Function of Cytoplasmic NAD-Dependent Malate Dehydrogenase from Rat Myocardium under Conditions of Ischemia

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NAD-dependent malate dehydrogenase activity decreased by 2.7 times in the myocardium of rats with experimental ischemia. Cytoplasmic NAD-dependent malate dehydrogenase from intact and ischemic rat heart was purified by 91.4 and 95.5 times. We compared kinetic characteristics and regulation of enzyme activity by Fe^{2+}, Cu^{2+}, Ca^{2+}, hydrogen peroxide, and glutathione under normal and pathological conditions.

Key Words: *rats; myocardium; ischemia; NAD-dependent malate dehydrogenase; properties*

Pathogenesis of cardiovascular diseases is associated with an imbalance between generation of reactive oxygen species (ROS) and activity of the antioxidant system [4,8,14]. It was hypothesized that the multilevel antioxidant protection system inhibits ROS generation by decreasing NADH supply to the mitochondrial electron-transporting chain [10]. It is interesting to study the function of NAD-dependent malate dehydrogenase (NAD-MDH, EC 1.1.1.37) catalyzing reversible conversion of malate into oxalacetate in the presence of NADH. Cytoplasmic NAD-MDH maintains activity of the transporting systems in the mitochondrial membrane and is involved in the transfer of reduced equivalents (NADH). Published data show that malate serves as a substrate of MDH and plays an important role in the biochemical adaptation to hypoxia [1].

Here we studied catalytic and regulatory properties of NAD-MDH from the cardiomyocyte cytoplasm of intact rats and animals with experimental ischemia.

MATERIALS AND METHODS

Experiments were performed on male albino rats weighing 250-300 g. Experimental myocardial ischemia was produced by 40-min occlusion of the left descending coronary artery [6]. Subcellular fractions of the myocardium were separated by differential centrifugation [9]. Activity of marker enzymes in the cytoplasm and mitochondria (lactate dehydrogenase and succinate dehydrogenase) was measured spectrophotometrically at 340 [3] and 600 nm [11], respectively. The degree of cross-contamination of the cytoplasmic and mitochondrial myocardial fraction was 12%. MDH activity was measured spectrophotometrically at 340 nm. Total protein concentration was estimated by the method of Lowry [12]. Cytoplasmic NAD-MDH was isolated from rat myocardium and purified in several stages: homogenization; separation of the cytoplasmic and mitochondrial fraction by differential centrifugation; gel filtration on a column packed with Sephadex G-25; ion-exchange chromatography on a column packed with DEAE-cellulose; and gel chromatography on a column packed with Sephadex G-150. Homogeneity of the enzyme preparation was verified by vertical electrophoresis in polyacrylamide gel (PAAG, method of Davis) [2]. Experiments were performed in 3-4 biological repetitions. Each sample was analytically assayed 2 times. The results were analyzed by standard statistical methods [5].

RESULTS

NAD-MDH activity is present in both cytoplasmic and mitochondrial fractions. Under normal and ischemic

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TABLE 1. Purification of Cytoplasmic NAD-MDH from the Intact and Ischemic Rat Myocardium (*M*±*m*)

Stage of purification, group	Activity, U	Protein concentration, mg	Specific activity, U/mg protein	Yield, %	Degree of purity
Cytoplasmic fraction					
normal	1.104±0.052	44.0±2.1	0.025±0.001	100	1
ischemia	0.451±0.030*	47.0±1.9	0.0096±0.0005*	100	1
Gel filtration on Sephadex G-25					
normal	1.03±0.05	29.70±1.32	0.035±0.001	93.3	1.4
ischemia	0.412±0.020*	28.50±1.27	0.014±0.001*	91.4	1.46
Ion-exchange chromatography on DEAE-cellulose					
normal	0.990±0.046	1.90±0.09	0.52±0.02	89.7	20.8
ischemia	0.381±0.018*	1.80±0.08	0.212±0.020*	84.5	22.08
Gel chromatography on Sephadex G-150					
normal	0.320±0.015	0.14±0.01	2.286±0.114	25.18	91.44
ischemia	0.110±0.005*	0.12±0.01	0.9167±0.0410*	24.4	95.49

Note. Here and in Table 2: *$p<0.05$ compared to normal.

conditions NAD-MDH activity in rat myocardium is mainly located in mitochondria (~75%); ~25% enzyme activity is localized in the cytoplasmic fraction.

NAD-MDH activity in the homogenate and subcellular fractions of rat myocardium during ischemia decreased by 2.7 times compared to normal (Table 1). Four-stage purification allowed us to obtain enzyme preparations of cytoplasmic NAD-MDH from intact and ischemic cardiomyocytes. NAD-MDH was purified by 91.4 and 95.5 times, respectively. Specific enzyme activity was 2.286 and 0.917 U/mg protein, respectively. Electrophoresis showed that fractions with maximum activity obtained after chromatography on Sephadex G-150 contain homogenous NAD-MDH. One protein band with R_f 0.65 (enzyme from the intact and ischemic myocardium) appeared in PAAG after protein staining. The study of purified enzyme preparations estimated kinetic characteristics of catalytic activity and regulatory properties of cytoplasmic NAD-MDH from intact and ischemic rat heart.

NAD-MDH from ischemic myocardium was characterized by a 50% change in K_m by oxalacetate and NADH (Table 2). Cytoplasmic NAD-MDH from intact and ischemic animals exhibited activity in a wide range of pH. It should be emphasized that NAD-MDH from ischemic myocardium had wider pH optimum (Table 2). Ischemic damage to the heart tissue is accompanied by activation of free radical oxidation (FRO) [7,13]. It can be accompanied by changes in enzyme conformation and properties.

