Regulatory Role of Supramolecular Alcohol Dehydrogenase and Lactate Dehydrogenase Complex in Cell Mitochondria

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The existence of a supramolecular alcohol dehydrogenase–lactate dehydrogenase complex was demonstrated. Enzyme activities were evaluated in a preparation of mitochondrial membranes from mouse liver. The role of the enzyme complex (alcohol dehydrogenase–lactate dehydrogenase) in the regulation of pyruvate and acetaldehyde metabolism in the cell was studied.

Key Words: *alcohol dehydrogenase; lactate dehydrogenase; mitochondria*

The mechanisms regulating cell metabolism is a key problem of biochemistry. One of the priority tasks in this problem is comprehensive study of the pathways of metabolism regulation at different levels of organization: individual enzymes, multienzyme complexes, subcellular organelles, and the whole cell.

According to modern concepts, metabolism regulation in the cell is realized by the sum of its enzymes and is not confined to modification of the rate of just one rate-limiting reaction in each metabolic cycle. The role of each enzyme in the regulation of the metabolic cycle cannot be strictly predicted from just characteristics of the purified enzyme, but is largely determined by its microenvironment, formation of dynamic supramolecular complexes with the cytoskeleton proteins, enzymes, cell organelle membranes, *etc.* [1,4,7]. The regulatory cascades consisting of numerous interrelated components in the cells form universal functional supramolecular ensembles, the primary element of these ensembles is the complex of regulator enzyme and regulated enzyme, *e.g.* mitochondrial supramolecular complex of alcohol dehydrogenase (ADH) and lactate dehydrogenase (LDH), in which ADH can act as a regulatory enzyme.

We studied the role of mitochondrial supramolecular enzyme complex (ADH–LDH) in the regulation of pyruvate and acetaldehyde metabolism in the cell.

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MATERIALS AND METHODS

Liver mitochondria of intact mice (n=10) were isolated by routine differential centrifugation in 0.01 M phosphate buffer (pH 7.4). The tissue (1 g) was homogenized in a 4-fold volume of the buffer. The mitochondrial precipitate was frozen three times in order to destroy the membranes of subcellular particles. After the procedure, "soluble" enzymes were removed by centrifugation of the samples for 30 min at 20,000g. The precipitate containing mitochondrial membranes was used for measuring ADH and LDH activities [3]. Enzyme activities were expressed in nmol of NADH/min×mg protein. Protein was measured by the method of Lowry [3].

The results were processed using Student's t test (BIOSTAT software). The differences were considered significant at $p<0.05$.

RESULTS

Activity of ADH in liver mitochondrial membrane preparation could be measured only in the inverse reaction (ethanol formation from acetaldehyde), while LDH was measured in the direct reaction (lactate transformation into pyruvate; Table 1). The interaction of LDH and ADH with subcellular organelle membranes

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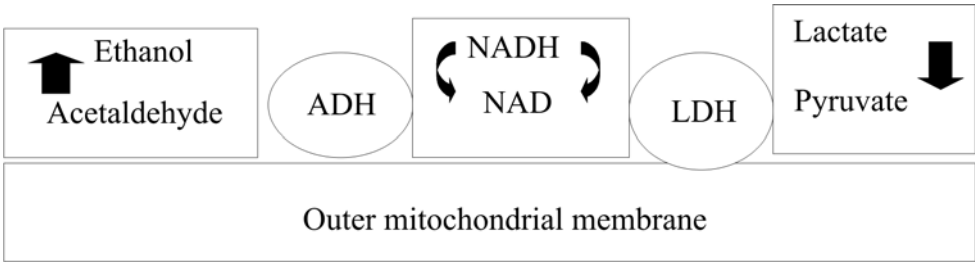

Fig. 1. Regulatory role of ADH and LDH interactions with cell mitochondrial membrane.

TABLE 1. Activities of LDH and ADH (nmol NADH/min×mg protein) in Liver Mitochondrial Membrane Preparation from Intact Mice (n=5; $M\pm m$)

Enzyme	Direct reaction	Inverse reaction
LDH	84.55±1.52	—
ADH	—	148.03±4.62

TABLE 2. LDH Activity (nmol NADH/min×mg protein) in Mitochondrial Membrane Preparation after Addition of Purified Enzyme (n=5; $M\pm m$)

Enzyme	Direct reaction	Inverse reaction
LDH	169.56±3.29*	453.57±11.26

Note. $p<0.05$ compared to value without LDH.

were described [5,6]. Presumably, oxidoreductase binding to membranes changes conformations of the enzymes leading to inhibition of direct ADH and inverse LDH reactions. The results of interactions between the membrane-bound enzymes (ADH, LDH) can be presented as a functional supramolecular complex (Fig. 1).

The scheme of regulation of ADH–LDH interactions (Fig. 1) attests to an increase in pyruvate content under these conditions. Pyruvate can be utilized by mitochondria in the Krebs cycle under aerobic conditions and for the maintenance of optimal concentration of acetaldehyde functioning as the endogenous regulator of the respiratory chain [2].

In our experiment, all soluble enzymes were removed from the membrane preparation; therefore, we carried out an *in vitro* experiment: purified LDH (soluble enzyme; Reanal) was added to the membrane preparation (1:1).

Addition of soluble enzyme to mitochondrial membrane preparation led to the appearance of catalytical activity of LDH in the inverse reaction (Table 2). Enzyme activity in the inverse reaction was 2.7 times higher ($p<0.01$) than in the direct reaction. Hence, in this case the enzyme was present in two forms: free "soluble", characterized by high activity of inverse lactate dehydrogenase reaction, and "membrane-associated" form with predominant activity of direct LDH reaction. Presumably, ADH is also present in two forms (free and membrane-bound) in intact cellmitochondria.

The findings confirm the existence of a regulatory supramolecular ADH-LDH complex, realizing metabolic regulation of pyruvate and acetaldehyde concentrations in the cell. Presumably, this enzyme complex is present in the subcellular organelles in two forms: free and membrane-bound. The membrane-bound ADH and LDH realize the catalytical process, mainly regulation of pyruvate and acetaldehyde content, thus increasing the bioenergetic potential of the cell.

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