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An Electrophoretic Study of LDH-Isozyme Distribution in Developing Embryonic and Adult Tissues of the Toad *Bufo arabicus*

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دراسة استشرادية لتوزيع ايسوزوم LDH في مراحل نمو جنين وأعضاء الضفدع العربي البالغ

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خلاصة: تم دراسة لاكتات الديهايدروجينيزات LDH باستخدام طريقة الاستشراد للنظر في الفروقات الايسوزيمية المتوقع وجودها في جميع أعضاء جنين الضفدع العربي في مراحل نموه المختلفة. تم تحليل الشكل التعبيري لايسوزومات اللاكتات الديهايدروجينية باستخدام هلام البولي اكريلامايد الاستشرادي غير المتغير الشكل (PAGE). تم ملاحظة خمسة ايسوزومات LDH في خمسة أعضاء للضفدع العربي البالغ حيث كان نشاط القطب السالب قويا، اما مراحل نمو الجنين فاظهرت الايسوزمات الاجابية أولا، حيث ظهرت اربعة LDH في مراحل الجنين من 1-16 (مرحلة الاخصاب الى مرحلة تكوين الذيل)، أما الايسوزوم الخامس فقد ظهر في مرحلة 17 وذلك بداية نشاط العضلات ويزداد كثافة عند التقدم في مراحل النمو ابتداءا من مرحلة 17 الى مرحلة 43.

ABSTRACT: Lactate (LDH) dehydrogenases were studied by electrophoresis to investigate the possibility of isozymic differences in different stages and organs of *B. arabicus* embryos, tadpoles and adults. The expression pattern of lactate dehydrogenase (LDH) isozymes was analysed using nondenaturing discontinuous polyacrylamide gel electrophoresis (PAGE). Five LDH isozymes were observed in five different adult tissues. The most cathodal isozyme, LDH-5 (A_4), showed the strongest activity. All embryonic stages tested displayed the anodic isozymes first. Four LDH isozymes were present in the stages 1-16 (fertilized egg-tail bud stage). The first appearance of the fifth isozyme was detected in stage 17 when the muscular response started. This isozyme showed gradual increase in intensity from stage 17 to stage 43.

KEYWORDS : Toad, embryos, tadpoles, skeletal muscle, LDH, *Bufo arabicus.*

1. Introduction

For a range of animal groups, 'model' species are now well characterized in terms of the morphology and molecular biology of their embryonic and early larval stages. For anuran amphibians, the best characterized species is *Xenopus laevis*. However, if we are to understand the ways in which development has diverged within animal groups, and adapted to different environments (Gilbert, 2001), we need to examine both morphology and molecular expression in a variety of related species.

Molecular analysis provides additional information on species identity in areas where mixed genetic types are present (Simovich and Sassman, 1986). During cell differentiation, genes play an important role in metabolism, so multilocus isozymes constitute probes for investigating the mechanisms of spatial and temporal gene regulation in different stages of development (Philipp *et al*., 1979). Thus the study of isozymes provides considerable insight into the structure and function of metabolic enzymes.

Lactate dehydrogenase (LDH) is considered a suitable system for studying several metabolic, genetic and ecological features (Coppes, 1990; Ropson *et al*., 1990; Almeida-Val *et al*., 1992). In most vertebrates, LDH is a tetrameric enzyme composed of two kinds of subunit polypeptides, A and B (Appella and Markert, 1961). LDH isozyme patterns are species and tissue specific and their expression patterns vary during development and several studies have been conducted on the electrophoretic distribution of LDH in amphibians (Mathews, 1975; Harintz and Diehl, 1999). This study aims to investigate the ontogeny of the enzyme lactate dehydrogenase (LDH) in some tissue of the *Bufo arabicus* embryos, tadpoles and adults.

