calculated K_m was 4.89 mM (Fig.2). The effect of ornithine on arginase activity was determined and complete inhibition was observed at a final concentration of 4.0 mM.

Ornithine carbamoyl transferase was detected in crude liver extracts and the specific activity value obtained was 2.28 $\mu\text{mols} \cdot \text{min}^{-1} \cdot \text{g}^{-1}$ of wet tissue under the experimental conditions.

The characteristics of arginase reported in this paper are similar to that observed in other animals so far described. The apparent K_m of 4.89 mM is inside the range of magnitude observed in other species. Arginase from *Clarias batrachus* is reported with K_m 15.38 mM at the optimal temperature of 37°C and pH of 9.5 and competitively inhibited by ornithine and leucine. Valine and isoleucine are described as non competitive inhibitor in this species (Sing and Sing, 1988, 1990). Ornithine is still reported as inhibitor at 7.5 mM and valine at 3.0 mM. Arginase from *Merluccius gayi* is reported with K_m of 1.7 mM at the optimum pH 9.5 (Carvajal *et al* 1989) and a K_m of 10.3 mM at pH 7.5 (Carvajal *et al* 1987). Proline is also reported as inhibitor for that arginase (Carvajal *et al* 1987).

Arginase described in the present paper seems to have similar affinity by arginine when compared to the others and the slight higher optimal temperature might reflect the environment from where come these fish.

The quite similar rates of urea excretion obtained from animals submitted to ornithine compared to the control let us to suppose two immediate possibilities for explaining them; the absence of ornithine carbamoyl transferase (OCT) or the inhibition of arginase by ornithine. Both assumptions were tested and the second is the most probable. The presence of OCT allow us to assume the ornithine transformation to citrulline in *H. unitaeniatus*. The strong inhibition of

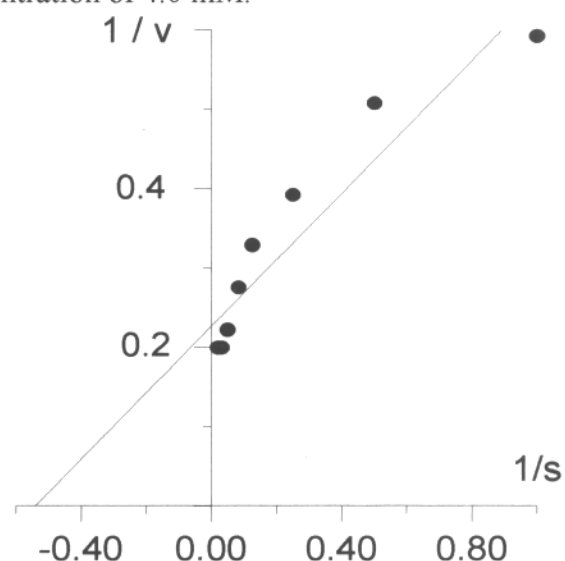


Figure 2. Kinetic characteristic of hepatic arginase of *H. malabaricus* (jeju). The K_m obtained for crude extract was 4,89 mM.

arginase by ornithine has permitted to understand about the similarity between urea excretion rates in animals submitted to ornithine against the control.

From the present results can be inferred the presence of all OUC enzymes in *H. unitaeniatus* specially when considered the urea excretion rate under citrulline administration. Such experiment has suggested that citrulline may be excreted through arginine succinate synthase (ASS) and arginine succinate lyase (ASL). The highest urea excretion rate under citrulline injection is difficult to be explained except by citrulline positive modulation of arginase.

The present paper reported the presence of OCT, the arginase characterization as well as the possibility of nitrogenous excretion through urea cycle in the tropical teleost fish *H. malabaricus*. Direct determination of ASL and ASS will allow us to confirm about that.

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