Separation of Lactate Dehydrogenase Isozymes from Various Mouse Tissues*

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The lactate dehydrogenase pattern of 10 tissues as well as that of serum and peritoneal fluid from mice was investigated by means of starch gel electrophoresis. Five lactate dehydrogenase isozymes were found, except for testicular tissue where another, positively charged, isozyme was found in addition to the five, negatively charged lactate dehydrogenase isozymes.

The central role of lactate dehydrogenase (L-lactate: NAD oxidoreductase, EC 1.1.1.27) in the metabolism of carbohydrates has rendered it the subject of extensive investigation. By means of modern techniques it has been established that the enzyme (henceforth called LDH) exists as several molecular species. Thus, Neilands [1,2] found that crystalline LDH from bovine heart could be separated into two active components. Vesell and Bearn [3] showed that on a starch block, human serum exhibited 3 zones which had LDH activity. Wieland and Pfleiderer [4] found that extracts from different organs upon electrophoresis divided into several components with LDH activity. In 1959, Markert and Møller [5] proposed the term, isozyme for various molecular species exhibiting LDH activity.

Methods for the fractionation of the isozymes of LDH comprise starch,[5] and agar gel electrophoresis,[6] paper electrophoresis,[7] and column chromatography.[8] By methods of the kind, it has been shown that there are 5 established forms of LDH, although more have been reported. Tentative explanations of the functions of isozymes have been offered as well.[9–13]

In relation to the present great activities in establishing various forms of isozymes, it was thought to be of interest to investigate LDH isozyme patterns of different mouse tissues.

MATERIALS AND METHODS

Tissue homogenates. The following organs were studied: brain, spleen, kidney, muscle, lung, liver, heart, thymus, ventricle, and testis. They were dissected immediately upon sacrification of the animals and homogenized by vibration in a Mickle homogenizer with 2 vol of 0.03 M borate buffer of pH 8.6 for 2 min at full speed. After storage for 30 min at 4°C, the homogenates were centrifuged at 14 500 g (15 min, 4°C).

Body fluids. Blood and peritoneal fluid were also examined. Blood was allowed to clot by standing in siliconized test tubes for 30 min at room temp.

Chemicals. The sodium salt of DL-lactic acid and the NAD were purchased from the Sigma Chemical Company (St. Louis, Mo.), phenazine methosulphate (PMS) and NADH from Nutritional Biochemicals Corp. (Cleveland, Ohio). Nitro-BT was obtained from Dajac Laboratories (Philadelphia, Pa.) and hydrolyzed starch from the Connaught Laboratories (Toronto, Ont., Canada).

Enzyme assays. For the assessment of LDH activity, 50 and 100 μl of the various samples were added to 3 ml of 0.1 M potassium phosphate buffer of pH 7.5, containing 1.5×10^{-4} M NADH and 3×10^{-4} M pyruvate. Enzymatic activity was measured in a spectrophotometer at 340 mμ. Sample dilutions of identical activities were next subjected to starch gel electrophoresis.

Electrophoresis. The starch gels were prepared according to the directions given by the manufacturer, using 0.03 M borate buffer of pH 8.6. The gel was connected with the buffer by means of filter paper wicks. 25 μl of the various samples, containing 15×10^{-1} units of LDH activity, were pipetted onto 9 by 4 mm pieces of Whatman No. 3 MM filter paper which were subsequently inserted into slots of the same dimensions in the gels, 1.5 cm from the cathode edge. 37 V/cm was then applied to the system for 5 h at room temp.

Following electrophoresis, the starch slab was sliced into two halves. One layer was incubated for the demonstration of LDH isozymes, the second was used for control experiments.

Demonstration of LDH isozymes. The LDH isozyme bands were demonstrated by incubation of the gels for 30 min (in the dark) at 37°C in a medium containing 26.5 ml of 0.1 M Tris—HCl buffer of pH 8.0; 1.5 ml of 2 M sodium lactate; 0.6 mg of phenazine methosulphate (PMS); 10 mg of Nitro—BT; and 18 mg of NAD.

Controls. For control purposes, gels were incubated as above, but in media devoid of a) sodium lactate; b) NAD; c) sodium lactate and NAD; d) PMS; and in the medium with NaCN added. As an additional control, gels were pre-incubated in formalin for half an hour before immersion into the reaction mixture.

RESULTS

Fig. 1 represents a photograph of stained gels after electrophoresis, showing the LDH isozyme patterns in specimens from 10 different organs, as well as those of serum and peritoneal fluid.

