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Mini Review

Does fish represent an intermediate stage in the evolution of ureotelic cytosolic arginase I?

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ABSTRACT

Arginase catalyses the last step of the urea cycle. At least two isoenzymes of arginase are known; cytosolic ARG I and mitochondrial ARG II. ARG I is predominantly expressed in liver cytosol, as a part of urea cycle in ureotelic animals. The second isoform ARG II is primarily responsible for non-ureogenic functions, expressed in mitochondria of both hepatic and non-hepatic tissues in most vertebrates. Most micro-organisms and invertebrates are known to have only one type of arginase, whose function is unrelated to ornithine–urea cycle (OUC). However, in ureo-osmotic marine elasmobranchs arginase is localized in liver mitochondria as a part of OUC to synthesize urea for osmoregulation. An evolutionary transition occurred in arginase enzyme in terrestrial ureotelic vertebrates, with the evolution of ARG I from a pre-existing ancestral mitochondrial ARG II. This cytosolic ARG I activity is supposed to have first appeared in lung fishes, but the 40% and 60% distribution of arginase I and II activity in liver and kidney tissue of *Heteropneustes fossilis* indicates reconsideration of the above fact.

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Introduction

Arginase (L-arginine ureohydrolase, EC 3.5.3.1) is a binuclear manganese metalloenzyme that catalyses the hydrolysis of L-arginine to form L-ornithine and urea [1,2]. Arginase plays a fundamental role in nitrogen metabolism. The presence of arginase in liver is known since the work of Kossel and Dakin [3]. Clementi was one of the first workers to study the distribution of arginase in vertebrate subphylum [4,5]. He concluded that arginase was present in the liver of vertebrates that possess ureotelic metabolism and was absent in vertebrates having uricotelic metabolism. In 1925, Hunter and Dauphinee [6] found very high arginase activity in the liver of animals that can synthesize urea from ammonia and amino acids. Krebs and Henseleit in their demonstration of the first cyclic pathway showed that arginine acted catalytically to produce urea in presence of arginase [7]. Arginase is the main enzyme responsible for cyclic nature of the urea cycle. It occurs in many vertebrate tissues but is especially abundant in liver of ureotelic animals, which synthesize urea via OUC as a mean for nitrogen excretion [2]. Such type of distribution is related to the role of arginase as a component of the OUC. This enzyme is not exclusive to the ureotelic organisms, but is widely distributed and found throughout the evolutionary spectrum of living organisms. Virtually all organisms synthesize arginine using four other enzymes of the urea cycle, but only those having arginase are able to carry out complete OUC. In organisms and tissues lacking OUC, arginase probably regulates cellular arginine and ornithine homeostasis.

Functions of arginase

Compared with other urea cycle enzymes, arginase is much widely distributed in tissues, which suggests its important physiological roles apart from the urea cycle. The extrahepatic arginase has a relatively low specific activity compared with the hepatic type arginase and more likely to have a housekeeping anabolic function than the liver arginase with its highly specific catabolic role in the urea cycle. These include potential role as a regulator of the synthesis of ornithine, urea, creatine, polyamines, glutamate, proline, nitric oxide and/or agmatine [8]. Arginase hydrolyses arginine to ornithine that in turn serves as precursor for polyamine biosynthesis via ornithine decarboxylase (ODC) and proline and glutamate via ornithine amino transferase (OAT) (Fig. 1).

Glutamate is a key player in ammonia metabolism and can be converted to glutamine by glutamine synthetase (GS) and participate in the ammonia transport throughout the body [9]. Glutamate is an important neurotransmitter in the central nervous system. It can act directly or can be converted into gamma-amino butyric acid (GABA) [10]. Therefore, it is proposed that arginase has a potential role in the metabolic control of neurotransmitter synthesis. Proline is an essential component of structural proteins like collagens. Collagen make up about a third of the mass of vertebrates, composed of repeated proline and hydroxyproline [11]. ODC, which catalyses the conversion of ornithine to putrescine in an

