PROPERTIES OF PURIFIED LACTATE DEHYDROGENASE (LDH) FROM THE BREAST MUSCLES OF DIFFERENT BIRDS ADAPTED TO VARIOUS HABITATS

M. PRABHAKARAM, J. KAUR, S. AGARWAL, A. AGARWAL, S. M. SINGH and S. N. SINGH
Department of Zoology, Banaras Hindu University, Varanasi 221 005, India.

ABSTRACT

Lactate dehydrogenase has been purified from the breast muscles of fowl, pigeon, duck and crow up to homogeneity. The purified enzyme of all the four birds migrated as a single band on polyacrylamide gels (both in specific enzyme activity stain and in general protein stain methods). However, the movement of the LDH of crow is greater towards cathode as against other birds. The optimum pH and temperature of crow LDH show variation in comparison with the LDH of other birds. It has also been observed that K_m for the LDH of fowl and duck and for crow and pigeon is alike. Similar pattern is observed for K_i (oxalate and oxamate). The significance of these results has been discussed in detail.

INTRODUCTION

Lactate dehydrogenase (LDH, L-lactate, NAD+ oxidoreductase, EC 1.1.1.27) is the key regulatory enzyme of anaerobic glycolysis. LDH is ubiquitous in all the vertebrate cells and occurs as a predominant M-type in anaerobic tissues like skeletal muscle, adrenal medulla etc and H-type in aerobic tissues like heart, adrenal cortex etc[1]. Both forms differ in their physical, catalytic, kinetic and immunological properties[2–4]. In the present study we compare the properties of LDH purified from the breast muscles of various birds adapted to different habitats. These selected birds include: flightless birds like fowl (*Gallus gallus*; terrestrial) and duck (*Anas anas*; semi-aquatic); and flight-adapted birds like pigeon (*Columba livia*) and crow (*Carvus splendins*). This work has been carried out to understand the adaptive role and physiological significance of LDH with respect to flight adaptation.

MATERIALS AND METHODS

The aforesaid birds were collected locally and maintained under standard laboratory conditions for a week prior to experimentation. All the chemicals used were of analytical grade. Biochemicals were obtained from Sigma Chemical Company, USA. DEAE A-50 and Sephadex G-200 were purchased from Pharmacia Fine Chemicals, Uppsala, Sweden. Triple distilled water was used for all the experiments. All the purification steps were carried out at 0 ± 2°C unless otherwise mentioned.

In brief, the purification procedure is as follows: Animals were killed and breast muscles were excised out and processed immediately for purification. Purification was carried out by employing ion-exchange chromatography on DEAE Sephadex A-50 column[5,6]. A 20% homogenate of each muscle tissue was made in 0.02 M tris-Cl^- buffer (pH 7.4) containing sterilized sea sand in a pre-chilled mortar and pestle. The homogenate was centrifuged at 700 *g* for 15 min at 0°C in a high speed refrigerated centrifuge (Janetzki K-24 Model) to remove nuclei and cellular debris. The post 700 *g* supernatant was further centrifuged at 18,000 *g* for 30 min and the clear supernatant was used as the enzyme source after filtering it through a plug of glass wool. To the supernatant obtained, solid ammonium sulphate (Sigma grade) was slowly added to attain 70% saturation followed by equilibration at 0°C for 30 min. The suspension was centrifuged at 18,000 *g* for 30 min and the pellet obtained was dissolved in 8 ml of tris-Cl^- buffer and dialyzed overnight against the same buffer. The dialysate was centrifuged at 18,000 *g* for 30 min and the supernatant was loaded on a DEAE Sephadex A-50 column (2.2 × 30 cm) which was pre-equilibrated against tris-Cl^- buffer (0.02 M, pH 7.4) and regulated at a flow rate of 30 ml/hr. M-type LDH was eluted in the equilibration buffer and the enzyme-rich fractions were concentrated by the addition of saturated ammonium sulphate (to give 35% saturation) and crystallized five times with an interval of one week for each crystallization. Crystalline enzyme was dissolved in 3–5 ml of potassium phosphate buffer (0.05 M,

pH 7.4); loaded on a Sephadex G-200 (1.2 × 40 cm) column; eluted with the phosphate buffer in void volume at a flow rate of 15 ml/hr and used for evaluating its properties.

