INTERDEPENDENCE OF SUBUNITS IN LACTATE DEHYDROGENASE TETRAMERS (RESULTS OF A QUANTITATIVE HISTOCHEMICAL INVESTIGATION)

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UDC 612.015.11:577.158.347.015.1

Investigation of relationships between the N and M subunits in lactate dehydrogenase (LDH) tetramers showed that the calculated values of type H LDH activity in heterotetramers from the spinal ganglia differ significantly from the observed values. This raises doubts about the validity of the assumption in the literature that the H and M subunits in LDH isoenzymes are mutually independent.

According to the tetramer theory [5, 13] each isoenzyme of lactase dehydrogenase (LDH) consists of four polypeptide H and M subunits. By their hybridization in vitro five molecularly heterogeneous isoenzymes are formed: LDH_1 (HHHH), LDH_2 (HHHM), LDH_3 (HHMM), LDH_4 (HMMM), and LDH_5 (MMMM) [12]. On the basis of this theory the enzyme activity dependent on the presence of the H (or M) subunit is calculated by the equation: $H = LDH_1 + 0.75\ LDH_2 + 0.50\ LDH_3 + 0.25\ LDH_4$ [8]. It is assumed that the activity of the subunits in the heterotetramers ($LDH_{2,3,4}$) is independent. Since the spectrum of LDH isoenzymes is relatively constant for each organ and since it changes reproducibly in the various states of that organ [8, 11, 15], determination of the LDH spectrum may be an important test to judge the state of oxidative metabolism. A special case of this problem is the study of enzyme activity dependent on the presence of the H or M subunit. Another equally important fact is that the LDH system is widely used as a model for the study of the mechanism of function of enzyme proteins [2, 7, 17]. For the reasons given above, verification of the assumption that the subunits in LDH heterotetramers are independent becomes a matter of great importance.

However, there is some evidence against the assumption that subunits in LDH tetramers are independent. First. LDH_1 and LDH_5 are known to change their enzyme activity under the influence of allosteric agents [7, 17]. Second, LDH isoenzymes possess a quaternary structure, and this is bound to be reflected in the conformation of the subunits and the enzyme activity of the tetramer [2, 9, 14]. It thus follows that the contribution of each subunit to the total activity of the tetramer is indeterminate. It has been shown [16] that the calculated values (relative activities of the heterotetramers calculated from LDH_1 activity in two different concentrations of pyruvate) differ from the observed values in a nonlinear manner.

Considering the importance of this problem it may prove useful to describe the results of observations concerning the assumption of mutual independence of the subunits in LDH heterotetramers.

EXPERIMENTAL METHOD

The activities of the LDH isoenzymes were determined by a histochemical method with nitro-BT after disk microelectrophoresis of homogenates of the spinal ganglia of Wistar rats and of single mechanoreceptors (Pacinian corpuscles) of the cat mesentery on polyacrylamide gel [10]. In parallel tests to determine type H LDH activity, 2M urea was added to the same incubation medium. The activity of LDH_5 in

Department of Histology, Kazan' Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR V. N. Orekhovich.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 74, No. 8, pp. 30-32, August, 1972. Original article submitted April 12, 1971.

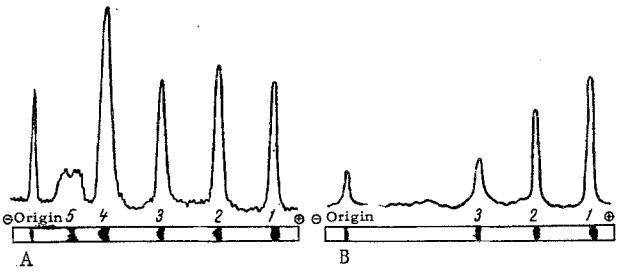

Fig. 1. Isoenzyme spectrum of LDH (A) and type H LDH (B) of spinal ganglia of Wistar rats. Above, densitograms; below, electrophoresis. Numbers denote serial numbers of isoenzymes.

