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AN ELECTROPHORETIC INVESTIGATION OF TISSUE-SPECIFIC ISOZYMES OF LACTATE DEHYDROGENASE IN SOME HOLOCENTRID FISHES FROM TAIWAN[1]

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Stephen C.M. Tsoi, Sin-Che Lee and Hin-Kiu Mok (1986) An Electrophoretic investigation of tissue-specific isozymes of lactate dehydrogenase in some holocentrid fishes from Taiwan. *Bull. Inst. Zool., Academia Sinica* **26**(2): 151-155. The tissue-specific isozymes of lactate dehydrogenase in ten different tissues of holocentrid fishes indicated that the major isozyme pattern can be attributed to three protein subunits under the control of three genes which is similar to other teleosts. The Ldh-A locus was expressed principally in white skeletal muscle. The Ldh-B locus showed expression predominant in heart muscle. However, the tissue expression of the Ldh-C gene was very broad in distribution with an anodal C_4 homopolymer detectable in extracts of eye, brain, heart, kidney and liver. This is in contrast to most advanced teleosts with C_4 activity restricted to nervous tissues only. All species exhibited two or three isozyme formation from LDH A-B subunits interaction with no indication of heterotetramers of B_3A and A_3B. It is probably due to the evolutionary divergence between the A and B subunits on the restriction of LDH A-B subunits interaction.

Early studies of fish lactate dehydrogenase isozymes revealed that three genes are produced related to complex pattern of fish zymograms. Generally speaking, LDH in most fishes is similar to the basic tetrameric structure found in other higher vertebrates (Pesce *et al.*, 1967; Baily and Wilson, 1968). An evolutionary study of fish LDH isozymes has shown that the homotetramer A_4 is the predominant isozyme appeared in white skeletal muscle and the homotetramer B_4 is abundant in tissues receiving a constant supply of oxygen: heart and brain (Whitt, 1970). However, a third locus designated as Ldh-C which is proposed by Shaklee and his co-workers (1973) occurs only in teleost. In most higher teleosts, the expression of Ldh-C gene is only restricted to neural tissues such as eye and brain or only appeared in liver (Shaklee *et al.*, 1973; Markert *et al.*, 1975). However, Markert and his co-workers (1975)

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found out that in most primitive bony fishes Ldh-C locus is expressed in a variety of tissues. Thus, among teleosts the general trend in evolution is clearly for specialization of the C gene and restriction of its expression to few tissues.

It was the purpose of this investigation to establish the tissue distribution pattern and subunit composition of the lactate dehydrogenase isozymes in order to discuss the position of holocentrid fishes and the order Beryciformes as a whole in relation to the evolutionary scheme proposed by Markert *et al.* (1975).

MATERIALS AND METHODS

Specimens were either obtained fresh from the fish markets located near at Chung-Chou, Ho-Pi-Hu and Tachi or brought alive from local aquariums, and carried under the liquid nitrogen to the laboratory immediately for dissection. Species identification were made according to Greenfield (1974) and Masuda *et al.* (1984). Ten different tissues including brain, eye, gills, gonad, heart, kidney, liver, white muscle, spleen and stomach were dissected and homogenized in two volumes of 50 mM Triethanol-amine-HCl, pH 7.5 with 10 mM $MgCl_2$, 5 mM EDTA and 50 mM 2-Mercaptoethanol at 4°C. The homogenates were then centrifuged at 45,000×g for 45 minutes at 4°C and stored at −70°C until electrophoresis.

Electrophoresis was performed on a horizontal gel apparatus with cooling system in an 12% electrostarch gel. The Tris-citrate buffer system was used according to Horowitz and Whitt (1972) with pH 7.0. Electrophoresis was carried out at 4°C for 4 hours at 175 volts. After electrophoresis the starch gel was sliced lengthwise. The top slice of the gel was incubated in an LDH staining solution at 37°C in the dark. The staining solution was prepared as described by Shaklee *et al.* (1973). The bottom slice of the gel was stained for non-specific reductants or 'nothing dehydrogenase' without putting the substrate, lactate, in the staining solution. All chemicals were brought from Sigma Chemical Company. Nomenclature of LDH isozymes, subunits and structural genes were made according to Shaklee *et al.*, 1973.