2. Materials and methods

Embryos and tadpoles of stages 1-18, 21-25, 29, 31, 35, and 43 (Ba-Omar *et al.* 2004) were obtained by induced fertilization. Adult toads were killed by pithing and the following tissues were collected skeletal muscle, heart, liver, lung and stomach (Ambusaidi, 2002). The embryos and tadpoles were first washed in distilled water, then frozen in liquid nitrogen and stored at -70 ºC (Beis *et al*. 1992). The adult organs were dissected out, kept in ice, and washed with distilled water prior to the protein extract being prepared.

With slight modifications, the method of Markert *et al.* (1998) was used to analyse LDH isozymes. Thawed embryos and tadpoles were homogenized in 250-500 µl of sterile de-ionized water in an ice bath with a tissue tearor (Biospec products, USA) for 3-5 minutes depending on the sample size. The same procedure was used for adult organs using 1.0 ml of sterile de-ionized water. The homogenate was then centrifuged at low speed (X 2000g) in a microcentrifuge (Eppendorf 5402, Germany) for 15 minutes at 4º C. The supernatant was then taken and centrifuged at high speed (X 16000g) for 30 minutes at 4ºC. The resulting supernatant was removed for LDH analysis by electrophoresis on a polyacrylamide gel.

LDH isozymes were separated using non-denaturing discontinuous polyacrylamide gel electrophoresis following the method of Markert *et al* (1998). LDH isotrol (Sigma Co, USA) was used as a marker. LDH isozyme activity was visualized by incubating the gel at 37° C in the dark, in a reaction mixture specific for LDH, consisting of 20 ml 0.35 M Tris-HCl (pH 8.0), 80 ml de-ionized water, 2 ml nicotinamide adenine dinucleotide (Sigma Co, USA) (10 mg/ml), 2 ml nitro-blue tetrazolium (Sigma Co, USA) (10 mg/ml), 0.1 ml phenazine methosulphate (Sigma Co, USA) (10 mg/ml) and 2 ml of (3.0 M) lactic acid syrup (BDH Co, UK) until bands appeared after about 15 minutes. To determine whether any enzyme activity resulted from the activity of alcohol dehydrogenase, control gels were incubated in the absence of the substrate (lactate).

3. Results

The electrophoretic pattern of the LDH isozymes of the toad *B. arabicus* was distinctly different from those of the LDH isotrol standards from human tissue (Figure 1 lane m). The bands revealed by LDH specific stain were due to LDH activity. In the absence of a lactate substrate, no bands appeared. This confirms that the observed bands were produced by LDH activity and not by alcohol dehydrogenase or impurities (Figures 1- 4).

