SOLUBILIZATION OF BOUND LACTATE DEHYDROGENASE BY NADH IN HOMOGENATES OF TROUT SKELETAL MUSCLE AS A FUNCTION OF TISSUE CONCENTRATION

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Received November 5, 1968

Solubilization of lactate dehydrogenase (LDH) bound to homogenized muscle tissue is accomplished by NADH, and to a lesser extent by NAD^+, at concentrations in the range used in assays and found in tissues. This solubilization increases with decreasing concentration of tissue in the homogenate. Under normal conditions of assay, one would expect LDH to be in a soluble form. At high tissue concentrations, which are probably more like conditions in situ, a considerable part of the LDH remains bound. Binding could affect the kinetics or equilibrium of the reaction.

Considerable disagreement exists as to whether lactate dehydrogenase (LDH) exists in situ in a bound or freely soluble state (see discussions in refs. 1 and 2). Because of the difficulties involved in showing by direct physical means whether LDH is bound or soluble in situ, we chose another approach, namely, to determine if the bound and soluble forms of the enzyme differed in kinetic properties. Any difference found might indicate a possible advantage to the cell in having the enzyme exist bound, soluble, or in equilibrium between the two phases. It was first necessary to establish the conditions which influence the distribution of the enzyme between soluble and bound phases. A report of the kinetic properties of LDH bound covalently to anion-exchange cellulose particles and sheets has recently appeared (3). The non-covalent bounding of LDH to cell particulates, however, may produce entirely different effects.

Experimental

The skeletal muscle of the brown trout (Salmo trutta Linnaeus) was used in this study. Freshly excised muscle was homogenized in distilled water in a blender and centrifuged for 30 min at 40,000 rpm in the No. 40 rotor of a Spinco Model L ultracentrifuge. The residue represented the LDH-rich particulate fraction while the supernatant fraction was discarded. The LDH-rich particulate fraction was rehomogenized in media under various conditions and centrifuged as above to test for the effect of these conditions in solubilizing the enzyme as described earlier (4). Ordinarily a 5% (weight of muscle/volume of medium) homogenate was prepared. Only one form of LDH was found in trout muscle by cellulose acetate electrophoresis which was less anodic than that prepared from trout heart. Presumably, this was the muscle isozyme.

Results and Discussion

The behavior of LDH from trout muscle was similar to that from chicken breast muscle (4) in that increasing pH and ionic strength led to greater solubilization of the enzyme; in addition, the LDH-rich particulate fraction contained a higher specific activity of LDH the lower the concentration of tissue in the homogenizing medium.

In examining the effect on solubilization of the substrates and products of the LDH-catalyzed reaction, we observed that NADH solubilized the enzyme at concentrations comparable to those used in the ordinary assay (5) (Table 1). The oxidized pyridine nucleotide, NAD^+, was considerably less effective. A similar relationship was seen with the reduced and oxidized forms of NADP. Several other metabolites including ATP, adenosine, and nicotinamide, produced little or no solubilization. Pyruvate produced little solubilization by itself but had a synergistic effect in the presence of either NADH or NAD^+. All compounds were examined under conditions of pH and ionic strength which would cause of themselves little solubilization. Similar effects of these compounds

Table 1. SOLUBILIZATION OF LDH FROM MUSCLE HOMOGENATES BY NADH AND NAD^+

NADH, Molarity	Percentage in Supernatant	NAD^+, Molarity	Percentage in Supernatant
0	7.8	0	8.6
2×10^{-5}	24.9	1×10^{-5}	18.3
5×10^{-5}	43.6	1×10^{-4}	39.4
1×10^{-4}	79.0	2×10^{-4}	44.8
1×10^{-3}	90.5	3×10^{-4}	47.2

Trout skeletal muscle was homogenized in distilled, deionized water at a 5% (w/v) tissue concentration. An LDH-rich particulate fraction was prepared by centriguging for 30 min. at 40,000 rpm in the No. 40 rotor of a Spinco Model L ultracentrifuge and discarding the supernatant fraction. The residue was rehomogenized in a 10 mM imidazole buffer, pH 6.7, solution containing NADH and NAD^+ at the indicated levels in a total volume equivalent to that before the first centrifugation. After centrifuging as above, the supernatant and residual fractions were assayed for LDH activity. The percentage in the supernatant fraction was obtained by dividing the total activity in this fraction by the total activity in the residual and supernatant fractions and multiplying by 100.

LDH activity was determined by the procedure of Wu and Racker (5).

were noted for inhibition of binding of soluble enzyme to LDH-free particles.

The above data were obtained at an equivalent tissue concentration of 5% (w/v). Although concentrations of NADH in these experiments were of the order of magnitude used in the assay, the amount of particulate suspension was much greater (some 500X) in the former. We wanted to determine the effect of NADH on solubilization of LDH under the conditions of assay, but this was not feasible due to the extremely low concentrations of enzyme (and particulate matter) present under assay conditions. As an approximation to the assay situation, we studied the re-

lease of LDH at tissue concentrations lower than 5%; it was possible to go as low as 0.5% with no experimental difficulties. We also examined levels of tissue concentrations higher than 5% as we felt that these may more accurately reflect conditions in situ. The results are given in Fig. 1 where solubilization is expressed as a function of NADH concentration at various tissue levels. Although of great importance (4), pH and ionic strength were not included in the present study but were held constant.