RESULTS

The electrophoretic LDH patterns of seven species of holocentrids are very similar

TABLE 1 Characteristics of lactate dehydrogenases in fishes in the family holocentridae[1)]

Fish species	A-B tetramers (No)	A-B tetramers RAM	Expression of the LDH-C locus B	E	G	Go	H	K	L	M	St	Sp
Family Holocentridae												
Subfamily Myripristinae												
Ostichthys japonica (2)	2	C>B>A	⫽	⫼	±	±	+	±	+	−	±	±
Myripristis adustus (2)	3	C>B>A	±	⫼		±	+	+	+	±	±	+
Myripristis kuntee (2)	3	C>B>A	⫽	⫼	⫽	±	+	⫽	±	±	−	⫽
Myripristis violaceus (4)	3	C>B>A	⫽	⫼	+	±	+	+	+	±	±	±
Subfamily Holocentrinae												
Flammeo sammara (2)	3	C>B>A	⫽	⫼	±	⫽	±	+	+	+	±	±
Sargocentron diadema (2)	3	C>B>A	⫽	⫼	+	±	⫽	⫽	+	±	±	±
Sargocentron caudimaculatus (1)	3	C>B>A	±	⫼	±	−	⫽	⫽	+	±	±	+
Holocentrus rufus (1)*	3	C>B>A	⫽	⫼	±	⫽	+	+	+	±	+	±

[1)]RAM=Relative anodal mobility
( )=the numbers in parentheses indicate the number of specimens examined.
Relative quantities of C subunits: ⫼, most abundant; ⫽ & +, intermediate abundant; ±, marginal presence; −, undetectable; blank, tissue not examined.
B, brain; E, eye; G, gills; Go, gonad; H, heart; K, kidney; L, liver; M, muscle; St, stomach; Sp, spleen
* Refer to Shaklee *et al.*, 1973.

to each other in their tissue-specificities. Electrophoretic analysis revealed that, in all the species examined, the B subunit is considered more negatively charged than the A subunit. The relative anodal mobility among three subunits is C>B>A (Table 1).

All species of holocentrids exhibit three LDH loci-A, B and C, with A_4 homopolymer predominated in white skeletal muscle (Figs. 1-4), the B_4 homopolymer predomonated in heart, brain, and stomach (Figs. 1-4). However, the C_4 tissue distribution was found very different from advanced teleosts. Not only the C_4 homopolymer predominated in eye and brain, all other tissues exhibited some degree of C gene activity (Table 1). However, in five out of ten tissues examined such as eye, brain, heart, kidney and liver, the C_4 activity is always visible after gel staining and appeared on the most anodal side of the gel. For the rest of the tissues examined, sometime only heteropolymers containing C subunits were observed (Figs. 1-4).

In most of the species examined the A-B subunits are not able to associate at random to form five isozymes A_4, A_3B, A_2B_2, B_3A and B_4 in all tissues. Only two or three

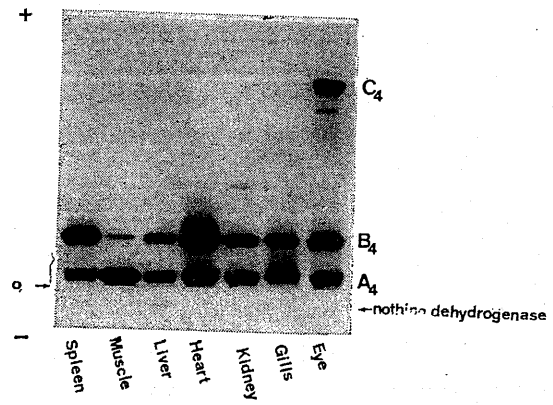

Fig. 1. LDH isozymes of *Ostichthys japonica*. Starch gel electrophoresis was performed using Triscitrate pH 7.0 buffer at 4°C for 4 hrs at 175 V.

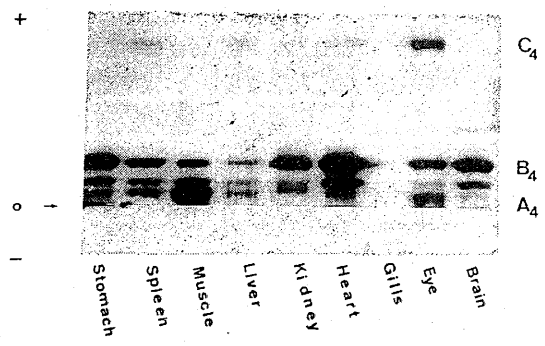

Fig. 2. LDH isozymes of *Myripristis adustus*. Starch gel electrophoresis was performed using Triscitrate pH 7.0 buffer at 4°C for 4 hrs at 175 V.

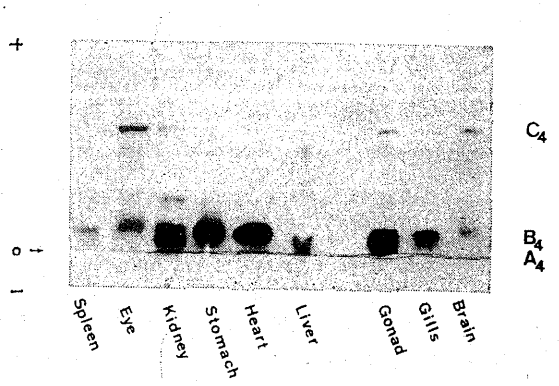

Fig. 3. LDH isozymes of *Flammeo sammara*. Starch gel electrophoresis was performed using Triscitrate pH 7.0 buffer at 4°C for 4 hrs at 175 V.

