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Activity of certain enzymes of amino acid metabolism in the liver and kidney of three amphibian species

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Abstract. Activity of arginase, GDH, AAT and ALAT have been studied in the liver and kidney of three amphibian species that differ in their habitat preferences ranging from aquatic, semiterrestrial to terrestrial modes of living. The enzyme levels in the liver tissue cannot be correlated to the habitat preferences of the species, but the kidney enzymes show some correlations, with aquatic species showing higher rates of enzyme activity. The kidney-to-liver ratio of enzyme activity is also high in aquatic species.

Keywords. *Bufo melanosticus*; *Rana tigrina*; *Rana cyanophlyctis*; arginase; glutamate dehydrogenase; aspartate aminotransferase; alanine aminotransferase.

1. Introduction

The capacity of urea synthesis, during the periods of restricted water availability, in the hepatic tissue of amphibians is considered to have played a key role in the transition of these animals to a terrestrial existence (Campbell 1973). Nitrogen excretion of a species has been classically related to the water availability in its environment (Balinsky 1970; Campbell 1973). According to Jungries (1976), the partition of excretory nitrogen depends upon the capacity of the enzyme systems involved in this metabolism rather than water availability to the species. Urea is a passive component of some unknown function and the excretory ammonia is produced in the kidney of amphibians mainly to conserve the blood sodium.

In view of the above suggestions, it was felt desirable to study the activity of certain enzymes involved in the amino acids catabolism in the liver and kidney of three amphibian species that differ in their habitat preferences ranging from fully aquatic (*Rana cyanophlyctis*), semiterrestrial (*Rana tigrina*) to terrestrial (*Bufo melanosticus*) mode of living (Daniel 1963, 1972).

Four enzymes, *viz* arginase (L-arginine ureohydrolase, EC 3.5.3.1), glutamate dehydrogenase (GDH; L-glutamate: NAD (P) oxidoreductase, EC 1.4.1.3), aspartate aminotransferase (AAT; L-aspartase-2-oxoglutarate aminotransferase, EC 2.6.1.1) and alanine aminotransferase (ALAT; L-alanine 2-oxoglutarate aminotransferase, EC 2.6.1.2), are chosen for the study because of their importance in both excretory and energy metabolism.

2. Materials and methods

The frogs, *Rana tigrina* and *Rana cyanophlyctis*, were collected from ponds around Kakatiya University, while the toads (*Bufo melanosticus*) were collected in the Campus

area. The animals were kept in wire-meshed cages in a constantly flowing water canal, leaving sufficient space and shade for animals to move in and out of the water. During captivity they were fed on cockroaches. Average size adult animals of either sex were used in the experiment. They were killed within a week by pithing and the tissues were removed to ice-jacketed containers. The tissues were processed for assay of their enzyme by the following methods.

Arginase was assayed by the method of Campbell (1961). Tissues were first cleaned and the blood clots removed in 0·02 M glycinate buffer (pH 9·5). They were homogenized in 0·1 % cetyl trimethyl ammonium bromide to a final concentration of 10 % (W/V). One ml of liver extract was diluted to 30 ml with 0·02 M glycinate buffer and 0·1 ml of the diluted extract was used for the enzyme assay. In the case of kidney, the extract was centrifuged at 3,000 rpm for 10 min and 0·1 ml of the supernatant was directly used for the enzyme assay, The assay mixture (one ml) contained 85 μmoles of L-arginine, 50 μmoles glycinate buffer (pH 9·5), 0·5 μmoles of $MnCl_2$ and 0·1 ml of the enzyme source.

After incubating for 30 min at 37°C, the reaction was stopped by 4 ml of 10 % perchloric acid. The contents were centrifuged and 1 ml of the clear supernatant was taken for urea estimation. Appropriate zero-time controls were maintained for all experiments. Urea was estimated by the method of Natelson (1957).