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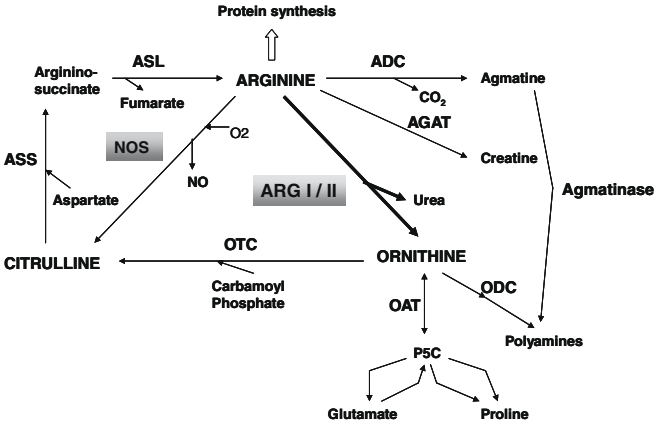

Fig. 1. The metabolic roles of the arginases. Abbreviations: ASS, argininosuccinate synthetase; ASL, argininosuccinate lyase; NOS, nitric oxide synthase; NO, nitric oxide; ARG, arginase; AGAT, arginine-glycine aminotransferase; ADC, arginine decarboxylase; ODC, ornithine decarboxylase; OAT, ornithine aminotransferase; P5C, 11-pyrroline-5-carboxylate; OTC, ornithine transcarbamylase.

irreversible reaction, is the key rate-limiting enzyme for the biosynthesis of polyamines (putrescine, spermidine and spermine), which plays a pivotal role in the control of DNA, RNA and protein synthesis during cell growth, differentiation and transformation of cells [12,13]. Role of arginase in controlling proline and polyamines synthesis would have wide ranging implications.

Many researchers have discussed the role of arginase in nitric oxide metabolism. Nitric oxide has been shown to play a fundamental role in many physiological processes falling into two broad categories: cell signalling and host defense mechanisms [14–16]. Nitric oxide is synthesized from arginine by nitric oxide synthase (NOS) with citrulline as a byproduct [17], which can be recycled to arginine by argininosuccinate synthase (ASS) and argininosuccinate lyase (ASL). It has been found that the endothelial nitric oxide (NO) synthesis depends on the activity of arginase in mitochondria and L-arginine carriers in cell membrane [18].

Arginase isoenzymes

It is a well substantiated fact that the arginase present in liver of ureotelic animals has different properties than the arginase found in the liver of uricotelic animals. An antibody to purified liver arginase cross reacts with the liver arginase of all ureotelic species so far, but it does not cross react with liver arginase of uricotelic animals. Moreover, it was found that the arginase of *Neurospora crassa* resemble the later more closely [19].

Arginase activity has been reported in several tissues of various animals [20]. Significant difference is found between arginases from mammalian hepatic and non-hepatic tissues based on isoelectric point (pI), immunological cross reactivity, charge and subcellular location. The above facts indicate the presence of another isoenzyme of arginase. The existence of multiple forms of arginase in eukaryotes suggests a complex regulatory role for this enzyme in the metabolism, development and maintenance of these organisms. The mammalian liver arginase is well characterized [21], however, the presence of a second form of arginase designated as ARG II has only been proven in 1996 [22–24] found to be present in low levels or absent in liver, but expressed in extrahepatic tissues. Although there are little disagreements between the tissue distributions of arginase genes among different mammalian species, type I arginase is generally found in hepatocytes [25], whereas type II arginase is widely expressed in virtually all mitochondria-containing non-hepatic cells and plays an important role in regulating synthesis of NO, polyamines and proline [26].

The comparative properties of ARG I and ARG II as given by Cederbaum et al. [2], it was hypothesized that the function of ARG I, found abundantly in liver cytosol was primarily in ureagenesis, while ARG II is mitochondrial and involved in many other functions like glutamate, proline and polyamine biosynthesis and modulation of NO synthesis in various organs. The cloning of three non-hepatic arginases in *Xenopus laevis* and the demonstration of their differential expression during metamorphosis is further evidence for a non-urea cycle role for arginase [27].

