

Quantitative and qualitative coding of Lactate dehydrogenase isoenzymes and their genes: Evolutionary implication in genus *Channa* (Channidae: Channiformes)

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Abstract

Lactate dehydrogenase (LDH: 3.1.1.27) is a tetrameric enzyme participates in carbohydrate metabolism by catalyzing the oxidation of lactate and reduction of pyruvate. The random tetramerization of LDH-A and -B subunits leads to the formation of five tetramers: LDH-B₄, LDH-A₁B₃, LDH-A₂B₂, LDH-A₃B₁ and LDH-A₄. Due to the biochemical and evolutionary significance of LDH isoenzyme characters or LDH genes, efforts are made here to propose a numerical coding method of quantifying the isoenzyme activities and demonstrate the presence of LDH loci or the changing levels of isoenzymes in different tissues of selected air-breathing teleosts *Channa punctata*, *C. gachua* and *C. striatus*. Polyacrylamide gel electrophoretic (PAGE) profiles of LDH isoenzymes were utilized and software analysis of gel scans was performed. Our results show a great variation in the quantitative as well as qualitative presence of isoenzymes or their genes among the selected freshwater congeneric species. Based on the obtained results, adaptive strategies of *Channa* species are discussed in relation to their evolutionary set up.

Introduction

Lactate dehydrogenase (LDH: 3.1.1.27) enzyme in fishes have always been the subject of much attention [1-4]. But, scarce reports are available from India dealing either with the expression of LDH genes /presence of loci or their quantitative changes in freshwater teleosts [5-7]. Lactate dehydrogenase is one of the chief enzyme of carbohydrate metabolism which catalyses the oxidation of lactate and reduction of pyruvate during anaerobic glycolysis. Usually two subunits -A and -B are present in different tissues as per their metabolic demands. Their random tetramerization results in the formation of two homotetramers: LDH-A₄ (muscle specific) and -B₄ (heart specific) and, three heterotetramers -A₁B₃, -A₂B₂, and -A₃B₁ [2, 8].

LDH genes in fish, LDH-A and LDH-B have been shown homologous with those of higher vertebrates and according to available reports gene duplication of a single ancestral form gave rise to these forms nearly five hundred million years ago, which is also the expected time of origin of fishes [1, 9-10]. A third gene LDH-C, which is presumed to be the result of LDH-B gene duplication also

shows an orthologous expression and has been demonstrated in the eye or liver of many teleosts [1, 3, 8].

It is evident that the LDH isoenzymes have also been utilized as an indispensable tool in systematic and phylogenetic studies due to either the quantitative/ qualitative differences or their differential expression [11-12]. Therefore, the significance of these important characters in evolutionary protocols cannot be ignored. In the present study, the presence/ absence of LDH isoenzymes or genes and their quantitative differences have been assigned Arabic numerals and utilized to understand the evolutionary genetics of some selected species of genus *Channa*: *C. punctata*, *C. gachua* and *C. striatus*. Channids, besides having accessory air-breathing organs, are of special interest due to their distinct adaptive lineage or evolutionary group.

Materials and Methods

Procurement of fish

Mature and live fish representing the mixed catch were brought to the laboratory from the fish markets of Aligarh

and Bareilly. They were identified and acclimatized in well-aerated water tanks prior further experimentation.

Preparation of tissue extract:

Following the sacrifice of *C. punctata*, *C. gachua* and *C. striatus*, their tissues namely brain, eye, heart, liver, skeletal muscle and kidneys were carefully dissected and homogenized in chilled 50 mM Tris-HCl buffer of pH, 7.5 as described earlier [13]. The extract was centrifuged at 4 °C and 15000 rpm for 20 min. The clear supernatant obtained after centrifugation was used for analysis by polyacrylamide gel electrophoresis (PAGE).

Protein estimation:

Standard method of Lowry et al. [14] was used for estimation of protein concentration in the supernatant of different tissues extract of selected fish species.

Electrophoresis and visualization of LDH isoenzyme bands:

Electrophoresis was essentially carried out according to the protocol outlined previously [13]. Initially, the runs were made in 1× upper gel buffer and later replaced with Tris-glycine buffer as the samples entered the lower separating gel.

