Scaling of Some Metabolic Enzymes in Liver of a Freshwater Teleost: An Adaptive Mechanism

Ghanashyam Tripathi*

Department of Zoology, J. N. V. University, Jodhpur-342 001, India

* Address for Correspondence and reprint requests: Dr. G. Tripathi, 7B Ratan Sadan, Vijay Nagar, New Pali Road, Jodhpur – 342 001, India

Z. Naturforsch. **54c,** 1103–1106 (1999); received July 12/September 1, 1999

Teleost, Liver, Scaling, Lactate Dehydrogenase, Malate Dehydrogenase

The activities of mitochondrial malate dehydrogenase (mMDH) and the total mitochondrial proteins increase as a function of body mass in the freshwater catfish, *Clarias batrachus*. It clearly indicates an increase in energy production in larger- sized individuals for various purposes including prey-predator interactions. The higher activity of lactate dehydrogenase (LDH) in larger fish may indicate more production of lactate for gluconeogenesis in the liver to meet emergency requirements of increased energy demand. However, the activity of cytoplasmic malate dehydrogenase (cMDH) decreases with the increasing body mass of the fish which reflects reduction in NADPH production and, in turn, reduced lipogenesis in liver of larger individuals. Thus, the present observations suggest an adaptive mechanism dealing with the higher energy budget, and reduced synthetic activities (lipogenesis) in the liver of larger-sized freshwater catfish. This type of biochemical scaling might be also supporting other metabolic pathways in order to adjust some physiological functions for survival in the aquatic environment.

Introduction

Liver is a major metabolic site for various important biochemical and physiological functions. The degree of functional requirements varies with the size of organisms. The investigations on size-dependent properties (i.e. scaling) of animals have been mainly focused on aerobic processes and the underlying structures supporting them (Schmidt – Nielsen, 1984; Berrioslopez *et al.*, 1996). The scaling of aerobic metabolism has a consistent pattern among different organs of fishes and tetrapods (Hochachka *et al.*, 1987; Ewart *et al.*, 1988; Somero and Childress, 1990). It is considered as an organism-wide reflection of the geometric characteristics which may impose constraints on oxygen uptake and ion and water transport. The precise factors that control general metabolic scaling are yet to be identified and explained. Some attempts have been made to explore the basic mechanism of aerobic scaling and the inferences are debatable (Calder, 1987; Heusner, 1987; Somero and Childress, 1990). The chase aspect of predatory-prey interactions among fishes is believed to be metabolically related to the scaling (Weihs and Webb, 1983; Tripathi, 1999). So far no report is available on the metabolic scaling in liver of any freshwater fish. Therefore, it was considered of an warrantable interest to study the biochemical scaling in a catfish.

Since lactate dehydrogenase (LDH : an indicator of lactate production for gluconeogenesis), mitochondrial malate dehydrogenase (m MDH : an ATP – supplying enzyme of oxidative processes) and cytoplasmic malate dehydrogenase (cMDH : an enzyme related to NADPH production for fatty acid synthesis) are clearly involved in the three different major metabolic processes, the scaling of these metabolic enzymes and proteins were studied in the liver of an economically important predatory catfish (*Clarias batrachus*) for elucidating enzymatic adaptations in larger-sized individuals.

Materials and Methods

Specimens of *Clarias batrachus* having 70–260 g (±8 g) body mass were collected during their reproductively regressed phase (November to February). The fish were sacrificed and livers were removed and frozen immediately in a deep freezer at −80 °C. There were no loss of enzymatic activities during tissue storage upto several months. The

0939–5075/99/1200–1103 $ 06.00 © 1999 Verlag der Zeitschrift für Naturforschung, Tübingen · www.znaturforsch.com · D

Dieses Werk wurde im Jahr 2013 vom Verlag Zeitschrift für Naturforschung in Zusammenarbeit mit der Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V. digitalisiert und unter folgender Lizenz veröffentlicht: Creative Commons Namensnennung-Keine Bearbeitung 3.0 Deutschland Lizenz.

This work has been digitalized and published in 2013 by Verlag Zeitschrift für Naturforschung in cooperation with the Max Planck Society for the Advancement of Science under a Creative Commons Attribution-NoDerivs 3.0 Germany License.