Distribution of arginase

Arginase has been studied in various groups of living kingdom and found to occur in almost all organisms as diverse as bacteria, yeast, plants, invertebrates and vertebrates except in some bacteria (*Escherichia coli*, *Streptomyces clavuligerous* and *Klebsiella aerogenes*), which are reported to have arginase family genes including agmatinase (Agmatine ureohydrolase), a probable agmatinase and a possible formiminoglutamate hydrolase, respectively. Agmatine is analogous to arginine; both produce urea as an end product. Region of homology between the two indicate a common active site structure [28]. The universal distribution of arginase suggests that it appeared very early in the process of biochemical evolution.

In animal kingdom, arginase activity is widely distributed in all ureogenic and non-ureogenic species and tissues of invertebrates and vertebrates. It is interesting to mention that uricotelic animals do not possess OUC and arginine biosynthetic pathway, instead they utilize purine biosynthetic pathway to excrete nitrogen [29]. Similarly, there are other organs that contain arginase and lack the capacity to synthesize arginine like brain, mammary glands, salivary glands, erythrocytes, etc. Significant arginase activity has been detected in a number of extrahepatic tissues which lack a complete urea cycle such as the kidney, heart, brain, placenta, lung, skeletal muscle, pancreas, spleen and testis [24]. This wide distribution in normal and pathological tissues supports the idea of many other functions for arginase beside its role in ureagenesis in the liver. Several studies have provided evidence that arginases from ammoniotelic animals and plants are localized in mitochondria [30–32]. In fishes the subcellular localization of arginase varies. In marine elasmobranchs *Squalus acanthias* arginase is mitochondrial in location [33]. In ammoniotelic fish rainbow trout, *Oncorhynchus mykiss* arginase activity is largely mitochondrial [34]. In avian species, arginase appears to be a mitochondrial enzyme [35]. In chicken mitochondrial arginase has been reported to be associated with either the matrix space [36] or with the intermembrane space [37]. In man and other mammals two forms of arginase exist, a cytoplasmic form located primarily in liver and a mitochondrial form expressed in lesser amounts in a larger number of organs, but especially kidney [38].

Evolution of arginase

In the beginning of life, living systems were relatively simple with small number of macromolecules for metabolic activities. Evolutionary process, subsequently introduced new macromolecules and metabolic pathways resulting in great biochemical complexity and variety of the existing living forms. Most micro-organisms and the invertebrates studied have only one type of arginase, whose function is unrelated to the OUC [39]. In lower vertebrates selective pressure for the integration of OUC for increased urea synthesis during the period of restricted water availability might have pre-adapted the early vertebrates for their transition from water to the land. The utilization of basic nutritional pathway of arginine synthesis for ammonia detoxification first took place in invertebrate animals [40,41] like in certain land planarians, earthworms