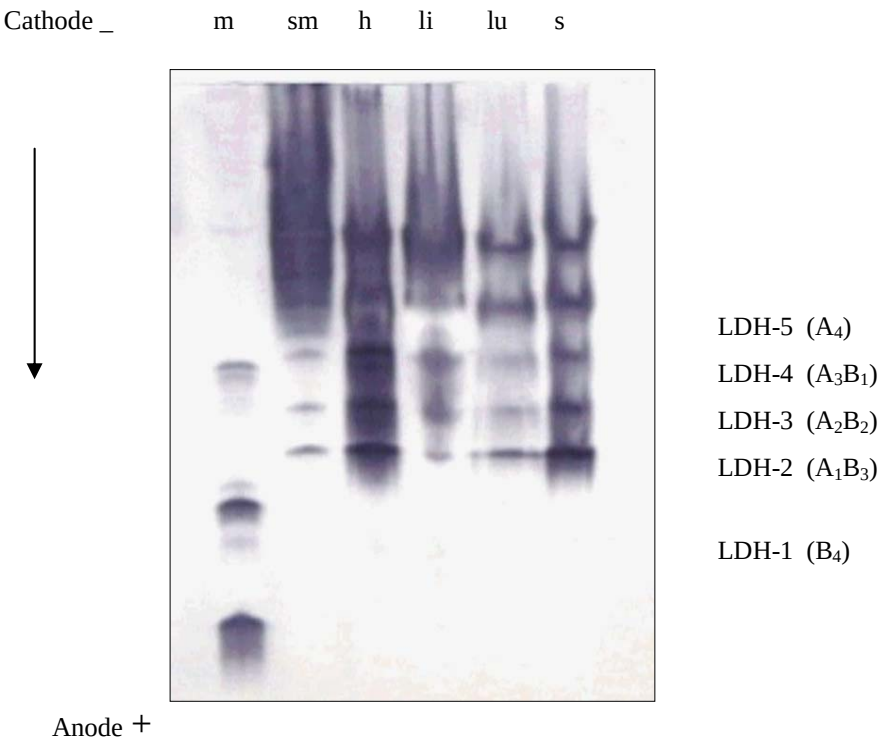

Figure 1. Electrophoretic patterns of LDH of *B. arabicus* adult from different tissues. Marker (m), skeletal muscle (sm), heart (h), liver (li), lungs (lu) and stomach (s).

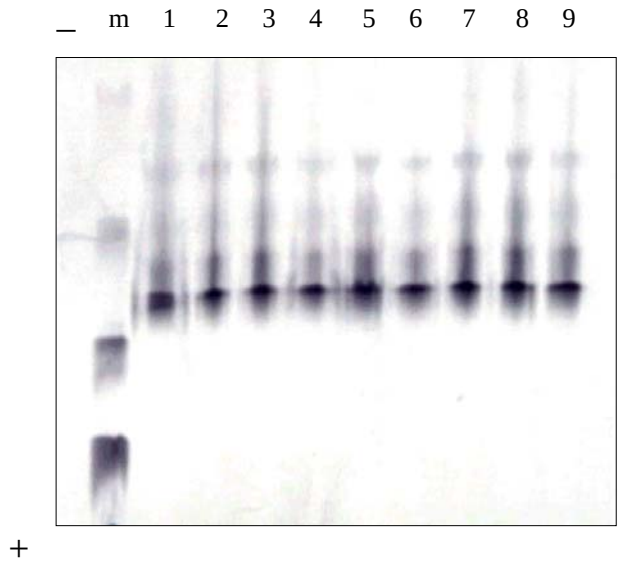

Figure 2. Electrophoretic patterns of LDH of *B. arabicus* embryos (stages 1-9). The numbers at the top refer to the developmental stages of *B. arabicus* embryos. Marker (m).

3.1 Electrophoretic pattern of LDH in adult tissues

LDH activity was detected in all tissues examined. Five distinct bands appeared on the polyacrylamide gel and these were numbered from 1-5 in their order of migration from the cathode to the anode (Figure 1). The most cathodic band was the homotetramer isozyme LDH-5 (A_4) and the most anodic band was the homotetramer isozyme LDH-1 (B_4). The three intermediate heterotetramers from cathode to anode were LDH-4 (A_3B_1), LDH-3 (A_2B_2) and LDH-2 (A_1B_3). All five isozymes LDH-5 to LDH-1 were observed in all adult tissues examined and the most cathodic isozyme (LDH-5 - A_4) showed the strongest activity.

In skeletal muscle and liver (Figure 1 lanes sm and li respectively) the most cathodal isozyme (LDH-5 - A_4) predominated and appeared as a very broad band, especially in skeletal muscle, followed by LDH-4 (A_3B_1). The other isozymes [LDH-3 (A_2B_2), LDH-2 (A_1B_3) and LDH-1 (B_4)] were only indicated by faint bands, especially in the skeletal muscle. The heart displayed all five bands with high intensity coloration (Figure 1 lane h). LDH-1 (B_4) was highly intense in the heart (Figure1 lane h) and secondly in the stomach (Figure 1 lane s), but it was only faintly indicated in skeletal muscle (Figure1 lane sm) and liver (Figure 1 lane li).