LDH isoenzyme bands were stained and visualized in the presence of L-lactate as the substrate according to the procedure of Shaw and Prasad [15]. Then the stained gels were fixed in 7% acetic acid (v/v) and documented via scanning on HP Deskjet F370 All-in-One computer assembly.

Quantitative/ qualitative analysis of LDH isoenzymes using softwares:

Quantitative estimates of LDH isoenzymes in the gelscans was carried out using GelPro software (Media cybernetics, USA), while their qualitative coding were made by Scion Imaging software (Beta release-4, Scion Corporation) respectively. Arabic numerals (0-4) were assigned to each isoenzyme activity as per the band peak or peak area corresponding to each LDH tetramer. But, while performing qualitative analysis numerals 1 and 0 were chosen to represent the presence or absence of a specific LDH gene/loci.

Results

In the investigated teleosts, typical zymograms show decreasing electrophoretic mobility from anode to cathode in the order of LDH-A₄, LDH-A₃B₁, LDH-A₂B₂, LDH-A₁B₃ and LDH-B₄. Since during the present study six tis-

ues namely brain, eye, heart, liver, skeletal muscle and kidneys were selected as the source of examining the LDH isoenzyme activities, on individual tissue basis their coding in Arabic numerals was performed (Table-1). It was observed that brain of *C. punctata* possessed very high activities of homotetramers LDH-B₄ and -A₄. While *C. gachua* demonstrated LDH-A₄ as the band of very high activity and *C. striatus* showed the detectable presence of it. In the eye of *C. punctata* LDH-B₄ predominated whereas in case of *C. gachua*, LDH-A₄ was detected as the major activity along with the detectable presence of LDH-C₄. This LDH-C₄ isoenzyme was unambiguously detected in the entire collection of *C. gachua* from local fish market of Aligarh or Bareilly district. Heart specific LDH-B₄ was observed as a band of very high activity in *C. punctata* while in other two sister species it was in detectable amounts. In the heart of *C. striatus* instead of -B₄, LDH-A₃B₁ was observed as the major activity.

Liver of *C. punctata* shows high activity of LDH-B₄ which was almost absent in *C. striatus* and detected as trace activity in *C. gachua*. Very high activity of LDH-A₄ was there in skeletal muscle of *C. punctata* as well as *C. gachua*. LDH-B₄ was the main activity in the kidneys of *C. punctata* while other two species were lacking it. Activity of LDH-A₃B₁ in *C. striatus* vary from very high to intermediate to again very high in liver, skeletal muscle and kidneys respectively (Table 1).

The present results also demonstrate the presence of three LDH loci in the investigated populations of selected teleosts. In the populations of *C. punctata*, *C. gachua* and *C. striatus* from Aligarh and Bareilly, consistent expression of LDH-A and -B genes was recorded. In addition to it, LDH-C gene was also observed in the populations of *C. gachua* only (Table-2).

Discussion

In subunit composition and expression of LDH-A or -B in a specific tissue, typical LDH zymograms of *C. punctata*, *C. gachua* and *C. striatus* are comparable to the reported electrophoretic profiles of several other teleosts [2, 9, 16]. Steady state patterns of *C. punctata* compared to its congeneric species, demonstrated the predominance of LDH-B₄ in adult tissues such as brain, eye, heart, liver and kidneys (Table-1). This may suggest that these tissues are comparatively aerobic, as reported previously [3, 8, 12]. However, the tissue restriction in LDH or its isoenzymes has been attributed to post translational modifications.

The presence of LDH-A₄ or increase in LDH activity has been correlated with anaerobiosis or hypoxic conditions. Hence, the presence of this specific homotetramer or its subunits in various tissues of *C. gachua* and *C. striatus* may reveal the increase in rates of LDH activity due to

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Table 1: Relative quantitative coding of lactate dehydrogenase isoenzymes in selected adult tissues of *C. punctata* (Cp), *C. gachua* (Cg) and *C. striatus* (Cs).