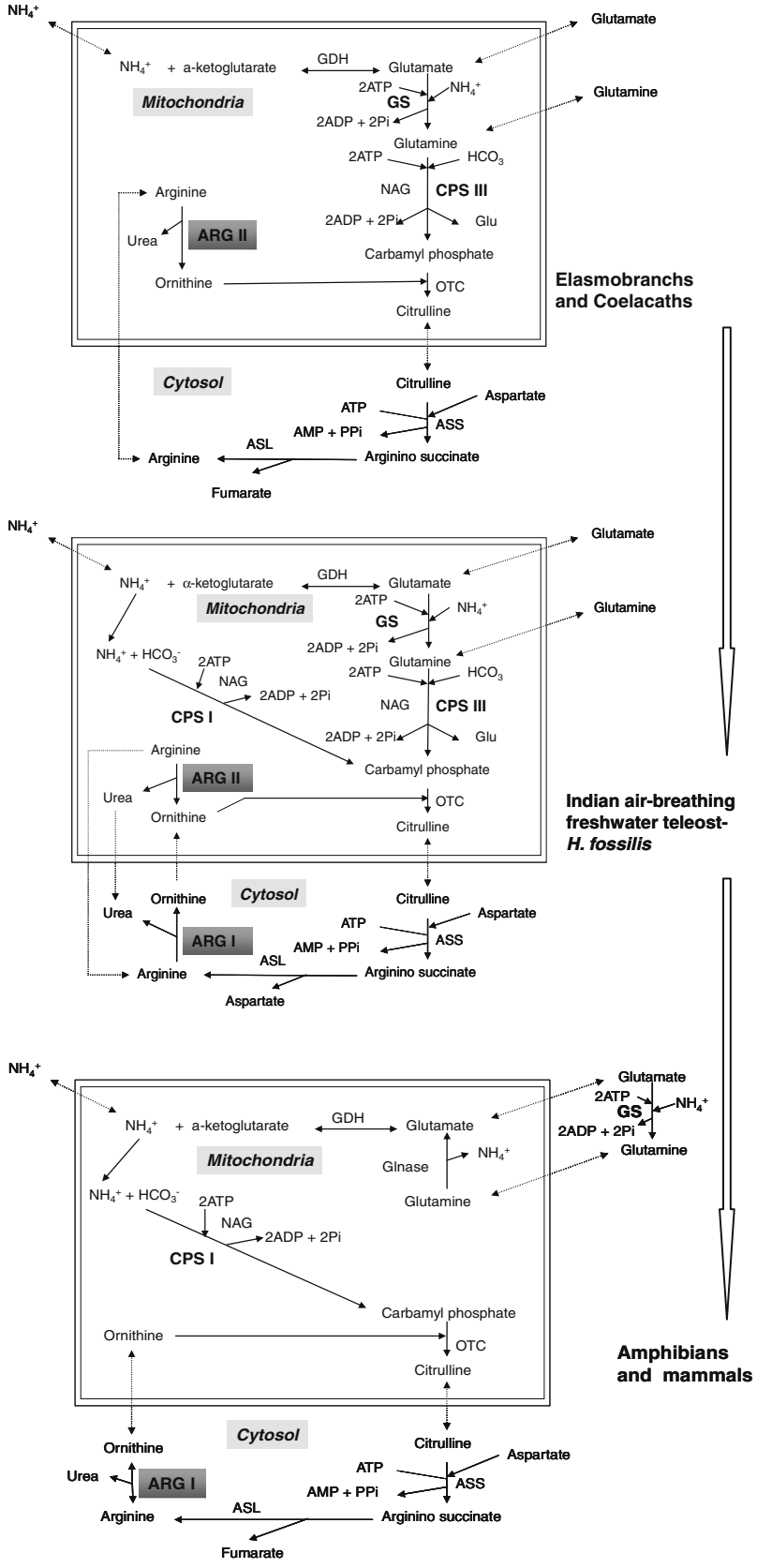

Fig. 2. Proposed changes in arginase isoenzymes and other OUC enzymes during ureotelic evolution in vertebrates.

[42] and snails [43,44] that synthesize excretory urea via OUC. The existence of a urea cycle is thought to keep ammonia levels low, preventing toxicity in ureotelic organisms by excreting excess nitrogen as urea, a neutral water-soluble molecule. Arginase

pathway and urea biosynthesis via OUC for ammonia detoxification and osmoregulation was common strategy in Paleozoic marine gnathostomes [45].

Elasmobranchs fishes, the coelacanth and estivating lungfish synthesize urea by the OUC; by comparison, urea synthetic activity is generally insignificant in teleostean fishes. In ureotelic species, ammonia generated or transported inside the mitochondria is detoxified to citrulline in the presence of carbamoyl phosphate synthetase I (CPS I) and ornithine trans-carbamoylase (OTC) [46,47] (Fig. 2). Citrulline is then transported out of mitochondria for ultimate conversion to ornithine and urea by three other OUC enzymes, ASS, ASL and ARG, located in cytosol. Ornithine enters the mitochondria to complete the cycle. The arginase from liver of spiny dogfish, *S. acanthias* is a mitochondrial enzyme [48] in contrast to the cytosolic liver arginase of ureotelic species associated with urea synthesis. The significance of localization of arginase in the mitochondria will be that the urea is formed inside the mitochondria. Mitochondrial CPS III and GS are significantly inhibited by physiological concentrations of urea [49,50] which could be of importance in osmoregulation, resulting in feedback inhibition of CPS III [51,52]. Another consequence of mitochondrial localization of arginase is that the ornithine availability for citrulline synthesis is dependent on the transport of arginine into mitochondria and its subsequent hydrolysis to ornithine by arginase. In *Squalus* arginine and ornithine both are equally permeable to the mitochondrial membrane. So ornithine can rapidly exit the mitochondria if not utilized for citrulline synthesis and since the arginase activity is sufficiently high, the formation of ornithine from arginine is not rate limiting for citrulline synthesis, thus mitochondrial localization of arginase does not appear to reflect a mechanism for regulating ornithine availability [33]. Coelacanths similar to marine elasmobranchs have functional OUC with mitochondrial arginase and retain relatively high levels of urea and trimethylamine oxide (TMAO) as a strategy for osmoregulation [53].