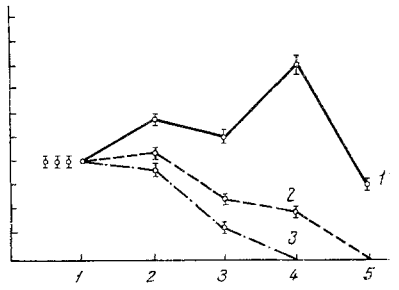

Fig. 2. Activity of LDH isoenzymes of spinal ganglia of Wistar rats: 1) observed activity of LDH isoenzymes; 2) calculated values of type H LDH; 3) observed values of type H LDH. Abscissa, LDH isoenzymes; ordinate, activity (in conventional units of optical density).

TABLE 1. Activity of Isoenzymes of LDH and Type H LDH in Spinal Ganglia after Temperature Stimulation of Skin (in conventional units of optical density)

Conditions of of stimulation (20 observations for each type of stimulation)	LDH_1	LDH_1H	LDH_2	LDH_2H	LDH_3	LDH_3H
30 min	K<0	K<0	K<0	K=0	K=0	K=0
60 min	K=0	K=0	K>0	K=0	K<0	K=0
30 min + 60 min rest	K=0	K=0	K=0	K=0	K=0	K=0

Legend: K) relative rest, 0) stimulation of the skin.

vitro is completely inhibited in this concentration of urea, whereas the activity of LDH_1 is slightly increased [6]. Homogenates of the spinal ganglis were studied in animals in a state of relative rest and after the following experimental procedures: temperature stimulation of the skin by alternation of baths at temperatures of 5 and 35°C for 30 and 60 min, and stimulation for 30 min followed by a rest of 60 min. The Pacinian corpuscles were tested in a resting state and 30-60 sec after stimulation by means of a pressure-sensitive element [3]. The activities of the LDH enzymes, converted in accordance with the tetramer theory to values for the content of the H subunit in each isoenzyme, were compared with the observed values for the type H isoenzymes. For comparison of the data, normal sampling was assumed [4] and the nonparametric criterion X [1] with levels of significance $\alpha = 5$ and 1% was used.

EXPERIMENTAL RESULTS AND DISCUSSION

The spectrum of the LDH isoenzymes of intact ganglia is shown in Fig. 1. The calculated values of activity dependent on the content of the H subunit in LDH_{1-4} and the observed values of the LDH and type H LDH activity are given in Fig. 2. Values of the activity of the isoenzymes during stimulation of the skin under different conditions are given in Table 1.

The Pacinian of the isoenzymes in homogenates of spectrum corpuscles (N = 34) in a state of relative rest was as follows: $LDH_1 : LDH_2 : LDH_3 : LDH_4 = 45.2 : 31.5 : 11.4 : 2.4$ (in conventional units of optical density) and 50.2 : 36.7 : 12.0 : 1.4 (in percent). Values of type H LDH did not differ from these figures by a statistically significant margin although after stimulation of the mechanoreceptors there was a significant increase in the activity of LDH_{1-2} (conventional units).

The experimental results suggest that the assumption that the subunits in LDH heterotetramers are independent is evidently incorrect. Under these experimental conditions (Fig. 2, Table 1), for instance,

the changes in activity of $LDH_{2,3}$ were evidently brought about by changes in activity dependent on the presence of the M subunit, which could not be established by the use of the accepted method of detecting activity of LDH isoenzymes and by subsequent calculation in accordance with the tetramer theory. On the contrary, it was necessary to postulate that the increase in activity took place on account of the H subunit, especially if it is taken into account that with the urea concentration used there is an increase in LDH_1 activity. In this respect the increase in activity of $LDH_{1,2}$, whose isoenzyme spectrum according to the tetramer theory consists practically entirely of H subunits (84%, activity of LDH_5 was absent in most cases) observed after stimulation of the Pacinian corpuscles, while at the same time the LDH_2 heterotetramer (HHHM) was detected histochemically in the homogenates of the Pacinian corpuscles and changed its activity depending on the functional state of the mechanoreceptor, also is demonstrative.

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