Dissociation–Reassociation of Lactate Dehydrogenase: Reversed Isozyme Migration and Kinetic Properties* (33792)

FRANCIS M. BUSH AND WILLIAM W. FARRAR
(Introduced by E. H. Ingersoll)

Department of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, Virginia 23219

Much of our knowledge of lactate dehydrogenase (LDH) has been derived from studies on avian tissues. In many species, the predominant isozyme, LDH-1, of heart migrates to a more anodic position than the predominant isozyme, LDH-5, of breast muscle (1–4). Exceptions are peafowl, *Pavo cristatus*, and Australian swamp quail *Synoicus ypsilophorus* (5). Their predominant isozymes of heart and breast muscle migrate in a position which is reversed to the more typical pattern.

This phenomenon of reversal for peafowl isozymes occurs in alkaline gels, but not in neutral gels. It appears to be independent of certain catalytic properties of the isozymes. As found for other vertebrates (2, 3), the predominant isozyme of peafowl heart attains optimal activity with a lesser pyruvate concentration and has a higher $NADH_L/NADH_H$ ratio[1] than that of the predominant isozyme of breast muscle. These variable electrophoretic properties in various buffers and a presumed relationship of electrophoretic and kinetic properties to tissue metabolism (2, 6) prompted us to examine the LDH in other avian tissues to determine the generality of these phenomena.

Virtually nothing is known of the LDH subunit structure in small birds because of the large number of specimens required to produce a purified sample of quantity suitable for analysis. This requirement can be overcome by dissociation–reassociation experiments, permitting enzyme analysis of individual birds. We now describe the isozyme and kinetic properties of somatic tissues of the small altricial species of house sparrow, *Passer domesticus*.

Methods. Tissue homogenates obtained from 22 females and 22 males were prepared in cold 0.07 *M* Tris [tris(hydroxymethyl)aminomethane]-HCl buffer (7) pH 8.6 followed by centrifugation at 31,000*g* for 30 min. Samples of supernatant fluids were assayed spectrophotometrically (3), at 25° with concentrations of pyruvate ranging from 1.0×10^{-4} to 1.0×10^{-2} M. Part of the remainder of each fluid was mixed with 1 *M* NaCl (8) in 0.1 *M* phosphate buffer, pH 7.0, followed by equal mixing of any two homogenates with each other. Samples were frozen overnight at —20°, thawed slowly to effect dissociation, diluted to approximately 50 LDH activity units whereby each unit equals a change in OD of 0.100/min (9). They were subjected to vertical starch gel electrophoresis (10) and then the LDH isozymes localized histochemically by an oxidation–reduction reaction (11).

Results. The house sparrow is another bird whose predominant LDH isozymes of ventricle and pectoralis muscle undergo a reversal in migration at an alkaline pH (Fig. 1). The predominant LDH isozymes of either pectoralis muscle or cerebrum migrate to a

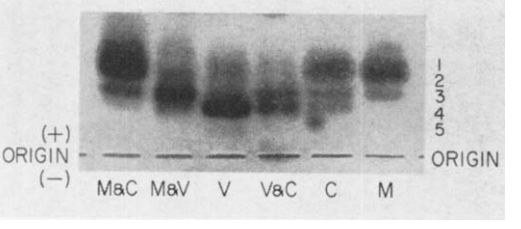

FIG. 1. Isozymogram of LDH in house sparrow pectoralis muscle (M), ventricle (V) and cerebrum (C) prepared separately and which are mixed equally with one another for dissociation–reassociation. Total activity and the quantity of diluted homogenate subjected to electrophoresis are approximately the same for each tissue. LDH-5 isozyme of ventricle stains faintly.

* Supported by NIH Grant GM 13649-01.