The enzyme activity was measured[7] in a Unicam SP-500 spectrophotometer at 25°C by monitoring the decrease in absorbance of NADH at 340 nm and represented in International Units. Protein content was measured[8] using BSA as the standard. Polyacrylamide gel electrophoresis (PAGE) was performed[9] on 5.3% slab gels instead of 7.5% gels due to better resolution. Specific LDH staining was done[10] with lithium lactate as the substrate and general protein staining was carried out[11].

RESULTS

Table 1 shows the purification protocol of LDH of different birds. The fold of purification and the specific activity are higher in the fowl followed by duck, pigeon and crow in decreasing order. PAGE of the purified enzymes of the four muscle tissues on slab gel of all the four birds is shown in figure 1a (specific enzyme activity stain) and figure 1b (general protein stain). A distinct, single cathodic band is observed in all the four birds on both gels. However, migration of crow LDH is greater towards the cathode in comparison with the other birds. Co-migration of the pigeon and duck LDH is also observed followed closely by fowl LDH. A broad range of pH optimum is seen for crow LDH (6.5–8.5) in comparison with the other birds (6.0–7.5 for fowl LDH and 7.5 for pigeon and duck LDH). Likewise crow LDH is resistant to higher temperatures (>60°C) as compared with the LDH of birds (figure 2).

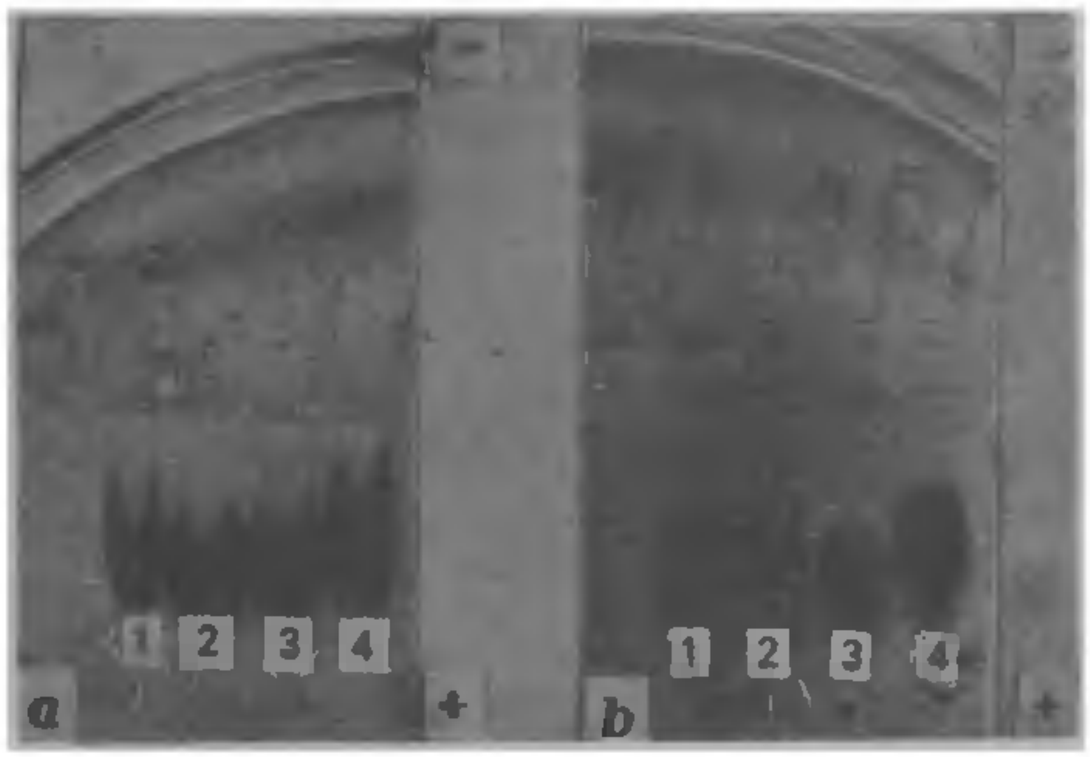

Figure 1 Polyacrylamide gel electrophoresis of LDH purified from the breast muscle of different birds on 5.3% slab gels. **a.** Specific LDH activity stain; **b.** General protein stain. **1.** fowl; **2.** pigeon; **3.** duck; **4.** crow.

Kinetic data (K_m for pyruvate and K_i for oxalate and oxamate) of the purified M-LDH of different birds are shown in table 2. It is evident that K_m for pyruvate is higher in fowl LDH followed by duck, pigeon and crow LDH. The K_i for oxalate (non-competitive) and oxamate (competitive) in the presence of pyruvate is more or less similar for fowl and duck LDH, and for pigeon and crow LDH.