3.2 Ontogeny of LDH in developing Bufo arabicus

All embryonic stages tested displayed first the anodic isozymes (Figures 2, 3, and 4). A gradual increase in the expression of more cathodic isozymes was observed in the embryos and tadpoles at various developmental stages. Stages 1-16 (fertilized egg-tail bud stage) showed four stained bands: LDH-1 (B_4), LDH-2 (A_1B_3), LDH-3 (A_2B_2) and LDH-4 (A_3B_1). LDH-3 showed the lowest activity, while LDH-1 (B_4) showed the highest activity (Figure 2 lanes 1-9; Figure 3 lanes 10-16). The first appearance of the fifth isozyme band was in stage 17 (Figure 3 lane 17). This isozyme showed a gradual increase in intensity from stage 17 to stage 43 (Figure 3 lane 17; Figure 4 lanes 21-43). This electrophoretic pattern persisted to stage 43 (Figure 4 lane 43). It is apparent that band 5 [LDH-5 (A_4)] was the strongest, followed in intensity by bands 4, 1, 3 and 2. No differences could be observed in stages 29 - 43 regarding the number and intensity of bands (Figure 4 lanes 29 - 43).

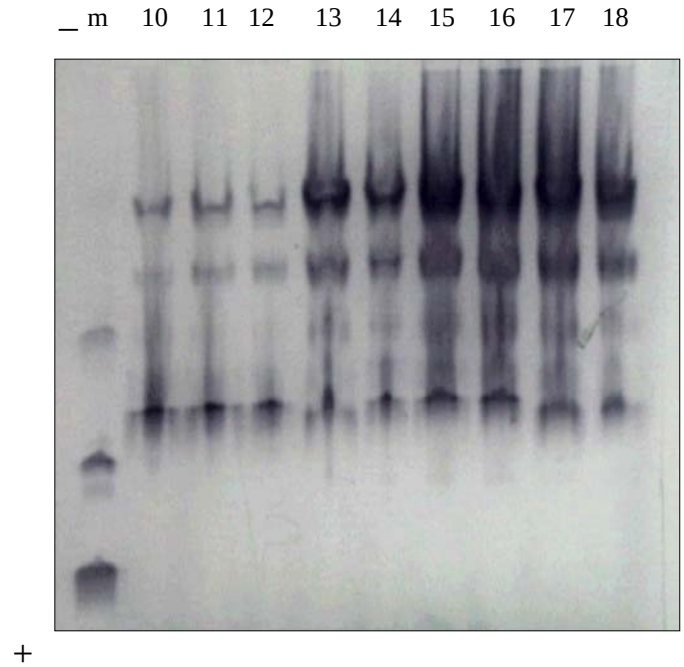

Figure 3. Electrophoretic patterns of LDH of *B. arabicus* embryos (stages 10-18). The numbers at the top refer to the developmental stages of *B. arabicus* embryos. Marker (m).

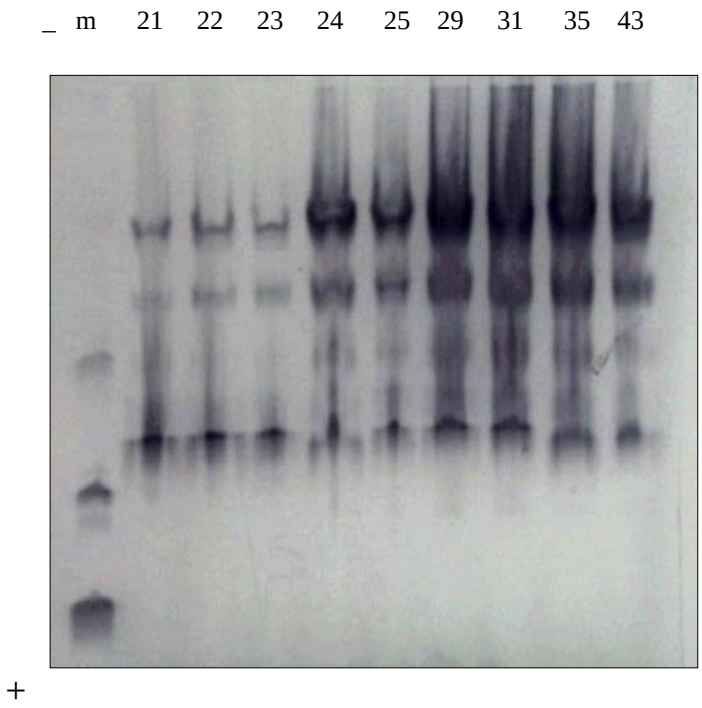

Figure 4. Electrophoretic patterns of LDH of *B. arabicus* embryos and tadpoles (stages 21-25, 29, 31, 35 and 43). The numbers at the top refer to the developmental stages of *B. arabicus* embryos and tadpoles. Marker (m).

