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IMP–GMP 5′-nucleotidase in reptiles: Occurrence and tissue distribution in a crocodile and three species of lizards

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Abstract

IMP-hydrolyzing activity (which is reactive with goose anti-pig lung IMP–GMP 5′-nucleotidase (c-N-II: EC.3.1.3.5) serum) was detected in extracts from several tissues (liver, heart, kidney, spleen, stomach, lung and skeletal muscle) from constitutively uricotelic reptiles: a crocodile (*Crocodylus siamensis*), and three species of lizard (*Furcifer oustaleti*, *Tupinambis rufescens* and *Varanus gouldi*). The activities were markedly high in the livers: 3.0 units/g in the crocodile and 1.4–2.9 units/g in the lizards. These were similar to those previously reported for the livers from chicken and snakes (also constitutively uricotelic), and 4- to 10-fold higher than those in ammoniotelic or ureotelic vertebrates. These findings suggest that the high activity of IMP–GMP 5′-nucleotidase in the liver is a feature of constitutive uricotelism, and that the enzyme may participate in the production of uric acid as an end product of amino acid catabolism.

Keywords: 5′-Nucleotidase; c-N-II; Crocodile; IMP; Liver; Lizard; Reptile; Uricotelism

1. Introduction

In reptiles, the detoxification of ammonia and the excretion of its end products appear to be closely related to the availability of water in the environment (for a review, see Campbell, 1995). It has been reported that crocodiles excrete mainly ammonia or uric acid (Cragg et al., 1961), some Testudines (turtles and tortoises) excrete mainly urea or even ammonia, and others excrete mainly uric acid (Baze and Horne, 1970). Uric acid excretion prevails in land snakes and lizards (Khalil, 1948a,b, 1951).

The first committed reaction of IMP degradation to uric acid is thought to be catalyzed by a cytoplasmic 5′-nucleotidase (5′-ribonucleotide phosphohydrolase, EC.3.1.3.5). This enzyme is effectively controlled by metabolic adaptation and allosteric regulation, and preferentially hydrolyses 6-hydroxypurine nucleotides over the other purine or pyrimidine nucleotides. Its lower K_m value for IMP compared with those of the other 5′-nucleotidases, and its approximately 20-fold catalytic efficiency for hydrolysis of IMP over AMP (Itoh and Yamada, 1990), suggest that this enzyme could shunt IMP to inosine through the dephosphorylation of IMP (For a review see: Itoh, 1993). This enzyme was designated as c-N-II (cytoplasmic 5′-nucleotidase-II) by Zimmermann (1992), or IMP–GMP 5′-nucleotidase by Bontemps et al. (1989).

The existence of the IMP–GMP 5′-nucleotidase has been detected in mammals (human, rat, pig and calf), birds (chicken and goose), a frog (*Rana catesbeiana*), fish (teleosts and an elasmobranch), reptiles (a turtle, a tortoise, snakes) and in an invertebrate *Artemia* (For a review see: Itoh, 1993; Pesi et al., 1994; Itoh and Kimura, 2002, 2003; Pinto et al., 1986). The cDNA coding for this enzyme was cloned from mRNAs from human placenta (Oka et al., 1994) and calf thymus (Allegrini et al., 1997), the expression of which led to a protein identical to IMP–GMP 5′-nucleotidase (Allegrini et al., 1997; Spychala et al., 1999).

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In the hepatic tissues of birds and snakes, which lack a complete set of urea cycle enzymes and are constitutively uricotelic, IMP–GMP 5′-nucleotidase is much more abundant compared with ureotelic mammals, an elasmobranch, and ammoniotelic teleosts (Itoh et al., 1992; Itoh and Kimura, 2002, 2003). In Testudines (a turtle and a tortoise), which have a complete complement of hepatic urea cycle enzymes and are constitutively ureotelic, its activity in the livers is low (Itoh and Kimura, 2003).

Based on the observation described above, we hypothesized that the high activity of IMP–GMP 5′-nucleotidase in the liver is a feature of constitutive uricotelism. To verify the hypothesis, it is essential to confirm the presence of its high activity in uricotelic reptiles, under the order of Crocodylia and the suborder of Sauria. In the present study, we estimated its activity in extracts from several tissues of a crocodile (*Crocodylus siamensis*) and lizards (*Furcifer oustaleti*, *Tupinambis rufescens* and *Varanus gouldi*), using a specific antiserum against IMP–GMP 5′-nucleotidase (Itoh and Yamada, 1991).