GDH activity was estimated by the method of Lee and Lardy (1965) with slight modifications. Homogenates (1 %) of both liver and kidney were prepared in ice-cold 0·25 M sucrose and the homogenate was centrifuged at 3,000 rpm for 15 min. The supernatant (0·5 ml) was used for the assay. The assay mixture contained 40 μmoles of sodium glutamate, 0·1 μmoles NAD^+ (nicotinamide adenine dinucleotide), 2 μmoles of INT (2-(P-iodophenyl)-3-P-(nitrophenyl)-5-(phenyl) tetrazolium chloride), 100 μmoles of phosphate buffer (pH 7·5) and 0·5 ml of the enzyme source in a final volume of 2 ml. The reaction mixture was incubated at 37°C for 30 min and the reaction was stopped by adding 5 ml of glacial acetic acid. The formazan formed was extracted overnight in 5 ml toluene under cold condition (in a refrigerator maintained at 0–5°C). The colour intensity was measured at 490 mμ in a colorimeter.

AAT and ALAT were estimated according to the method of Reitman and Frankel (1957). Homogenate (10 %) of both liver and kidney was prepared in ice-cold 0·25 M sucrose. The homogenate was centrifuged for 15 min at 3,000 rpm. The supernatant was diluted 1 : 39 with ice-cold 0·25 M sucrose and aliquots (0·05 ml for AAT and 0·2 ml for ALAT) of diluted mixture were used for the enzyme assay. The reaction mixture in a final volume of 1 ml contained 0·5 mmoles of substrate (L-aspartic acid for AAT, DL-Alanine for ALAT), 2 μmoles α-ketoglutarate in 0·1 M phosphate buffer (pH 7·5) and the appropriate amounts of the enzyme source.

The contents were incubated at 37°C for 1 hr and the reaction was stopped by adding 1 ml of 2, 4-dinitrophenyl hydrozine (200 mg/100 ml of acid solution). The contents were mixed gently and after 20 min 10 ml of 0·4 N sodium hydroxide solution were added. The contents were mixed by inversion and after 5 min, the colour developed was read at 520 mμ. The activity is expressed as μmoles of pyruvate formed/g/hr.

3. Results and discussion

Arginase is one of the key enzymes in urea cycle. Although urea synthesis in amphibia is confined to liver, arginine hydrolysis is reported to take place in the kidney of

amphibians (Brodsky *et al* 1965). Table 1 shows higher arginase activity in the liver of terrestrial *Bufo melanosticus* and semiterrestrial *Rana tigrina* when compared to that of aquatic *Rana cyanophlyctis*. Kidney enzyme activity (table 2) is high in *Rana tigrina* and *Rana cyanophlyctis* when compared to that of *Bufo melanosticus*. Liver arginase activity cannot be correlated so much with the habitat preferences of the species and is reported to vary with diet. Kidney arginase, on the other hand, is reported to be more active in aquatic amphibians, when compared to that of terrestrial species (Carlisky 1970 and Boernke 1974). Although arginase activity in the kidney tissue of *Rana tigrina* is slightly higher over that of *Rana cyanophlyctis*, the kidney-to-liver ratio of enzyme activity is high in the order of *Rana cyanophlyctis* > *Rana tigrina* > *Bufo melanosticus* (table 3). This is in agreement with the role of active urea transport attributed to this enzyme in the kidney tissue of aquatic species (Boernke 1974) which in turn helps them in overcoming the hydration stress (Forster 1970).

GDH in amphibians is less specific and is capable of deaminating a number of L-aminoacids (Balinsky 1970). In liver, the enzyme participates in the removal of NH_4^+

Table 1. Levels of arginase, GDH, AAT and ALAT in the liver of three amphibian species.