4. Discussion

The ontogeny of lactate dehydrogenase (LDH) expression in the toad *B. arabicus*, embryos, tadpoles and some adult organs, shown by electrophoresis gives a five-band pattern. The expression of LDH genes is not tissue-specific in *B. arabicus* since all five isozymes appeared in all organs examined, although the isozyme A_4 predominated in skeletal muscle and the liver. Similar observations were also reported by Hranitz and Diehl, (2000) in their study of *B. woodhousii fowleri*. The isozyme A_4 always predominated in skeletal muscle, confirming its metabolic role in reducing pyruvate during the anaerobic glycolysis. Vonwyl (1983), in a study of *X. l. laevis, X. vestitus, X. borealis* and *X. wittei*, claimed that the multibanded pattern of LDH isozymes or the presence of secondary isozymes facilitates the identification of the species and contributes strongly to a species specific pattern .

B. arabicus exhibits anodal bands in early embryonic stages (stages 1-16). Moyer *et al*., (1968), Kunz (1973) and Vonwyl (1983) showed that the species they studied are characterized by the appearance of anodal bands in oocytes and early embryonic stages. In *B. arabicus*, the first appearance of the fifth isozyme (LDH-5, A_4) was at stage 17, which coincides with the onset of muscular contraction. This can be explained by the synthesis of the A_4 homotetramer isozyme at this stage. According to Kunz (1973), the LDH pattern from the newly fertilized egg to early tail bud (stages 1-24) is the same and a change is observed at stage 25, when isozyme IX appears and this may be correlated with the onset of contractility. Moyer *et al*. (1968) indicated that clear evidence of the new isozyme was found around stage 19 when the heart starts beating.

Basu *et al*., (1992) claimed that the initial predominance of B_4 over A_4 in fish up to the onset of muscular contraction (stage 17 in *B. arabicus* of ontogenesis) indicates that cell differentiation of the heart and kidney takes place earlier and retarded expression of A_4 may indicate that skeletal muscular cell differentiation and activity is somewhat delayed during ontogenesis. On the other hand, Goldberg *et al.*, (1969) described the

predominance of B subunits in early embryonic stages of lake and brook trout, with a gradual increase in the activity of A-containing isozymes as they develop. It seemed to be that LDH isozyme composition of unfertilized and cleavage embryos revealed that more B subunits than A subunits had been synthesized prior to or during these development stages.

It is possible that the synthesis of different isozymes is mediated by preformed stable mRNA during the process of oogenesis. Goldberg *et al.,* (1969) indicated that at gastrulation, the increase in proportion of LDH-5 suggests that new mRNA synthesis takes place providing more A subunits for tetramer assembly at a time when the synthesis of many molecules is accelerated in embryos.

In *B. arabicus,* it is possible that new synthesis of mRNA takes place when muscular contraction starts (stage 17) which provides more A subunits for the synthesis of the homotetramer LDH-5 (A_4).

5. References

AMBU-SAIDI, I. 2002. Staging of *Bufo arabicus.* M.Sc. Thesis. Sultan Qaboos University, Oman.

ALMEIDA-VAL, V.M., PAULA-SILVA, M.N., CARACIOLO, M.C., MESQUITA, L.; FARIAS, I.P. and VAL, A.L. 1992. LDH isozymes in Amazon fish-III. Distribution patterns and functional properties in Serrasalmidae (Teleostei: Ostriophysi). *Comp. Biochem. Physiol.* **103B:** 119-125.