2. Materials and Methods

2.1. Animals

C. siamensis (2200 g), was purchased from a local breeder in Shizuoka Prefecture, Japan. The animal was bred in Thailand, imported into Japan, and kept for six months in an aquatic environment. *F. oustaleti* (270 g), *T. rufescens* (1800 g) and *V. gouldi* (350 g) were purchased from commercial suppliers in Tokyo. They were used for the experiments immediately after purchasing.

2.2. Chemicals

5′-IMP was obtained from Yamasa Shoyu Co. (Choshi, Japan). All other chemicals were of reagent grade or of the highest quality available. Matrex Gel Blue A was from Amicon Inc. (Beverly, MA, USA).

2.3. Antibodies

A polyclonal antibody against IMP–GMP 5′-nucleotidase was prepared by immunizing a goose with a homogeneous preparation of the enzyme obtained from pig lung as described previously (Itoh and Yamada, 1991). Rabbit anti-goose IgG fraction was purchased from Nihon Biotest Laboratories Inc. (Kokubunji, Japan). Antibodies were thoroughly dialyzed against 0.05 M Tris–Cl (pH 7.5), containing 0.2 M NaCl before use.

2.4. Sampling

The animals were anesthetized with isoflurane inhalation, and killed by decapitating. Tissues were excised, washed with 0.9% NaCl, blotted dry and immediately utilized or stored at −80 °C. Because of the small numbers of the animals used ($n=1$), two samples of each tissues examined were obtained and assayed. The averages of the two measurements were reported.

2.5. Preparation of crude extract

Tissues were homogenized with 4 vol. of 0.05 M Tris–Cl (pH 7.5), containing 0.2 M NaCl, 10 mM 2-mercaptoethanol and 1 mM EDTA (Buffer A) with a high-speed homogenizer (Polytron; Kinematica, Luzern, Switzerland). The homogenates were centrifuged at 100 000×*g* at 4 °C for 60 min. The supernatant was dialyzed for approximately 3 h against 100 vol. of Buffer A: this first dialysis was followed by the second one for approximately 17 h. The dialyzed extracts were stored at −80 °C until use.

2.6. Partial purification of IMP–GMP 5′-nucleotidase in crude extracts on Matrex Gel Blue A

The IMP–GMP 5′-nucleotidase in crude extracts was partially purified using the Matrex Gel Blue A column as described previously (Itoh and Kimura, 2002). Tissue extracts of 2 ml each from *C. siamensis* liver, *V. gouldi* liver and *T. rufescens* kidney in Buffer A were loaded on a Matrex Gel Blue A column (0.8×4 cm). After the column was rinsed with 20 ml of Buffer A, the adsorbed IMP-hydrolyzing activity was eluted with 0.05 M Tris–Cl (pH 7.5), containing 10 mM 2-mercaptoethanol, 1 mM EDTA and 4.5 M NaCl.

2.7. Assay of IMP-hydrolyzing activity

IMP-hydrolyzing activity was assayed in the presence of 50 mM $MgCl_2$ and 500 mM NaCl at pH 6.5 by measuring the Pi released from IMP as described previously (Itoh, 1981). One unit of activity was defined as that required to hydrolyze 1 μmol of IMP per min at 37 °C.

2.8. Protein determination

Protein was determined by the method of Lowry et al. (1951) with crystalline bovine serum albumin as a standard.

2.9. Quantitative immunotitration

Immunotitration of IMP–GMP 5′-nucleotidase in tissue extracts was performed as described previously (Itoh and Yamada, 1991; Itoh and Kimura, 2002). Briefly, increasing amounts of goose anti-IMP–GMP 5′-nucleotidase serum were mixed with a constant volume of crude extracts. After the incubation for 2 h at 20 °C, rabbit anti-goose IgG, adequate to precipitate the goose IgG, was added as the secondary antibody. After incubation for 1 h at 20 °C, the mixtures were centrifuged and the IMP-hydrolyzing activity