As seen from the figure, there were regularly 5 bands present, all of them displaced towards the anode. However, extracts from testes exhibited an additional band, which was displaced towards the cathode in relation to the point of application of the original sample.

The various tissues may be divided into three groups according to their contents of fast moving, intermediary, and slowly migrating LDH isozyme forms. As a representative of the first group, heart is the most typical tissue, although brain and kidney belong to this group as well. Lung and ventricle belong to the intermediary group, whereas liver, muscle, and spleen largely contain slowly migrating LDH isozymes.
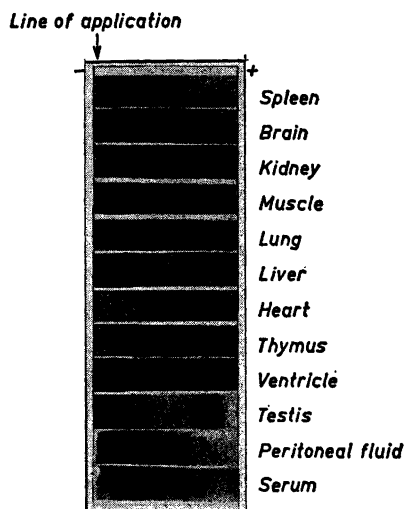
Line of application

Fig. 1. Stained electrophoretograms of 10 different mouse organs and of serum and peritoneal fluid of the mouse.

When incubation of the gels in the reaction mixture was prolonged, the LDH bands tended to overlap, their individuality thereby becoming less distinct.

The control experiments showed that removal of any of the components of the reaction mixture caused an inhibition of the reaction leading to formazan deposition, whereas the addition of NaCN accelerated formazan deposition.

Preincubation of the gels in formalin before incubation in the reaction mixture caused complete inhibition of LDH activity.

DISCUSSION

Several explanations have been proposed for the existence of five or more isozymes of LDH. Some investigators are of the opinion that endogenous components of the respiratory chain are responsible for the creation of LDH with different electrophoretic mobilities.[14] However, also the experimental conditions may be of importance in relation to the number of isozymes which can be demonstrated.[13]

In the work here presented, the occurrence of the LDH isozymes in the various mouse tissues and body fluids was investigated by means of starch gel electrophoresis, using borate buffer of pH 8.6. For the visualization of the LDH isozyme bands, a modification of the tetrazolium salt method introduced by Markert and Møller [5] was employed, enabling the visualization of the different LDH isozyme bands in a very simple manner.

Upon the omission of any one of the components of the reaction mixture, no isozyme band appeared. This is explained on the basis of the reactions involved:

a) Lactate $+$ NAD$^+$ $\rightleftharpoons$ NADH $+$ pyruvate $+$ H$^+$
b) NADH $+$ PMS $+$ H$^+$ $\longrightarrow$ NAD$^+$ $+$ reduced PMS
c) Reduced PMS $+$ Nitro$-$BT $\longrightarrow$ Reduced Nitro$-$BT $+$ PMS

The addition of NaCN to the reaction mixture caused an acceleration of the appearance of the LDH isozyme bands. This is due to cyanide combining with pyruvate to form cyanhydrine. Hence, the equilibrium in the reaction a) above is displaced to the right. The increased formation of NADH accelerates the irreversible reactions b) and c), resulting in an accelerated rate of formation of formazan.

Preincubation of the gels in formalin caused a denaturation of the LDH isozymes, thereby inhibiting enzymatic activities.

As seen in Fig. 1 at most 5 different isozymes were detected in all samples except for the testicular tissue which contained six, whereof one isozyme migrated towards the cathode. This is of interest in relation to the observation by Blanco and Zinkham[11] of an unusual X-form of LDH in homogenates of human testicular tissue. This X-form, however, was found to migrate towards the anode with a mobility between those of LDH_3 and LDH_4, according to the system of numbering the isozyme bands introduced by Apella and Markert,[15] where the form of LDH with the fastest mobility towards the anode is designated LDH_1, while the isozyme with the slowest electrophoretic mobility is termed LDH_5.

As yet, little is known about the physiological roles of the different forms of LDH, and even less when it comes to explaining the altered LDH isozyme pattern found in certain pathological states, although suggestions have been made with regard to different functions of LDH_1 and LDH_5.[16,17] Thus, the LDH_5 seems to be less sensitive to changes in the concentration of pyruvate than LDH_1. Hence, the LDH_5 predominates in tissues where glycolysis is the main source of energy, i.e. in skeletal muscle where the immediate oxygen supply is limited. Conversely, LDH_1 predominates in tissues characterized by an aerobic metabolism, as heart and brain. The findings here presented are in agreement with this pattern.

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