Zum 01.01.2015 ist eine Anpassung der Lizenzbedingungen (Entfall der Creative Commons Lizenzbedingung „Keine Bearbeitung") beabsichtigt, um eine Nachnutzung auch im Rahmen zukünftiger wissenschaftlicher Nutzungsformen zu ermöglichen.

On 01.01.2015 it is planned to change the License Conditions (the removal of the Creative Commons License condition "no derivative works"). This is to allow reuse in the area of future scientific usage.

chemicals were purchased from Sigma Chemical Company (USA) and Sisco Research Laboratories (India). The substrates (oxaloacetic acid and pyruvic acid) and coenzyme (ß-NADH) were prepared at every 3 hr whenever required. The liver homogenates (10%) were prepared in ice-cold 0.25 M buffered sucrose solution (0.1 M sodium phosphate buffer, pH 7.4, containing 0.25 M sucrose) and centrifuged at 700×*g* for 15 min in a high speed refrigerated centrifuge. The supernatant was decanted and centrifuged at 12,100×*g* for 20 min to obtain the mitochondrial pellet. The subsequent supernatant thus obtained was recentrifuged at 30,000×*g* for 30 min and the resulting supernatant was taken as cytoplasmic fraction for the assay of lactate dehydrogenase (LDH) and cytoplasmic malate dehydrogenase (cMDH). The mitochondrial pellet was washed three times in 0.25 M ice-cold buffered sucrose (pH 7.4) and each washing was followed by a centrifugation at 12,100×*g* for 15 min. The preparation of mitochondrial malate dehydrogenase (mMDH) was done according to the method as described earlier (Casado *et al.*, 1980). The marker enzymes, SDH and LDH were assayed in cytoplasmic and mitochondrial fractions respectively and less than 5% of their total activities were detected which were considered negligible.

The malate dehydrogenese isozymes (cMDH and mMDH, l-malate: NAD^+-oxidoreductase, EC 1.1.1.37) and lactate dehydrogenese (LDH, l-lactate : NAD^+-oxidoreductase, EC 1.1.1.27) were assayed according to the methods described by Ochoa (1955) and Kornberg (1955), respectively, with slight modifications. The optimum concentrations of substrates (oxaloacetate and pyruvate), coenzyme (NADH) and enzymes were used and readings were taken against a blank containing all components except substrate(s) and coenzyme. One unit of enzyme activity was taken as the amount of an enzyme catalysing the oxidation of one µmol of NADH per minute under the above specified conditions. The protein contents were measured by folin method (Lowry *et al.*, 1951) and regression analysis was done as described by Croxton (1953).

Results and Discussion

The activities of lactate dehydrogenase (LDH) and mitochondrial malate dehydrogenase (mMDH) increased as a function of body mass (Fig. 1, Table I). The scaling coefficient of LDH ($r = 0.951$) was positive and statistically significant ($t = 6.85$, $P < 0.001$) showing an important relationship of LDH with the fish body mass. The higher activity of LDH in larger fish may indicate more production of lactate in the liver to meet emergency requirements of increased energy demand. Similarly, the mMDH scaling coefficient ($r = 0.980$) was strongly positive and highly significant ($t = 121.582$, $P < 0.001$) justifying a significant relationship of mMDH with body mass. This size-dependent strongly positive scaling of mMDH (an ATP-supplying enzyme) further indicates an increase in energy production in larger-sized fish for various metabolic purposes in liver tissue. Since liver is a major metabolic site, the scaling in an ATP- suppling enzyme (mMDH) of TCA cycle may be to fulfil a greater need of energy for ana-

Table I. Regression equations relating the body mass of *Clarias batrachus* (weighting between 70 and 260 g) to its liver lactate dehydrogenase (LDH), mitochondrial malate dehydrogenase (mMDH), cytoplasmic malate dehydrogenase(cMDH) and mitochondrial protein (mP).