Table 2 *Kinetic properties of purified breast muscle LDH of different birds*

	Fowl	Duck	Pigeon	Crow
K_m (x 10^{-4}M pyruvate)	3.6	3.1	1.7	1.4
K_i (a) Oxalate (mM)	0.60	0.55	0.25	0.30
(b) Oxamate (mM)	0.11	0.12	0.15	0.14

Table 1 *Purification protocol of LDH (M-type) purified from the breast muscle of different birds*

	70% Ammonium sulphate step	Ion-exchange column chromatography	V Crystallization
Activity (units)			
Fowl	13355	9064	2886
Duck	5924	5785	3036
Pigeon	6570	5698	726
Crow	3915	3020	596
Specific activity (units/mg protein)			
Fowl	13.79	20.66	1187.65
Duck	9.29	15.05	803.17
Pigeon	9.61	12.56	290.40
Crow	7.25	10.91	248.33

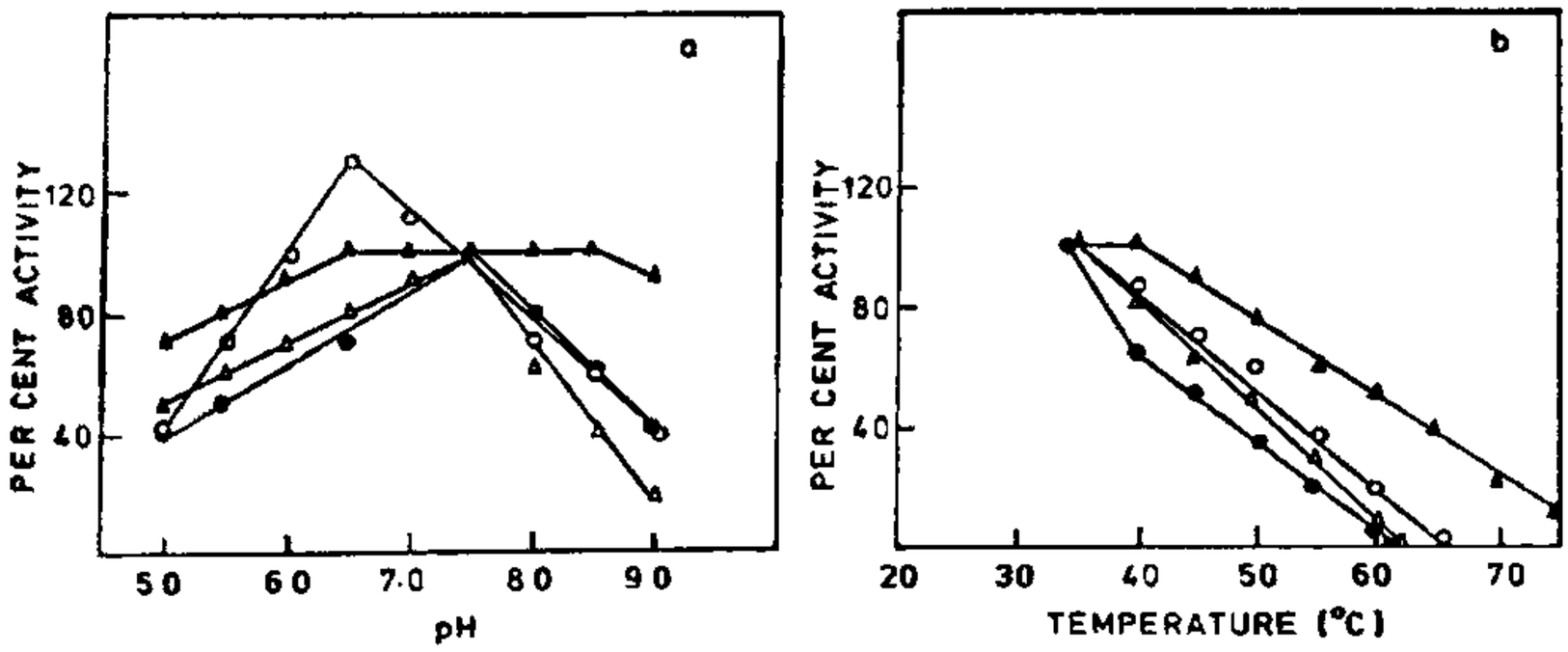

Figure 2. Effect of pH and temperature on purified LDH of different birds. ○—○ fowl; △—△ pigeon; ●—● duck; ▲—▲ crow.