[1] Ratio of the activity with NADH at a low concentration of pyruvate ($3.3 \times 10^{-4} M$) to that at a high concentration of pyruvate ($1.0 \times 10^{-2} M$).

more anodic position than the predominant LDH isozymes of ventricle. Assignment of numbers to LDH isozymes presents some problem. Ventricle homogenates exhibit five LDH isozymes; however, the least anodic stains weakly in all adult patterns examined. LDH-4 is the predominant isozyme in homogenates of ventricle; LDH-2, in homogenates of pectoralis muscle; and LDH-1 and LDH-2, in homogenates of cerebrum.

Dissociation–reassociation experiments indicate that the LDH isozymes are composed of subunits as the predominant LDH isozyme of the mixture with ventricle and pectoralis muscle is different from the two homogenates electrophoresed separately (Fig. 1). The reassociated form appears to be LDH-3. New isozymes are not generated by the freeze–thaw cycle in the other mixtures. LDH patterns for the separate homogenates prepared with NaCl resemble patterns of separate homogenates prepared without NaCl. The presence of five bands suggests that the LDH isozymes are tetramers, composed of two different kinds of subunits. The close spacing of LDH isozymes indicates that there may be similar charges on the subunits. Hence, this makes detection of a variant form difficult.

The LDH isozymes of ventricle reach optimal activity at a lower pyruvate concentration than do the LDH isozymes of pectoralis muscle as shown by the assay (Fig. 2). There is more inhibition of activity produced by high pyruvate concentration for ventricle

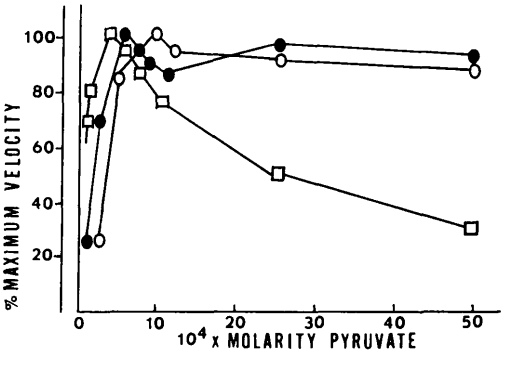

FIG. 2. Pyruvate inhibition of LDH activity at 25° of house sparrow ventricle (□), pectoralis muscle (○) and cerebrum (●) homogenates.

than for the other tissues. The pyruvate concentration required for optimal activity of ventricle differs little from that required for cerebrum.

Pyruvate inhibition of LDH activity, expressed by the $NADH_L/NADH_H$ ratio, shows homogenates of ventricle have a value of 2.74, while homogenates of pectoralis muscle and cerebrum have values of 1.50 and 1.17, respectively.

Discussion. An explanation for reversal of predominant isozymes is not apparent from our results. Evidence shows that the type of buffer affects direction of isozyme migration in this species. All isozymes migrate anodally in Tris–citrate gels and all migrate cathodally in borate gels at the same pH (12); this indicates that the buffer alters the charge on the subunits to the same degree. A higher histidine content for the breast muscle isozyme of several birds, including chicken (13), has been hypothesized as contributing to reversal in peafowl (5). There is some objection to this hypothesis simply from results obtained for purified chicken LDH. Although LDH-1 of breast muscle has a higher histidine content than LDH-5 of heart, the relative isozyme mobilities remain nearly the same in both alkaline and neutral gels. No reversal occurs in predominant isozymes. Such variability among birds raises the question if there is any particularly selective advantage associated with more negatively charged H subunits, as the house sparrow is a less primitive species than either chicken or peafowl (14), or in the more negatively charged M subunits common to isozymes of other higher vertebrates.