APPELLA, E. and MARKERT, C.L. 1961. Dissociation of lactate dehydrogenase into subunits with guanidine hydrochloride. *Biochem. Biophys. Res. Commun.* **6:** 171-176.

BA-OMAR, TA; AMBU-SAIDI, I.; AL-BAHRI, S. and AL-KHAYAT, A(2004). Embryonic and larval staging of the toad *Bufo arabicus*. *Zoo .Mid. East*. **32:** 47-55.

BASU, S.K., GANGULY, S., SARKAR, S.K. and BASU, T.K. 1992. Ontogenetic and comparative analysis of LDH isozymes in the three species on Indian major carp. *Ind. J. Exper. Biol.* **30:** 90-93.

BEIS, A., LAZOU, A. and KONTOGIANNI, E. 1992. Changes in plasma membrane Protein composition during early development of the frog *Rana ridibunda*. *Cellular and Molecular Biol.* **38:** 513-523.

COPPES, Z.L. 1990. Divegence of duplicate genes in three Scianid species (perciformes) from the South coast of Uruguay. *Comp. Biochem Physiol.* **96B:** 33-39.

COPPES, Z.L. 1984. Isozymes of lactate dehydrogenase in fishes of the superorder Acanthopterygii: an update. *Comp. Biochem. Physiol*. **97B:** 1-8.

GILBERT, S.F. 2001. Ecological developmental biology: developmental biology Meets the real world. *Develop. Biol*. **233:**1-12.

GOLDBERG, E., CUERRIER, J.P. and WARD, J.C. 1969. Lactate dehydrogenase ontogeny, paternal gene activation and tetramer assembly in embryos of brook trout, lake trout and their hybrids. *Biochemical Genetics.* **2:** 335-350.

HRANITZ, J.M. and DIEHL,W.J. 2000. Allozyme variation and population genetic structure during the life history of *Bufo woodhousii fowleri* (Amphibia: Anura). *Biochem. Syst. and Ecol.* **28:** 15-27.

KAPLAN N, O. 1964. Lactate dehydrogenase: structure and function. *Brookhaven Symp. Biol.* **17:** 131-153.

KUNZ, Y.W. 1973. Changes in lactate dehydrogenase isozyme pattern during the development of *Xenopus laevis* (Daudin). *Rev. Suisse Zoo.* **80(2):** 431-446.

MARKERT, C.L., AMET, T.M. and GOLDBERG, E. 1998. Human testis-specific lactate dehydrogenase-C promoter drives over expression of mouse lactate dehydrogenase-1 cDNA in testes of transgenic mice. *J. Exp. Zoo.* **282:** 171-178.

MATHEWS, T.C. 1975. Biochemical polymorphism in populations of the Argentine toad *Bufo arenarum*. *Copeia.* **3:** 454-465.

MOYER, F.H., SPEAKER, C.B. and WRIGHT, D.A. 1968. Characteristics of lactate dehydrogenase isozymes in amphibians. *Ann. N. Y. Acad. Sci.* **151:** 650-669.

PHILIPP, D.P., CHILDERS, W.F. and WHITT, G.S. 1979. Evolution of patterns of differentialgene expression: a comparison of temporal and spatial patterns of isozyme locus expression in two closely related species

(northern large mouth bass, *Micropterus salmoides salmoides* and small mouth bass, *M. dolomieui*). *J. Exp. Zoo.* **210:** 473- 488.

ROPSON, I.J., BROWN, D.C. and POWERS, D.A. 1990. Biochemical genetics of *Fundulus heteroclitus* (L). VI. Geographical variation in the gene frequencies of 15 loci. *Evolution.* **44:** 16-26.

SIMOVICH, M.A. and SASSAMAN, C.A. 1986. Four independent elecrophoretic markers in spade foot toads. *J. Heredity.* **77:** 410-414.

VONWYL, E. 1983. Expression of the lactate dehydrogenase genes of the *Xenopus* species and interspecies hybrids during early development. *Comp. Biochem. Physiol.* **76B:** 17-21.

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