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Lactate Dehydrogenase Isozyme Pattern, Fiber Type Composition and Myofibrillar ATPase in the Skeletal Muscle of Certain Vertebrates with Different Modes of Locomotion

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The distribution pattern of LDH isozyme and of their subunits indicate the metabolic profile of the muscle. The fiber type composition of different muscles indicate the varying metabolic requirements. The muscles which are fast contracting and fatigue rapidly in response to their mode of locomotion (swimming, hopping, sprinting) had predominantly M-type LDH isozyme pattern but there was a shift to H-type in muscles with capacity for endurance locomotion pattern (flying). The present study has elucidated the specific correlation of LDH isozyme pattern, myofibrillar-ATPase (m-ATPase) and fiber type composition of muscle with the locomotor capacity of animals.

Key Words: Vertebrate locomotor muscles, LDH Isozyme, Myofibrillar ATPase, Muscle fiber types, Electrophoresis

Introduction

Muscle metabolism (aerobic or anaerobic) and the fiber types, a muscle contains, are generally related to the locomotor patterns of different vertebrates. The anaerobic energy production is enhanced by both the amount and isozymes of lactate dehydrogenase (LDH) present (Hochachaka et al. 1983) as they serve to impose amplification of flux as well as fine control by increasing glycolysis to two fold during maximum activity (McGilvery 1975). LDH isozymes, metabolic indicators of glycolytic activity, have been widely studied in different vertebrates (Dawson et al. 1964, Kaplan & Goodfriend 1964, Markert 1984, Baldwin 1988).

The varying fiber type composition of major locomotor muscles (gastrocnemius, pectoralis, myotomal) accounts for the different metabolic requirement thus affecting the specific distribution of LDH isozymes. There are very few reports relating LDH isozyme pattern to the locomotor habits of animals (Wilson et al. 1963, Brush 1968, Gutierrez et al. 1973) and these too are mainly confined to mammals and birds. The work we report in this paper relates the distribution of LDH isozymes, fiber type composition and biochemical study of m-ATPase to the functioning of gastrocnemius, pectoralis and myotomal muscles of different representative vertebrates. m-ATPase activity of muscle fiber is related to the speed of muscle shortening (Barany 1967).

Materials and Methods

Animals were purchased from local suppliers and were anaesthetised. Muscles from the anaesthetised animals were excised free of blood vessels and kept in cold petri dishes. Small pieces of muscles were frozen by immersion in liquid N_2 and

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sectioned (10 μm) in a cryostat. Sections were stained for SDH and m-ATPase according to the methods of Nachlas et al. (1957) and Guth & Samaha (1970) respectively. Fiber type population was counted in different muscles. For m-ATPase assay, myofibrils were prepared according to Perry and Grey (1956). Assay was performed at 37°C in a reaction mixture containing (mM) Tris HCl (*p*H 7.5): KCl 40 and $CaCl_2$10. (Talesara & Goldspink 1978). Values were expressed as μ moles of Pi liberated/mg protein/min. Protein content was measured by the method of Gornall et al. (1949). Isozymes were separated and stained for LDH by 7% PAGE according to Davis (1964). Samples were prepared in chilled saline EDTA buffer (*p*H 8.0). Isozymes were visualised by using NBT as an electron acceptor. Gels were stored in 7% acetic acid and scanned in cecil (CE 570) gel scanner.

Results

Histochemical and biochemical studies on SDH and m-ATPase show that fish myotomal, frog, lizard and rabbit gastrocnemius muscles have predominantly Fast glycolytic (FG) fiber types (figure 1, A-F). Rat, squirrel and goat gastrocnemius muscles have predominantly Fast Oxidative Glycolytic (FOG) fiber types (figure 1 J-M). Sparrow, bat pectoralis and rat soleus muscles have predominantly slow oxidative (SO) fiber types (figure 1 N-R). The muscles with predominantly fast fiber types (FG or FOG) have very high m-ATPase activity as studied biochemically (table 1). Muscles with high glycolytic activity have predominantly M-type LDH isozyme pattern whereas more oxidative muscles have predominantly H-type isozyme pattern as revealed by the study of LDH isozyme patterns of different muscles of locomotion (figure 2). The number of bands and the intensity of corresponding subunits vary in different muscles. Fish myotomal, frog, lizard, rabbit, rat and squirrel gastrocnemius muscles show predominantly M-type LDH isozyme pattern (figure 2 A-G) where LDH M_4 peak is very high for fish myotomal and rabbit gastrocnemius muscles. Squirrel gastrocnemius has more of H_4 and H_4M subunits than the rat gastrocnemius (figure 2 F, G). Goat gastrocnemius has high level of M_2H_2 in addition to high M_4 (figure 2 H). Pectoralis muscles of pigeon and bat show H-type LDH isozyme pattern (figure 2 J,K). LDH-Isozymic pattern of rat soleus closely resembles that of bird pectoralis, i.e. H-type (figure 2 I).