Differences in isozymic patterns for ventricle and pectoralis muscle, in $NADH_L/NADH_H$ ratios, and in variable susceptibility to excess pyruvate fit the hypothesis that tissues with predominate isozymes most inhibited by excess pyruvate (2, 6) or with high $NADH_L/NADH_H$ ratios (2) undergo aerobiosis, whereas tissues with predominant isozymes least inhibited by excess pyruvate or with low ratios undergo anaerobiosis. The relationships fit in spite of the reversal observed for the house sparrow. But the variability of

cerebrum offers an interesting deviation from the general statement that brain is aerobic as is the heart (15). The kinetic and electrophoretic studies suggest that cerebrum LDH is more consistently like LDH of anaerobic muscle than of aerobic ventricle, with exception of pyruvate concentration required for optimal activity. This is another example of a lack of association in type of metabolism and in predominant isozymes that has been shown before for bovine lens fibers and for human platelets (16). The pyruvate concentration is similar to the 3.3×10^{-4} *M* giving optimal activity for purified LDH-1 of chicken heart (3), but the ratio is more similar to the 0.85 and 0.75 of chicken and peafowl breast muscles, respectively, than the higher ratios for heart of these species (5). Optimal activity for pectoralis muscle is reached at a slightly lesser concentration than the 1.0×10^{-3} *M* for purified LDH-5 of chicken breast muscle (3). The ratio agrees exactly with a published value for house sparrow muscle (17). If any metabolic relationship exists, ventricle ought to be more aerobic than either cerebrum or pectoralis muscle. But house sparrow ventricle may be less aerobic and pectoralis muscle less anaerobic than these similar tissues of chicken and peafowl.

Summary. Pyruvate saturation curves and $NADH_L/NADH_H$ ratios show that the predominant LDH isozymes of avian ventricle and pectoralis muscle are catalytically similar to those of several other vertebrates, including man. The hypothesized relationship between type of metabolism and predominant LDH isozymes and between type of metabolism and degree of susceptibility to excess pyruvate are incongruous; our results suggest that neither hypothesis may be consistently correct. This is supported by observations on reversal in predominant isozymes of some avian tissues, and on differences in electrophoretic and kinetic properties of house sparrow ventricle and cerebrum, as well as published exceptions.

We thank J. I. Townsend and E. S. Kline for advice.

1. Markert, C. L. and Møller, F., Proc. Natl. Acad. Sci. U. S. **45**, 753 (1959).

2. Cahn, R. D., Kaplan, N. O., Levine, L., and Zwilling E., Science **136**, 962 (1962).

3. Lindsay, D. T., J. Exptl. Zool. **152**, 75 (1963).

4. Appella, E. and Markert, C. L., Biochem. Biophys. Res. Commun. **6**, 171 (1961).

5. Rose, R. G. and Wilson, A. C., Science **153**, 1411 (1966).

6. Latner, A. L., Siddiqui, S. A., and Skillen, A. W., Science **154**, 527 (1966).

7. Vesell, E. S. and Brody, I. A., Ann. N. Y. Acad. Sci. **121**, 544 (1964).

8. Markert, C. L., Science **140**, 1329 (1963).

9. Markert, C. L. and Ursprung, H., Develop. Biol. **5**, 363 (1962).

10. Poulik, M. D., J. Immunol. **82**, 502 (1959).

11. Zinkham, W. H., Blanco, A., and Clowry, L. J., Jr., Ann. N. Y. Acad. Sci. **121**, 571 (1964).

12. Bush, F. M. and Farrar, W. W., Proc. Intern. Union Physiol. Sci., **6**, 68 (1968).

13. Pesce, A., Fondy, T. P., Stolzenbach, F., Castillo, F., and Kaplan, N. O., J. Biol. Chem. **242**, 215 (1967).

14. Mayr, E. and Amadon, D., Am. Museum Novitates **1496**, 1 (1951).

15. Lindy, S. and Rajasalmi, M., Science **153**, 1401 (1966).

16. Vesell, E. S., Science **150**, 1735 (1965).

17. Wilson, A. C., Cahn, R. D., and Kaplan, N. O., Nature **197**, 331 (1963).

Received Sept. 17, 1968. P.S.E.B.M., 1969, Vol. 131.