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Catalytic potential of arginase in selected tissues of frog *Rana hexadactyla* during chronic starvation

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Abstract. The catalytic potential of arginase has been studied in the liver and kidney of frog *Rana hexadactyla* during prolonged starvation. Starvation involves the alterations of Michaelis-Menten constant and the maximal velocity of arginase in both the tissues. Increased activation energy values for the enzymes of both the tissues during starvation indicated decreased catalytic potential.

Keywords. *Rana hexadactyla* ; starvation; arginase .

1. Introduction

Activities of several cellular enzymes were found to be altered during diverse stress conditions (Knox *et al* 1956). The effect of starvation on the activities of number of enzymes has been reviewed recently (Krebs 1972; Segal 1973). Though most enzymic changes during starvation occur in liver, enzyme activities of other organs are also altered especially if the starvation is prolonged and severe (Szepsi 1976). The protein degradation is high during starvation (Schimke 1962; Durand 1973) resulting in the increased free amino acids which are subjected to transamination and deamination thereby altering the nitrogen metabolism of the tissue. Concomitantly the enzymatic systems associated with ammonogenesis and ureogenesis may also show altered activity potentials (Schimke 1962). Hence, an attempt has been made in the present investigation to study the detoxification mechanisms operating in the liver and kidney of frog by analysing the kinetic parameters of arginase under chronic caloric stress.

2. Materials and methods

The liver and kidney tissues of medium-sized normal and starved male frogs, *Rana hexadactyla* (Lesson) weighed about 35 ± 3 g were used in the present study. Two batches of frogs were maintained in separate clean glass aquarium tanks. One batch was used as normal and was fed with cockroaches *ad libitum* to prevent starvation and the other batch was starved with total food deprivation. After

6 months the tissues from the normal and starved frogs were quickly excised, weighed and placed in a pre-chilled petridish and used for the estimation of arginase (EC3.5.3.1) activity levels. Arginase activity was estimated by the method of Campbell (1961). 2% (W/V) homogenates of liver and kidney tissues were prepared in ice-cold 0·1% CTB (cetyl trimethyl ammonium bromide) (Brown and Cohen 1959), centrifuged at 1000 g for 15 min and the supernatants were dialyzed overnight in a dialysis bag at 0° C against CTB. The dialysed supernatants were used for enzyme assay. The reaction mixture in a total volume of 2 ml contained different concentrations of L-arginine (substrate) varied from 1 mM to 20 mM, 50 μmol of sodium glycinate buffer (pH 9·5), 0·5 μmol of $MnCl_2$ and 0·2 ml of enzyme extract. The incubation was carried out at 37° C for 30 min and the reaction was arrested with the addition of 2 ml of 0·5 M perchloric acid. The contents were centrifuged and an aliquot of the supernatant was taken for urea estimation by diacetyl monoxime method as described by Natelson (1971). The enzyme activity was expressed as μmol of urea formed/mg protein/hr. The maximal velocities (V_{max}) and Michaelis-Menten constants (K_m) were determined from the Lineweaver-Burk plots. The activation energy values were determined as given by Dixon and Webb (1964). The direct and reciprocal plotting of substrate *versus* velocity was done by taking the average value of six observations at each concentration of substrate to minimize the deviation. After due standardization an enzyme concentration of 4 mg, 30 min of incubation time and 37° C incubation temperature were selected for the present study to ensure maximal velocity of the enzyme catalysis in both the liver and kidney tissues.

3. Results and discussion

Substrate concentration *vs* velocity plots for liver and kidney arginase of both the normal and starved animals revealed that the enzyme activity was linear with the substrate concentration following a first order reaction upto 5 mM and from then onwards the reaction had entered zero-order phase showing the substrate-independent activity. The Lineweaver-Burk plots for both the liver and kidney arginase activity in the control and starved animals showed variation in the kinetic parameters like V_{max} and K_m values (table 1). The (V_{max}) values for liver arginase were decreased by 57·6 and 54·6% (table 1) in liver and kidney respectively during starvation indicating a reduction in the velocity potential due to decreased active site density of the enzyme content. The decrease of arginase activity may be due to degradation of enzyme protein in both liver and kidney tissues during prolonged starvation. In evidence to this an increased degradation of proteins has also been reported during starvation in several animals (Tsyganov 1971; Adibi 1972).

The decrement in the arginase activity of both liver and kidney tissues can also be attributed to the marked depletion in the arginine content, a substrate for this enzyme (Jones and Mayer 1973) or may be due to the decreased half life of this enzyme during starvation which implied that the concentration of a specific enzyme will vary under this caloric stress (Gray *et al* 1960; Jones and Mayer 1973).

The decreased arginase velocity observed during starvation is well in line with the low levels of urea in the plasma and its decreased excretion in the starved

animals (Jungreis 1970; Jungreis and Hooper 1970). This gains further support from the observations of Schimke (1964) who reported that the stability and turnover of arginase was altered significantly during starvation. The K_m values which measure the affinity of the enzyme for substrate as well as the rate of breakdown of E–S complex, form the basis for catalytic potential and these values were found to increase by 98 and 102% in liver and kidney respectively during starvation (table 1) suggesting the decreased affinity between the enzyme and substrate and also accounting for the low catalytic potential. Since the percent decrement in K_m is similar for both the liver and kidney enzymes, the observed inactivity is in general order with no specificity.

Table 1. Substrate dependent kinetics of arginase in liver and kidney tissues of normal and starved frogs.