Discussion

The results show a strong correlation of m-ATPase muscle fiber types (table 1, figure 1) and different LDH isozyme patterns (figure 2, table 1) to the modes of locomotion of different animals studied. Rabbit and frog, which have sprinting and hopping type of locomotion, respectively (Sperry 1981, Schnurr & Thomas 1984), have powerful hindlimb extensor muscle which works sporadically and requires quick bursts of energy for its movements. Lizard is a sit and wait predator which extends less energy on locomotor activity and has greater capacity for burst activity (Gleeson et al. 1980). Present results show that LDH isozyme distribution pattern parallels the functional response of muscle as frog and lizard leg muscles show almost a single peak of LDH M_4 isozyme and rabbit muscle shows very high LDH M_4 peak compared to other subunits (figures 2B, 2C, 2D, table 1). These muscles are composed of predominantly FG fiber types (figure 1 A-F). LDH activity study on individual muscle fibers of different types have shown that higher activity in FG, lower in FOG and least in SO fiber types (Takekura & Yoshioka 1987). Fish myotomal muscle shows rapid movements of short duration (Johnston 1981). It has predominantly FG fiber type (figure 1A) and shows high peak of LDH M_4 which seems to be more important for glycolysis (Wilson et al. 1975). It has been suggested that electrophoretic homologies cannot be drawn between fish and mammals as mobility of LDH subunits in fish is opposite to that of mammals (Markert & Faulhaber 1965, Talesara & Kiran 1984).

Densitometric study shows that anodic enzymes H_4 and H_3M are more in squirrel than in