Enzyme/ Protein	Estimating equation ($Y=a+bX$)		Regression coefficient (r)	Number of sample (N)	Value of 't'	Level of significance
	a	b				
LDH	23.089	0.862	0.951	7	6.850	P<0.001
mMDH	0.560	0.038	0.980	7	121.582	P<0.001
cMDH	86.679	0.729	0.951	7	6.638	P<0.001
mP	1.404	0.038	0.905	7	4.755	P<0.01

Y is the enzyme activity (units x g wet mass^{-1}) or protein content (mg x g wet mass^{-1}) and X is the wet body mass (g) of the fish. The rates of LDH, mMDH and cMDH were found to be 180, 52 and 288 µmol·mg protein^{-1} . h^{-1}, respectively. The 't' values are the results of t – test performed to test the significance of regression coefficient.

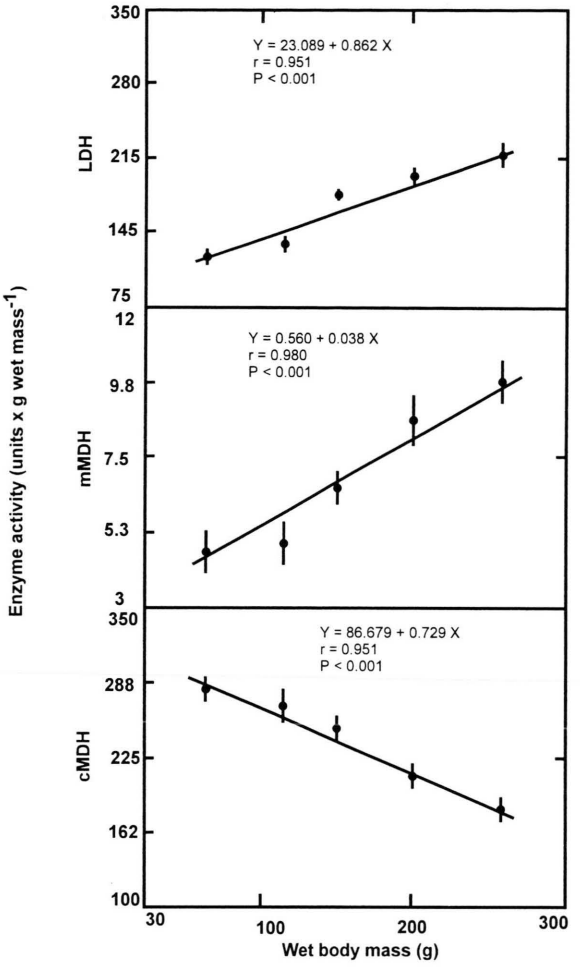

Fig. 1. Scaling of activities of lactate dehydrogenase (LDH), mitochondrial malate dehydrogenase (mMDH) and cytoplasmic malate dehydrogenase (cMDH) in the liver of the catafish, *Clarias batrachus.*

bolic and catabolic activities in a larger subject. In contrast, the activity of cytoplasmic malate dehydrogenase (cMDH) showed a negative relationship as a function of increasing body mass (Fig. 1, Table I). The value of scaling coefficient was 0.951 with the t value of 6.638 and the level of significance of $P < 0.001$. This again showed a strong relationship of body mass with the cMDH activity in liver tissue. The decreased activity of cMDH may indirectly reflects reduction in lipogenesis in liver of larger individuals. The present observations are more or less in agreement to the findings of other workers (Dobson *et al.*, 1987; Somero and Childress, 1990).

The mitochondrial protein contents showed a parallel scaling effect to that of the mMDH actitivity. Like mMDH, the scaling coefficient (r = 0.905) of the total mitochondrial protein concentration was positive and statistically significant (t = 4.755, $P < 0.01$). The similar results have also been reported by Somero and Childress (1990). However, the total cytoplasmic protein concentration did not exhibit the scaling property in liver of the freshwater catfish, *Clarias batrachus* (Fig. 2, Table I). Thus inspite of changes in the activities of LDH and cMDH, there was no net effect of scaling on the total cytoplasmic protein content.

It is now evident that the similar scaling patterns of LDH, mMDH and mitochondrial proteins are indicative of adaptive modifications for a higher energy budget as a function of increasing body mass. Further, the scaling trend of cMDH reflects reduced NADPH production in order to lessen various synthetic activities including lipogenesis in larger individuals for a better survival. Therefore, it may be suggested that the scaling of metabolic enzymes and protein in liver is an adaptive mechanism to cope up with the biochemical and physiological machineries in the larger-sized fish.