	Kinetic parameters			
	V_{max} (μmol of urea/mg protein/hr)		K_m (mM)	
	Normal	Starved	Normal	Starved
Liver	12·73	5·40	0·75	1·49
% Change		(−57·58)		(+ 98·67)
Kidney	8·08	3·67	1·20	2·44
% Change		(−54·57)		(+103·33)

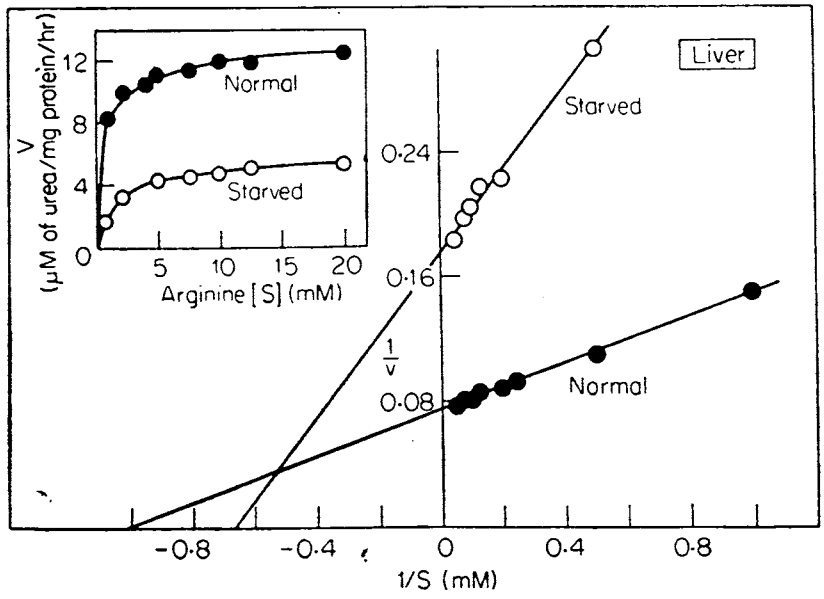

Figure 1. Double reciprocal plots of substrate (arginine) *versus* velocities of arginase in the liver of normal and starved frogs at pH 9·5 along with the substrate *versus* velocity curves.

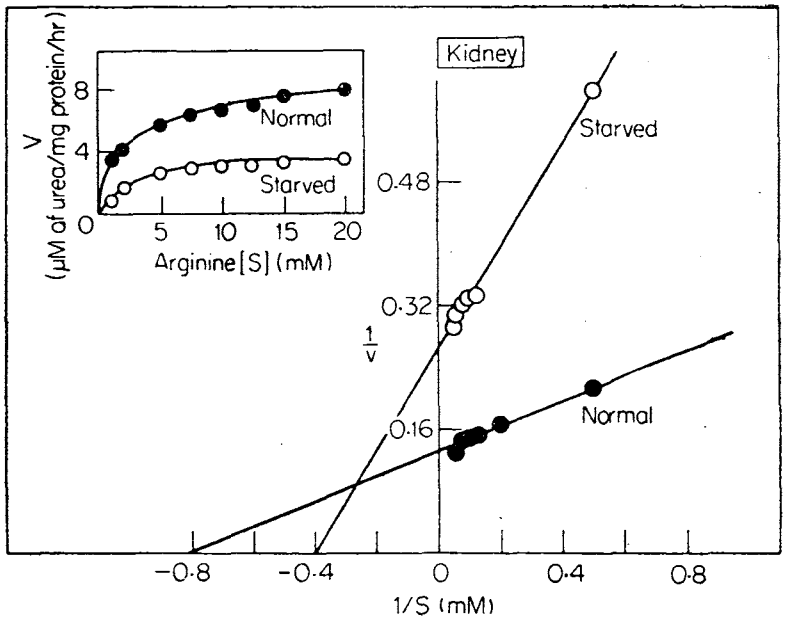

Figure 2. Double reciprocal plots of substrate (arginine) *versus* velocities of arginase in the kidney of normal and starved frogs at pH 9·5 along with the substrate *versus* velocity curves.

Table 2. Activation energy values of arginase in liver and kidney tissues of normal and starved frogs.

Temperature range °C	Activation energy (Cals/mol)			
	Liver		Kidney	
	Normal	Starved	Normal	Starved
20–25	8430·48	10827·70	5074·27	6952·15
% Change		(+28·44)		(+37·01)
25–30	6817·50	9164·40	3503·81	4148·38
% Change		(+34·42)		(+18·39)
30–35	6123·90	8148·13	2493·97	3442·03
% Change		(+33·05)		(+38·01)

The activation energy values showed an increment for both the liver and kidney enzymes during starvation (table 2). The per cent increase observed in the activation energy values was found to be almost similar at all temperatures studied except for kidney arginase at 25–30° C. The catalytic efficiency is often measured in terms of activation energy values. Increase in the activation energy values

indicate lower efficiency of the enzyme and *vice versa*. The present finding showed that the activation energy barrier for both the liver and kidney arginase was increased by starvation phenomenon. The decreased catalytic potential of arginase might be responsible for increased levels of ammonia in the liver of starved animal (Aoki *et al* 1974). The decreased catalytic potential of arginase indicates decreased utilization of ammonia for the ureogenesis. The present study reveals that the operation of detoxification system is affected during the starvation stress thereby making the tissues more vulnerable to the toxic effect of hyperammonemic condition.

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