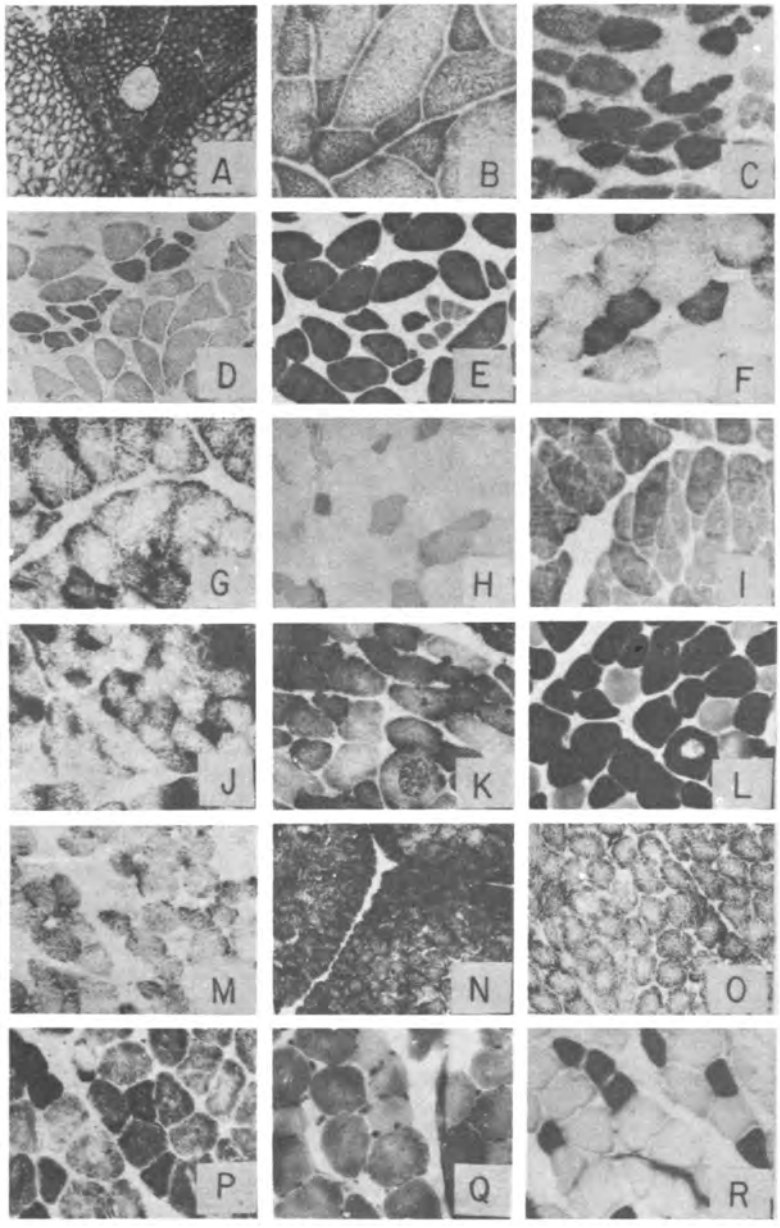

Figure 1 **A-R** T S of certain locomotor muscles of vertebrates stained for SDH (**A, B, C, F. G, J, K, M, N, O, P**), m-ATPase preincubated at acidic (**D, H, Q**) and alkaline *p*H (**E, I, L, R**) respectively × 120)

A Fish myotomal; **B** Frog gastrocnemius; **C, D, E** Lizard gastrocnemius; **F** Rabbit gastrocnemius; **G, H, I** Turtle gastrocnemius; **J** Rat gastrocnemius; **K,L** Squirrel gastrocnemius; **M** Goat gastrocnemius; **N, O** Sparrow and bat pectoralis; **P, Q, R** Rat soleus