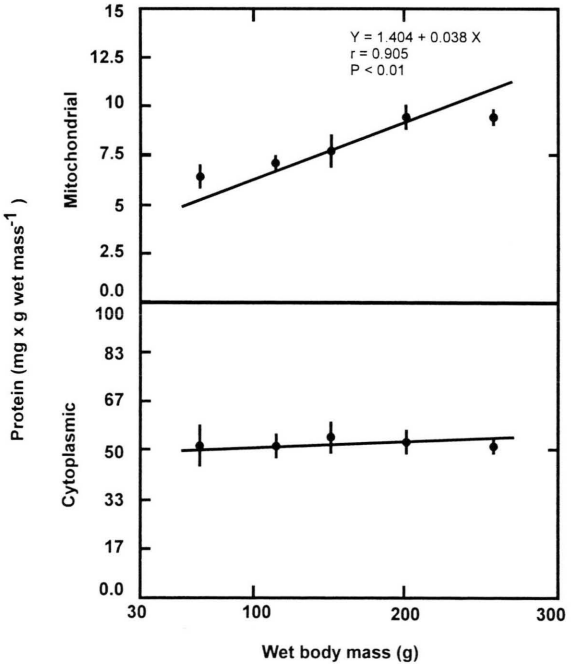

Fig. 2. Scaling of mitochondrial and cytoplasmic proteins in the liver of the catfish, *Clarias batrachus.*

Berrioslopez M., Lewis A. R. and Hensley, D. A. (1996), Scaling of skeletal mass to body mass in fishes. J. Morphol. **227**, 87–92.

Calder W. A. (1987), Size, Function and Life History. Harvard University Press, Cambridge, USA, 431 pp.

Casadó F., Cortés A. and Bozal J. (1980), Extraction and kinetic characteristics of chicken liver mitochondrial malate dehydrogenase. Int. J. Biochem. **11**, 437–447.

Croxton, F. E. (1953), Linear correlation of two variables. In: Elementary Statistics with Applications in Medicine and the Biological Sciences. Dover Publications, Inc., New York, pp. 109–147.

Dobson G. P., Parkhouse W. S. and Hochachka P. W. (1987), Regulation of anaerobic ATP generating pathways in trout fast-twitch skeletal muscle. Am. J. Physiol. **253**, R186-R194.

Ewart H. S., Canty A. A. and Driedzic W. R. (1988), Scaling of cardiac oxygen consumption and enzyme activity levels in sea raven (Hemitripterus americanus). Physiol. Zool. **61**, 50–56.

Heusner A. A. (1987), What does the power function reveal about structure and function in animals of different size? Annu. Rev. Physiol. **49**, 121–133.

Hochachka P. W., Emmett B. and Suarez R. K. (1987), Limits and constraints in the scaling of oxidative and glycolytic enzymes in homeotherms. Can. J. Zool. **66**, 1128–1138.

Kornberg A. (1955), Lactate dehydrogenase of muscle. In: Methods in Enzymology (S. P. Colowick and N. O. Kaplan, ed.). Academic Press, New York, Vol. **1**, pp. 441–443.

Lowry O. H., Rosebrough N. J., Farr A. L. and Randall R. J. (1951), Protein measurement with the folin phenol reagent. J. Biol. Chem. **193**, 265–275.

Ochoa S. (1955), Malic dehydrogenase from pig heart. In: Methods in Enzymology (S. P. Colowick and N. O. Kaplan, ed.). Academic Press, New York, Vol. **1**, 735–736.

Schmidt-Nielsen K. (1984), Scaling: Why is Animal Size so Important?, Cambridge University Press, Cambridge, England, 241pp.

Somero G. N. and Childress J. J. (1990), Scaling of ATP-supplying enzymes, myofibrillar proteins and buffering capacity. In: Fish Muscle : Relationship to Locomotory Habit. J. Exp. Biol. **149**, 319–333.

Tripathi, G. (1999), Scaling of cytoplasmic and mitochondrial enzymes and proteins in skeletal muscle of a catfish. J. Anim. Physiol. Anim. Nutr. (In press).

Weihs D. and Webb P. W. (1983), Optimization of locomotion. In Fish Biomechanics (Weihs, D. and Webb, P. W., ed.). Praeger, New York, pp.339–371.