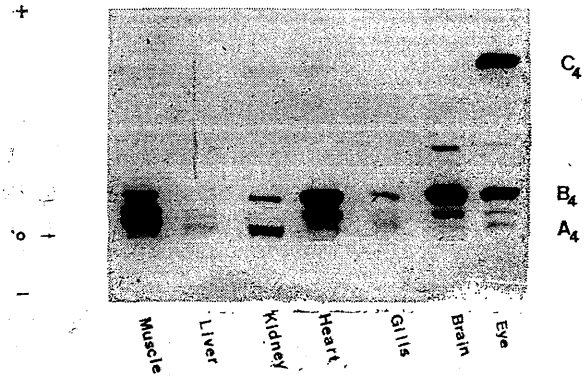

Fig. 4. LDH isozymes of *Sargocentron caudimaculatus*. Starch gel electrophoresis was performed using Triscitrate pH 7.0 buffer at 4°C for 4 hrs at 175 V

banded pattern related to the formation of A_4, A_2B_2 and B_4 was observed (Table 1; Figs. 1-4).

DISCUSSION

According to Markert and his co-workers (1975), the restriction assembly of heteropolymers containing A and B subunits could be due to the evolutionary divergence of subunits A and B. In almost all the species examined from the present study, the subunits A and B only give rise to three isozymes. Whenever Ldh-A gene and Ldh-B gene are both active in the same tissue, this phenomenon is observed. It is unlikely that the epigenetic mechanisms operate in order to restrict the association of subunits. Our data supports the above proposal given by Markert *et al.*, 1975. Among fishes, the restriction of subunit assembly between LDH A-B subunits seems to be related to the phylogeny of teleosts (Toledo and Ribeino, 1978). Until recently, Buth (1984) suggested that the dervied condition which has been observed in most higher teleosts, the loss of the ability to form heteropolymers, can be of systematic value and judged as a character state. In the present study, we consider the inability to form all five isozymes observed in all the species of holocentrids as an advanced character.

Extensive evolutionary surveys involving over 150 species (Markert *et al.*, 1975) have revealed that nearly all bony fishes express the LDH-C_4 isozyme. In primitive forms (Acipenseriformes, Amiiformes, Anguilliformes, etc.) the LDH-C_4 appears relatively unspecialized in its tissue expression; generally present in several to many different tissues (Markert *et al.*, 1975; Whitt *et al.*, 1975). Whitt and his co-workers (1975) proposed that this relatively undifferentiated state of the Ldh-C locus is precisely that which could be expected soon after its origin by gene duplication and can be considered as an ancestral character state. Our data of tissue expression of Ldh-C locus suggests that all the seven species of holocentrids represented by four genera *Ostichthys*, *Myripristis*, *Flammeo*, and *Sargocentron* exhibited primitive character with a board C_4 tissue distribution (Table 1). Our finding is also comparable to one species of Atlantic holocentrid, *Holocentrus rufus*, studied by Shaklee *et al.*, 1973. In this species of fish, the eye-band (C_4) is detectable in extracts of eye, brain, gills, liver, testis, and kidney with heteropolymers containing C subunits in muscle, heart, stomach, and spleen extracts (Shaklee *et al.*, 1973).

It is not suprising to find both of the derived and primitive biochemical characters in the species of the family Holocentridae, since holocentrids belong to one large group of fishes within the order Beryciformes which Nelson (1984) mentioned a polyphyletic taxon among beryciforms. Upon other biochemical characters are studied in the future from the families within the beryciforms, the phylogenetic relationships among beryciform fishes will be more clear to support the above hypothesis.

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以電泳方法探查若干臺灣產金鱗科魚類乳酸去氫同功異構酶在各組織之分佈特性

蔡昌明　李信徽　莫顯蕎

本文記述金鱗魚的乳酸去氫同功異構酶在十種不同組織之分佈特性。由研究結果顯示金鱗魚之乳酸去氫同功異構酶之基因與一般眞骨魚類相同分別由三種基因控制同功異構酶蛋白分子(protein subunits 之製造。Ldh-A 基因在骨肌表現出較顯著的特性，Ldh-B 基因在心肌特別活躍，Ldh-C 基因則廣泛地在各組織內都有一定程度上之活性，尤其在眼、腦、心肌、腎及肝等組織特別顯著。此類組織上之分佈狀況與一般高等眞骨魚類有很大的差異。另外由所研究的金鱗魚材料中發現只有二種或三種乳酸去氫同功異構酶係由 LdhA-B 蛋白分子交互作用而產生的，並無任何 B_3A 及 A_3B 之異質四合體 (heterotetrameres)。此種現象可能是由於A與B蛋白分子間在演化之差異而限制其交互作用之進行。