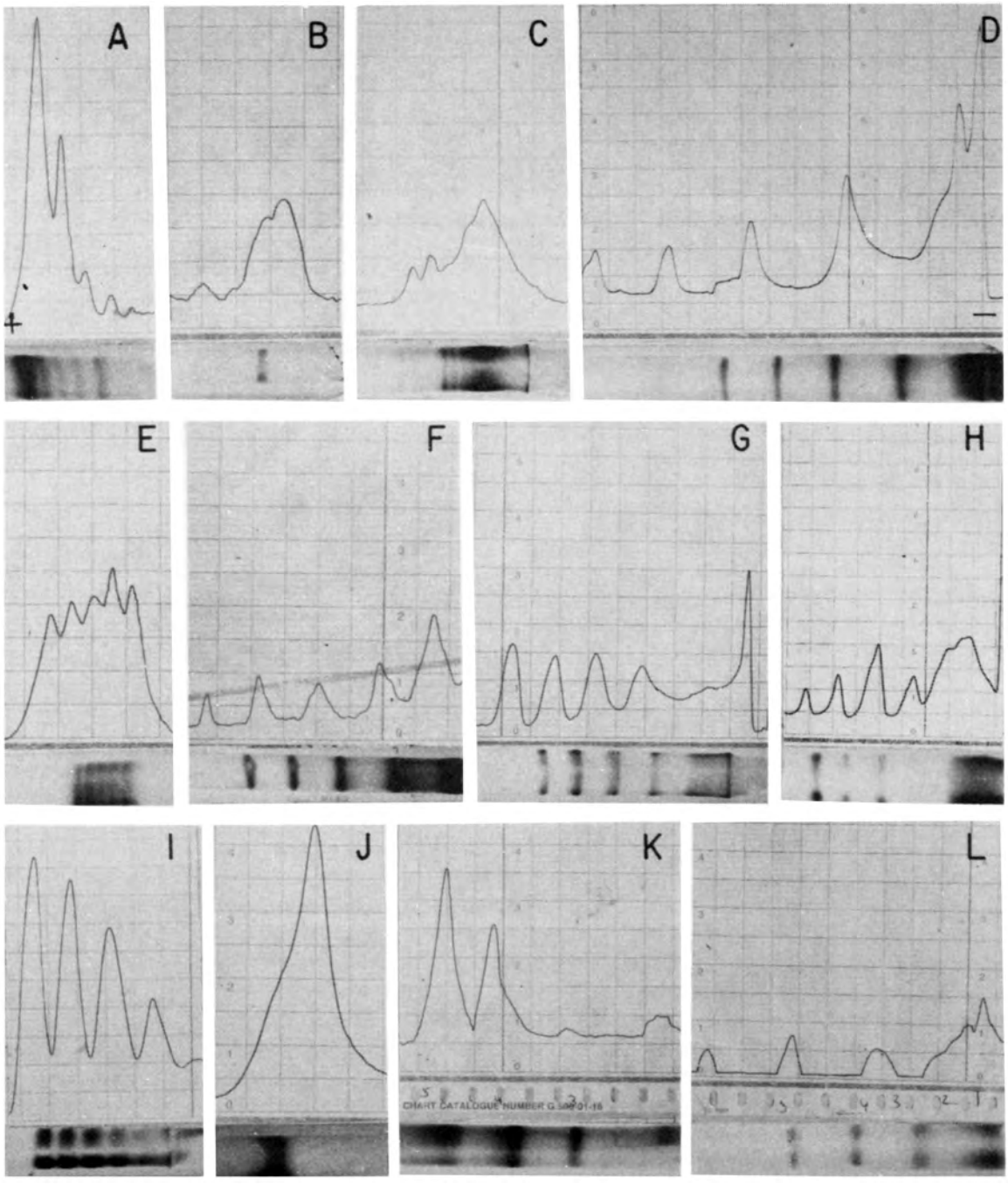

Figure 2 A-L LDH isozyme pattern and the densitometric scans of certain locomotor muscles **A** to **L** Fish myotomal; Frog gastrocnemius; Lizard gastrocnemius; Rabbit gastrocnemius; Turtle gastrocnemius; Rat gastrocnemius; Squirrel gastrocnemius; Goat gastrocnemius; Rat soleus; Sparrow pectoralis; Bat pectoralis; Bat gastrocnemius respectively

Table 1 *Fiber type composition, m-ATPase activity and LDH-isozyme pattern of certain vertebrate locomotor muscles*

Animal/muscle	Percentage composition of fibre types			m-ATPase activity (μmoles Pi/mg protein/min)	Amount of LDH subunits based on densitometry					Locomotion patter
	FG	FOG	SO		H_3	MH_3	M_2H_2	M_3H	M_4	
Fish (myotomal) (*H. fossilis*)	90	8	2.0	0.401 ± 0.022	+	+ +	+ + +	+ + + +	+ + + + +	Swimming
Frog (gastrocnemius) (*Rana tigrina*)	54	25	21	0.2859 ± 0.092	−	−	−	+	+ + + + +	Hopping
Lizard (gastrocnemius) (*Hemidactylus flaviridus*)	71	8	21	0.318 ± 0.028	−	−	+	+	+ + + + +	Running
Rabbit (gastrocnemius) (*Oryctologus cuniculus*)	55	30	15	0.335 ± 0.021	+	+ +	+ + +	+ + + +	+ + + + +	Sprinting
Turtle (gastrocnemius) (*Lessymus punctata*)	34	42	24	0.179 ± 0.062	+	+ +	+ + +	+ + + +	+ + + + +	Swimming
Rat (gastrocnemius) (*Rattus noroegicus*)	28	54	18	0.238 ± 0.025	+	+ +	+ + +	+ + + +	+ + + + +	Running
Squirrel (gastrocnemius) (*Funambulus Sp.*)	25	60	15	0.175 ± 0.024	+ + + +	+ + +	+ + +	+ +	+ + + + +	Running
Goat (gastrocnemius) (*Capra Sp.*)	35	40	25	0.09 ± 0.012	+	+ + +	+ + + +	+ +	+ + + + +	Walking
Sparrow (pectoralis) (*Passer domesticus*)	—	100	—	0.311 ± 0.02	+ + + + +	−	−	−	−	Flying
Bat (pectoralis) (*Pipistrellus Sp.*)	—	100	—	0.251 ± 0.02	+ + + + +	+ + + +	+ + +	+ +	−	Flying
Rat (soleus)	—	18	82	0.150 ± 0.016	+ + + + +	+ + + +	+ + +	+ +	+	Standing