LDH isoenzymes	Brain			Eye			Heart			Liver			Muscle		
	Cp	Cg	Cs	Cp	Cg	Cs	Cp	Cg	Cs	Cp	Cg	Cs	Cp	Cg	Cs
LDH-B ₄	4	1/0	0	4	0	0	4	1	1	3	1/0	0	1	0	0
LDH-A ₁ B ₃	2	0	0	2	0	0	3	0	0	3	0	0	0	0	0
LDH-A ₂ B ₂	2	0	2	2	0	1	3	0	0	1	0	0	0	0	0
LDH-A ₃ B ₁	2	0	1	1	0	1	1	1	4	0	0	4	1	1	2
LDH-A ₄	4	4	1	3	2	1	1	1	1	1	1	1	4	4	1
LDH-C ₄	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0

4= very high activity; 3= high activity;
 2= intermediate activity; 1= detectable presence; 1/0=
 present in traces and
 0= absence.

Table 2: Qualitative coding of LDH loci/ gene in mixed samples of *C. punctata*, *C. gachua* and *C. striatus* populations collected from Aligarh and Bareilly district. Where, 1 = presence and 0 = absence.

LDH loci/ gene	<i>C. punctata</i>	<i>C. gachua</i>	<i>C. striatus</i>
LDH-A		1	
LDH-B		1	
LDH-C		1	

either of the conditions described above which had been reported earlier in other fish species [8,17]. Therefore, the information on quantitative coding of these isoenzymes provided here may offer an explanation of the adaptive strategy adopted by the *C. gachua* and *C. striatus* under state of respiratory stress. While, higher levels of LDH-B in *C. punctata* seems to have favored least against the hypoxia/asphyxia induced alterations as an adaptive trait [13].

LDH zymograms as explained under results show the presence of two detectable activities in *C. punctata* and *C. striatus* that typically correspond to two loci of LDH namely LDH-A and LDH-B (Table-2). The inferred homotetrameric composition of the representative LDH activities of A and B loci and that of their random heterotetramers (-A₁B₃, -A₂B₂, -A₃B₁) is also confirmed by heat inactivation (results not shown here). Published evidence also reveals the existence of a third locus 'C' that is temporally expressed in fishes [18]. Previously it was referred to as E locus because it was known to predominantly function in regions of nervous system concerned with vision, but subsequently an orthologous expression was found in other tissue such as the liver and thus renamed as 'C' [1]. It is estimated that C-locus might have evolved as a result of duplication of B [2-3,8] whereas predecessor of A and B was a single gene of more remote origin.

Whitt and Maeda [19] have also reported that blind cave fish, *Anoptichthys jordani*, also lack the LDH-C gene functioning specially in the nervous system because of loss of visual structures and function in this fish. However, other closely related species of characins and some other teleosts have been reported to lack function of this gene [19-20] that strongly suggests that LDH-C₄ isoenzyme is not an essential component for vision. Therefore, the lack of LDH-C gene in *C. punctata* and *C. striatus* may indicate that evolutionarily they are not of recent origin. They may be phylogenetically more close to the fishes belonging to super order Osteriophysii; and the species of Characiformes and Siluriformes that lack the functioning of LDH-C gene. However, unambiguous presence of LDH-C in the eye of *C. gachua* elucidates its evolutionary closeness with species of bony fishes belonging to class Acipenseriformes, Amiiformes, Anguilliformes, Myctophiformes, Perciformes, Pleuronectiformes, etc. where the existence of this gene has been correlated with the metabolism of photoreceptor cells in the retina [1-2].

Out of the three species of Channids selected here, *C. gachua* is a polyploid with 2n=78 [21]. As polyploidization is an important sympatric speciation process which marks an important evolutionary step- an autapomorphy for newly formed species [22]. Therefore, it may be proposed that during the course of evolution with the event like polyploidization or eventual synapomorphy, *C. gachua* might have evolved as a fish of generalized existence against the selective or stochastic forces similar to few other groups of bony fishes. Reports are also available where due to differed evolutionary protocols, variations in the level of gene expression have been demonstrated to contribute dissimilarity in individual tissue enzyme/ isoenzyme levels [12,16]; hence these variations can be utilized to understand the mode of evolution of regulatory genes controlling the steady state levels of these enzyme molecules and other related metabolic processes in the selected air-breathing teleosts [16]. Now, it appears that the significance of these characters (isoenzyme coding proposed here) in estimating the genetic divergence or evolution of different fish species at tissue level may be of great impact and further elaborative studies should be designed in this direction to explore the survivorship of air-breathing teleosts against the unknown selective forces acting during the course of evolution.

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