DISCUSSION

Due to their different habitats, variation in the metabolic and physiological status of the individual tissue or organism may occur to meet the energy requirements. During evolution birds adapted to various modes of life (terrestrial, aquatic and aerial) may have influenced their metabolic status. In the present study, we have compared the properties of LDH purified from the breast muscles of fowl and duck (non-flying birds) and pigeon and crow (flying birds). This is to elucidate the mechanism of alteration in the enzyme molecule as a function of habitat. Physicochemical properties of the LDH of a number of birds have been analyzed in detail[12–14].

It has been observed that the elution pattern of LDH of fowl, duck and pigeon showed similarity in comparison to that of crow LDH. The enzyme yield is greater in fowl followed by duck. pigeon and crow. Crystalline enzyme has not shown any variation among the four species in the initial stages (up to three crystallization). However, upon storage for one month (at 2°C) the LDH of the crow yielded long cylindrical crystals with no major difference among the LDH of other birds (amorphous crystals). On PAGE all the enzymes migrated as a sharp band. The migration of crow LDH is greater towards cathode in comparison to others. Similar results have been obtained; either the gels stained for specific enzyme stain or general protein stain. However, a molecular weight of 140,000 ± 5,000 has been obtained on SDS gels (12.5% separating gels) which is similar for all the four enzymes and also on Sephadex G-200 gel filtration column which is in accordance with reported values[15]. The optimum pH of duck and pigeon LDH is similar in comparison to fowl/crow LDH. The optimum temperature of crow LDH is also different in comparison to other birds. In addition the LDH of crow is more resistant to higher temperatures than the others and this is the property of H-LDH[6]. The broad optimum pH and resistance to higher temperature of the crow LDH, in comparison to other birds signify that it has some subtle conformational differences which may be responsible for its uniqueness.

Kinetic data of the fowl and duck show resemblance like that of pigeon and duck. The value of K_m obtained for LDH of pigeon and crow is approximately half of that for fowl and duck and in good agreement with reported values[12]. Similarly, K_i for oxalate of fowl and duck is also approximately double that of pigeon and crow. However, no apparent differences have been observed in the K_i for oxamate.

The present data prove that LDH of flying birds is altered in comparison to the LDH of non-flying birds in some properties. The role of LDH in non-flying birds and flying ones may be correlated as follows: Since most of the non-flying birds consist of white muscle, they are dependent on anaerobic glycolysis to yield energy in the form of ATP. In addition, during rigorous muscular activity Cori-Cori cycle operates well in these birds. Hence, the stored glycogen is used for this purpose in white muscle. In contrast, the red muscle which is rich in mitochondria, myoglobin and occurs in flying birds, attains ATP via TCA cycle to fulfil the higher energy requirements during flight. Synthesis of more M-subunits by heart cells in culture and their inhibition in presence of oxygen in chicken has been

reported[16–18]. Thus it seems plausible to conclude that alterations in enzymes may occur as a function of adaptation and habitat of the organism.

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NEWS

MRI AND MRS

..."During the past few years, magnetic resonance imaging (MRI) has become a widely accepted diagnostic tool for physicians. By subjecting a patient's body to strong magnetic fields, the presence of certain chemical elements, notably hydrogen, which indicates water in body tissue, can be imaged. Unlike computerized tomography, MRI readily distinguishes different materials in the body—bone, muscle, fat—without x-rays or dye injections. In order to examine a wider range of chemical elements than can normally be imaged with MRI, and determine the amount of chemicals present in parts of the body, researchers are turning to a complementary technique—magnetic resonance spectroscopy (MRS)—that may render MRI an even more powerful diagnostic tool. MRS does not produce images, but it generates spectra that show the distribution of an element within the body. The technique would allow researchers and physicians to examine such elements as phosphorus, lithium, sodium, potassium, fluorine, and carbon, whose properties aid in diagnosing heart disease and cancer. Research at the General Electric Research & Development Ctr. (Schenectady, New York) under Paul Bottomley has led to the development of a whole body imaging and spectroscopy research system. A similar system has been built at the Massachusetts Inst. of Technology... Bottomley's best results so far have occurred in phosphorus spectroscopy of the heart. Spectra obtained in minutes can help determine whether a patient's symptoms indicate a heart attack or a minor ailment."

[(Hugh Aldersey-Williams in *High Technology* 7(2):62, February 1987). Reproduced with permission from Press Digest, *Current Contents*[00], No. 12, March 23, 1987. Published by the Institute of Scientific Information[00], Philadelphia, PA, USA].