Species	Arginase (15)	GDH (10)	AAT (12)	ALAT (10)	ALAT/AAT
Bufo melanosticus	9230·25[a] ±479·02	102·61 ±25·35	7955·88 ±2689·30	864·76 ±141·23	0·109
Rana tigrina	9320·59[a] ±937·34	52·16 ±18·67	3080·38 ±1030·38	1473·64[b] ±635·88	0·478
Rana cyanophlyctis	6758·97 ±1358·42	75·304 ±20·797	4662·74 ±636·30	1067·06[b] ±184·64	0·229

Variation of enzyme activity is statistically significant (P values range from $< 0{\cdot}05$ to $< 0{\cdot}001$) except in case of arginase, [a] between *Bufo melanosticus* and *Rana tigrina* and ALAT [b] between *Rana tigrina* and *Rana cyanophlyctis*.

Values represent the mean ±S.D. of μmoles of product formed/g wet wt/hr; No. of observations are given in parenthesis.

Table 2. Levels of arginase, GDH, AAT and ALAT in kidney tissue of the three species.

Species	Arginase	GDH (10)	AAT (10)	ALAT (10)	ALAT/AAT
Bufo melanosticus	124·46 (10) ±15·65	24·35 ±4·33	3649·59 ±376·96	830·00 ±158·33	0·227
Rana tigrina	487·61 (8) ±12·66	65·80 ±14·43	2873·53 ±894·61	1534·12 ±566·97	0·534
Rana cyanophlyctis	438·82 (10) ±58·34	99·66 ±20·41	5383·31 ±1413·65	2219·41 ±312·77	0·412

Variation of enzyme activity of all the four enzymes amongst the three species is statistically significant (P values range from $< 0{\cdot}05$ to $< 0{\cdot}001$).

Values represent the mean ± S.D. of the μM of product formed/g wet wt/hr; No. of observations are given in parenthesis.

Table 3. Kidney-to-liver ratio of enzyme activity in three species.

Species	Arginase	GDH	AAT	ALAT
Bufo melanosticus	0·013[a]	0·24[a]	0·46[a]	0·96[c]
Rana tigrina	0·052[a]	1·26[c]	0·93[c]	1·04[c]
Rana cyanophlyctis	0·065[a]	1·32[b]	1·16[c]	2·08[a]

[a, b] Variation of enzyme activity between the liver and kidney is statistically significant ([a]$P < 0.001$, [b]$P < 0.05$)

[c] The variation is not significant at $P = 0.05$.

Values are computed from the mean values of tables 1 and 2.

from the tissue. In kidney, it is involved in ammonia excretion (Janssens 1972). Results presented in tables 1 and 2 indicate that GDH activity in liver is high in the order of *Bufo melanosticus* > *Rana cyanophlyctis* > *Rana tigrina*. In kidney the order is *Rana cyanophlyctis* > *Rana tigrina* > *Bufo melanostocus*. A direct relationship exists between the kidney GDH activity and habitat preferences of the species. Aquatic species show higher kidney-to-liver ratio of enzyme activity (Balinsky 1970). The kidney-to-liver ratio of the enzyme activity and the kidney enzyme activity in the present investigation shows high activity in the order of aquatic > semiterrestrial > terrestrial species. The liver enzyme does not show this pattern. GDH activity is regulated by the energy status of cells (Hochachka and Somero 1973). In an early investigation (Lakshmipathi and Ramana Rao 1980) it was shown that SDH activity in the liver of *Rana tigrina* is very high. It can be suggested that the liver GDH activity is probably under the regulation of energy status of these cells.