FG, Fast glycolytic; **FOG**, Fast oxidative glycolytic; **SO**, Slow oxidative
+ + + + + very high, + + + + high, + + + moderate, + + low, + least, − absent

rat muscle (figure 2 F-G). This difference in rat and squirrel LDH isozyme pattern is explained by the arboreal-terrestrial habitat of squirrel which demands running and climbing type of locomotion. This is confirmed by the presence of more oxidative and energy efficient FOG fiber types in squirrel (figure 1 J&K, table 1). Less peak of LDH M_4 in rat and squirrel compared to rabbit is explained by their increased sustainability for running. Turtle gastrocnemius with predominantly FOG fiber types (figure 1 G-I) shows M type LDH pattern suitable for its survival during long anaerobic periods of submergence or diving in water (figure 2E). Relative gastrocnemius areas may also play important role in determination of relative and absolute lactate threshold during running (Atomi et al. 1987).

There is a shift to H-type LDH isozyme pattern in muscles of animals with increased capacity for endurance locomotion. Goat shows slow walking and prolonged standing for its grazing activity. Thus, it has higher levels of LDH M_2H_2 in addition to LDH M_4 subunits (figure 2H, table 1). This is in correlation with the histochemical study which shows more oxidative fiber types in its muscle (figure 1M, table 1). The fast flapping fliers like sparrow and bat (insectivorous) show sustained flight as they have long foraging period during which time they are continuously on wing (Vaughan 1970), where leg muscles are used occasionally for hanging in bat but perching in sparrow. The flight muscles show predominantly H_4 LDH isozyme pattern (figure 2 J&K) as these muscles depend on the energy efficient lipolytic pathway, whereas leg muscle shows M_4 LDH isozyme pattern (figure 2L). This is consistent with the histochemical study which shows the homogeneous composition of FOG fibers and complete absence of FG fibres in flight muscles (figure 1 N&O). FG fiber types contribute to M_4 and M_3H LDH isozyme (Van Wijhe 1964).

Muscles with high glycolytic activity showing a single or high peak of LDH M_4 in gastrocnemius (frog, lizard, rabbit) and myotomal muscles (fish) have high m-ATPase activity (table 1) showing a correlation between m-ATPase and glycolytic activity. This is in agreement with the earlier reports (Talesara & Kiran 1984, Laborde et al. 1985, Briand & Briand 1986). However, the bird and bat flight muscles show a reverse relationship as they show high m-ATPase and low glycolytic activity but high oxidative capacity. This is the result of characteristics of individual muscle fibers as FOG fibers of flying animals have attained the highest oxidative capacity among locomotor muscles favouring sustained work by efficiently producing ATP.

Histochemical study also shows the uniform and dense distribution of mitochondrial enzyme, SDH in the muscle fibers (figure 1 N&O) which reflects the extreme metabolic specialisation achieved in the flight muscle. In FOG fiber type of some mammals, oxidative and glycolytic capacities are intermediate to SO and FG fiber types (Ashmore & Doerr 1971, Peter et al. 1971, Reichmann & Pette 1981). The present data reveal that FOG fiber types are the most adaptable in vertebrate muscles because of their dual capacity for oxidative and glycolytic activities. Muscles predominant in SO fiber type such as soleus (figure 1P, Q, R) shows H-type LDH pattern (figure 2 I) but those predominant in FG fiber types have M-type pattern. However, bird pectoralis and goat gastrocnemius muscles predominant in FOG fiber types show H-type pattern as they are more oxidative than glycolytic but M-type if vice versa in the gastrocnemius of turtle, rat, squirrel and bat. The results confirm that fiber type composition of a muscle is the most important determinant of locomotor capacity as skeletal muscle contractility, oxidative and glycolytic capacity stem from different fiber types present. The properties of LDH are important determinants of interplay between anaerobic glycolysis and oxidative phosphorylation.

The correlation between tissue enzyme pattern and function affords a sound basis to evaluate the role of isoenzyme. Modifications of LDH isoenzymes, distribution pattern in tissues, during ontogenesis (Markert & Ursprung 1962), in tissue culture studies (Kaplan & Goodfriend 1964) and changes in tissue patterns correlated to

the changes in metabolic requirement (Hochachaka et al.1988, Parkhouse 1988) constitute meaningful evidence in favour of physiological roles assigned in LDH M_4 and H_4. In this context correlation of LDH isozymes in muscle with locomotion modes of certain vertebrates in the present study is highly significant.

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