While determining the subcellular location of arginase in a variety of fish species Mommsen and Walsh [54] proposed that cytosolic arginase first appeared in the lungfish. However, few representatives of fishes of the family Batrachoididae (*Opsanus beta*, *Opsanus tau* and *Porichthys notatus*) and Heteropneustidae (*Heteropneustes fossilis*) showed the expression of both mitochondrial and cytosolic activities of arginase in the liver [55,56]. These fishes are primarily ammoniotelic, becomes facultative ureotelic when exposed to stressful environmental situations like high ammonia concentrations or extended exposure to air [57–59]. This indicate that urea can potentially be generated by arginase activity within both cytoplasmic (analogous to the ureotelic terrestrial vertebrates, where arginase is localized in cytosol) and mitochondrial (analogous to ureo-osmotic marine elasmobranchs, where arginase is exclusively localized in mitochondrial matrix) compartments.

Early biological evolution required creation of new metabolic machinery encoded in new deoxyribonucleic acid (DNA), but the subsequent course of evolution was depended primarily upon the modification and elaboration of pre-existing DNA [60,61]. According to Dykhuizen–Hartl effect gene duplication occurred due to evolutionary forces, involved random mutations fixed in one daughter gene under relaxed purifying selection, which occurs by reduced functional constraint provided by genetic redundancy. These fixed mutations later induce a change in gene function when the environment is altered. It is probable that the original role of arginase was a biosynthetic one in micro-organisms. The two forms of arginase in terrestrial ureotelic vertebrates are encoded by separate genes [62,63]. Cloning of these two genes for the arginases demonstrated that they were different from one another [25], but certainly of common origin [64]. These two enzymes are approximately the same size, share more than 50% of their amino acid residues, with 100% homology in areas critical to enzymatic function [22]. Three-dimensional crystal structures of the two isoforms are identical [65]. A II appeared to be the ancestral gene, with A I evolving in amphibians as urea cycle became the predominant pathway for ammonia detoxification in land dwelling animals. The appearance of two distinct arginase genes in *Xenopus* and mammals is thought to be due to a gene duplication event occurred before the emergence of amphibians and mammals from their most recent common ancestor [27,20,66]. From this point the two genes evolved independently.

The activity of arginase is quiet high in five species of freshwater air-breathing teleosts, namely *H. fossilis*, *Clarias batrachus*, *Channa punctatus*, *Anabas testudineus* and *Amphinuous cuchia* [67,68]. *H. fossilis* is freshwater air-breathing teleosts capable to tolerate temporary dehydration when kept out of water, showed considerable tolerance to ambient ammonia and induced OUC enzyme activities to permit conversion of accumulated ammonia to urea in vivo [59,67]. This fish unusually indicated that the localization of both CPS I and III along with GS are mitochondrial and arginase is both mitochondrial and cytosolic in liver and kidney [56]. The mitochondrial location of arginase is known in ureosmotic elasmobranchs and toadfishes while, the conversion of ammonia to citrulline in the mitochondria and then to arginine in the cytosol in *H. fossilis* resembles that of ureotelic species. This freshwater air-breathing catfish with its unique ammonia-metabolizing features can be used as an excellent experimental model to unravel the mystery of the evolution of ureotely in vertebrates. The biochemical and molecular characterization of ARG I and ARG II protein and the encoding gene will help to ascertain whether ureotelic evolution was really monophyletic.

Concluding remarks

Since, arginase is found throughout the primary kingdoms of life, it has been postulated that all arginases are derived from a universal common ancestor before the divergence into archaea, eubacteria and eukarya [69]. Hence, understanding the metabolic roles of two major arginases in fishes like *H. fossilis* and *Opsanus* sp. could provide an insight into evolutionary branch point where the primordial arginase evolved into an essential component of the urea cycle.

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