AAT activity in liver is high in the order of *Bufo melanosticus* > *Rana cyanophlyctis* > *Rana tigrina*, but in kidney it is in the order of *Rana cyanophlyctis* > *Bufo melanosticus* > *Rana tigrina*. ALAT activity is high in the liver of *Rana tigrina* followed by *Rana cyanophlyctis* and *Bufo melanosticus*. In kidney it is high in the order of *Rana cyanophlyctis* > *Rana tigrina* > *Bufo melanosticus*. The kidney-to-liver enzyme activity ratio of both AAT and ALAT is high in the order of *Rana cyanophlyctis* > *Rana tigrina* > *Bufo melanosticus*. The variation between the kidney and liver enzyme activity is significant, in the case of AAT, in *Bufo melanosticus*, and in case of ALAT in *Rana cyanophlyctis*. The high AAT activity was related to the increase in urea production and the enzyme activity was shown to increase during metamorphosis and in aestivation. ALAT activity, on the other hand, decreases during metamorphosis (Balinsky 1970 for references). Balinsky (1970) recorded evidence that the ratio of ALAT to AAT and the GDH were both high in *Xenopus laevis* when compared to that of *Rana angolensis*. *Xenopus laevis*, kept out of water, was shown to increase its AAT to ALAT ratio (Janssens 1964). High AAT, low ALAT and low GDH activity in *Bufo melanosticus* (tables 1 and 2) can be attributed to its terrestrial mode of living. *Rana tigrina* shows low activity of AAT both in liver and kidney, when compared to that of *Rana cyanophlyctis*. Since GDH regulates the levels of transaminases by controlling the levels of glutamate (Hochachka and Somero 1973) it can be stated that low transaminase activity in this species is due to low GDH activity. *Rana cyanophlyctis*, on the other hand, shows a high GDH and high transaminase activity. The ratio of kidney-to-liver ALAT activity is double in this species. Since alanine deamination through GDH is the principal route of excretory ammonia

formation in the kidney of aquatic species (Balinsky 1970), the high ALAT and GDH activity in kidney of *Rana cyanophlyctis* can be attributed to this deamination process. The ALAT/AAT ratio in this species is, however, low when compared to that of *Rana tigrina* (tables 1 and 2). The ALAT/AAT ratio was shown to remain constant in aestivation in *Xenopus laevis* (Balinsky 1970) indicating that it is not the ratio of ALAT/AAT, but the relative activity of transamination coupled with GDH activity, particularly in the kidney, that can be correlated with the habitat preferences of the species. The high ALAT activity in kidney in the order of *Rana cyanophlyctis* > *Rana tigrina* > *Bufo melanosticus* is in accordance with the habitat preferences of the species.

Comparative studies of the levels of GDH, AAT and ALAT in the liver tissues of the three species present an interesting picture. The activity of both GDH and AAT is high in the order of *Bufo melanosticus* > *Rana cyanophlyctis* > *Rana tigrina*, but a reverse relationship exists in case of ALAT where the activity is in the order of *Rana tigrina* > *Rana cyanophlyctis* > *Bufo melanosticus*. AAT and GDH form enzyme-enzyme complexes and both glutamate and aspartate as the substrates enhance the binding of these enzymes (Fahien and Kmiotek 1979). The general correlation in the activity of GDH and AAT indicates that the two enzymes are probably involved in the complex formation. A reciprocal relationship between ALAT and GDH activity was reported in bovine liver tissues (Tomkins *et al* 1961). A low molecular monomeric forms of the enzyme proteins promote L-alanine-pyruvate interconversion, while high molecular polymeric forms promote GDH activity. Bitensky *et al* (1965) reported that factors influencing the state of aggregation of the molecules effect changes in the activities of the enzyme. The reciprocal relationship observed in the activity of GDH and ALAT in the liver tissue of the three species suggests the qualitative reaction mechanisms of ALAT and GDH to be similar in both beef liver and frog liver. Earlier investigators (Wiggert and Cohen 1965; Balinsky 1970) reported differences between the two enzymes but suggested that a closely linked enzymatic site is probably involved in the oxidation of both L-alanine and L-glutamate in frog livers. The absence of this reciprocal relationship in the kidney of the three species indicates that the two enzymes are different. Janssens (1972) demonstrated differences in electrophoretic and kinetic properties of liver and kidney enzymes and suggested that they are either of different origin or of different aggregation.

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