The marked dependence of solubilization of LDH by NADH on tissue (and hence enzyme) concentration indicates that an equilibrium is involved in this process. It further indicates that with concentrations

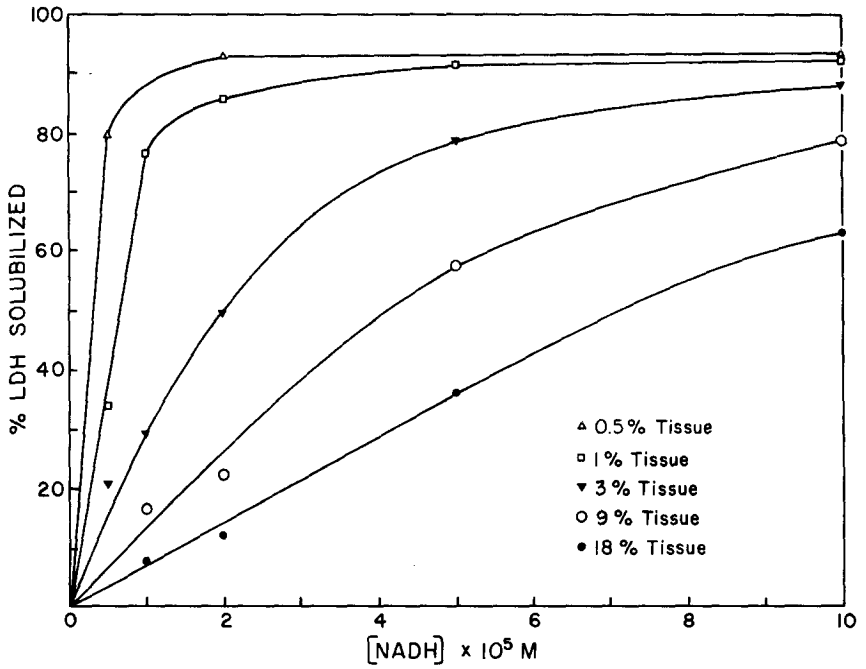

Fig. 1. Solubilization of LDH in muscle homogenates by NADH at different tissue concentrations. LDH-rich particulate fractions were prepared from tissue homogenized at concentrations from 0.5 to 18% (w/v) and centrifuged at 40,000 r.p.m. for 30 min. They were rehomogenized in a solution equal in volume to the discarded supernatant fractions and containing imidazole buffer (10 mM, pH 6.7) with increasing levels of NADH. They were then centrifuged as above, and LDH activity was determined in the supernatant and residual fractions.

of NADH normally used for assay the enzyme is soluble even when the pH (6.7) and ionic strength (0.010) are adjusted to prevent solubilization. This means that kinetic properties of bound and soluble enzyme cannot be studied under these conditions. To maintain the enzyme bound at concentrations of NADH used in the assay and found in muscle tissue (6), high tissue (and enzyme) concentrations would be required. Techniques utilizing rapid measurements would be necessary to measure the activity of LDH in this case, i.e., where the molar concentration of enzyme approaches that of its substrates. Many enzymes are, in fact, present in much higher concentrations in the cell than used in assays, often by 3 or 4 orders of magnitude (7).

As an alternative to the hypothesis that binding changes the kinetic characteristics of LDH, it may be that there is present in the cell enough LDH to rapidly utilize substrates as they become available. In the case where the enzyme concentration is high compared to its substrates, it may not be the rate of reaction that is important since this would always be very fast but rather the apparent equilibrium. Control mechanisms could depend, therefore, on changes in apparent equilibria brought about by coupled enzyme systems, compartmentation, or differential binding of substrates and cofactors. We suggest that differential binding of substrate and cofactor molecules may be determined by whether the LDH is bound to subcellular structures. Conformational changes in the enzyme induced by this binding might alter the capacity of LDH to bind its substrates. Binding more or less of its substrates may influence the apparent equilibrium constant of the reaction by changing the ratios of the reacting constituents in the soluble phase in muscle the same way that the concentration of LDH can affect the apparent equilibrium (8). Alternatively, the site on the enzyme which binds NADH may be the same as that which binds to the subcellular structures; in this case, one would expect binding of the enzyme to the particles to competitively inhibit binding of NADH and, hence, LDH activity. We regard the possibility that NADH

causes solubilization by binding to sites on the particulate structures as less likely than binding to the enzyme since solubilizing effectiveness is related to the dissociation constants of the enzyme and the oxidized and reduced NAD (9). The synergistic effect of pyruvate also is indicative of an effect on the enzyme itself.

Although these suggestions are speculative, the high solubilizing effectiveness of the substrate of LDH at concentrations found in tissues suggests a metabolic role for the equilibrium between the bound and soluble forms. It also indicates that data obtained at non-physiological conditions must be viewed with caution when attempting to extrapolate to conditions in situ. Solubilization of hexokinase by products or substrates of the reaction catalyzed by this enzyme has been reported and a physiological role suggested for the phenomena (10, 11).

Acknowledgments

This work was supported in part by grant GM-12064 from the National Institute of General Medical Sciences and by graduate educational grant 14-17-0007-276(G) from the U.S. Department of the Interior, Bureau of Commercial Fisheries.

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