Fe^{2+}, Cu^{2+}, Ca^{2+}, and ROS (*e.g.*, hydrogen peroxide) play an important role in FRO activation. We studied the effect of these agents on activity of MDH from intact and ischemic myocardium. Fe^{2+} inhibited this enzyme under normal and pathological conditions. It is important that activity of MDH from ischemic myocardium decreased upon exposure to the effector in a lower concentration (Fig. 1, *a*). Cu^{2+} also decreased enzyme activity. After treatment with Cu^{2+} in concentrations of 0.01-0.40 mM enzyme activity from the ischemic tissue decreased more significantly compared to the control (Fig. 1, *b*). Changes in the sensitivity of NAD-MDH to metal ions probably determine the regulation of enzyme activity during ischemia and oxidative stress produced by ischemic tissue damage. Ca^{2+} in a concentration below 0.1 mM decreased enzyme activity under normal and pathological conditions. Increasing the concentration of these ions was

TABLE 2. Catalytic Activity of Cytoplasmic NAD-MDH from the Intact and Ischemic Rat Heart (*M*±*m*)

Group	pH optimum	K_m, mM	
		oxalacetate	NADH
Normal	7.2-7.8	0.040±0.003	0.050±0.003
Ischemia	7.0-8.2	0.050±0.004*	0.075±0.004*

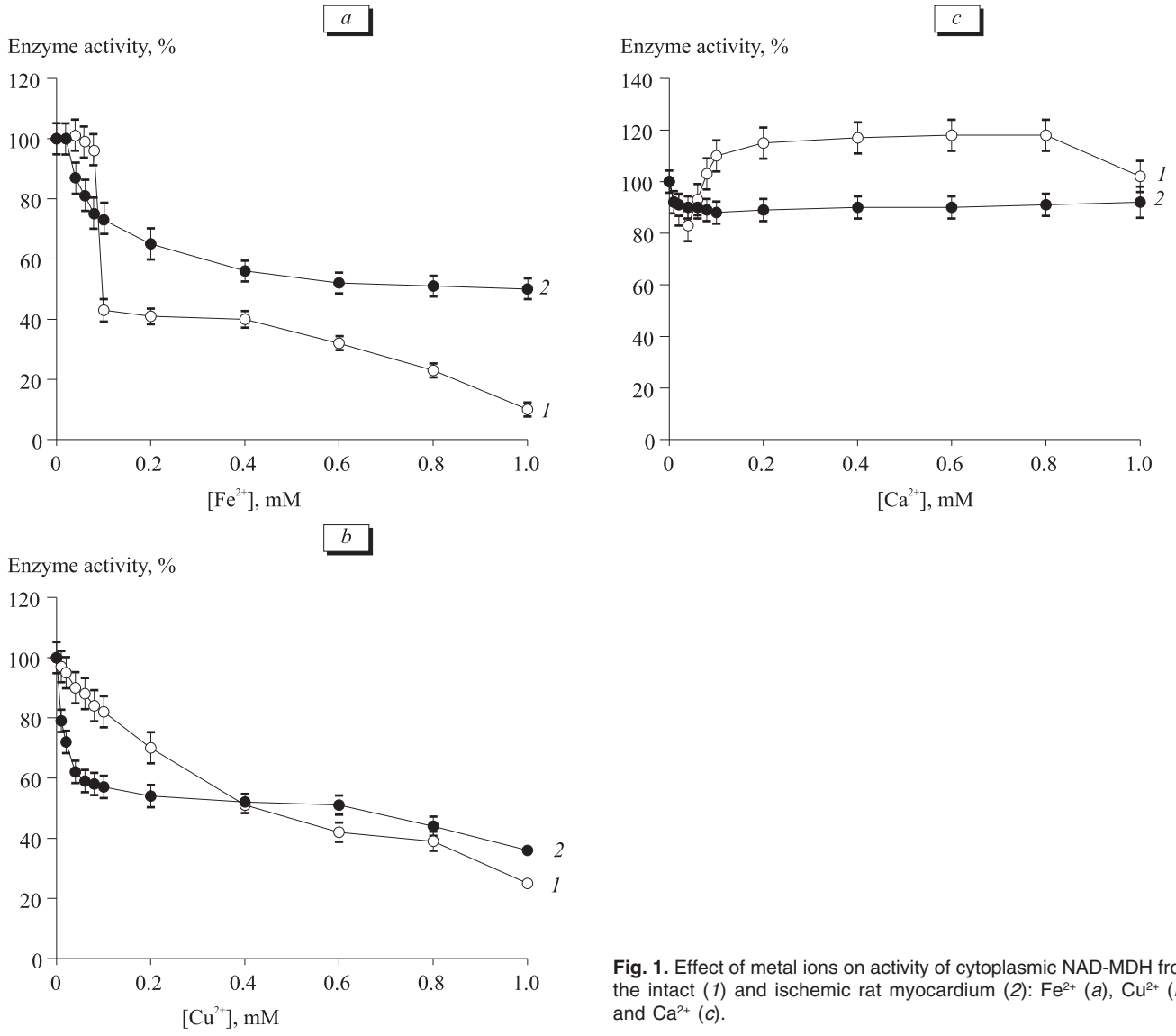

Fig. 1. Effect of metal ions on activity of cytoplasmic NAD-MDH from the intact (*1*) and ischemic rat myocardium (*2*): Fe^{2+} (*a*), Cu^{2+} (*b*), and Ca^{2+} (*c*).

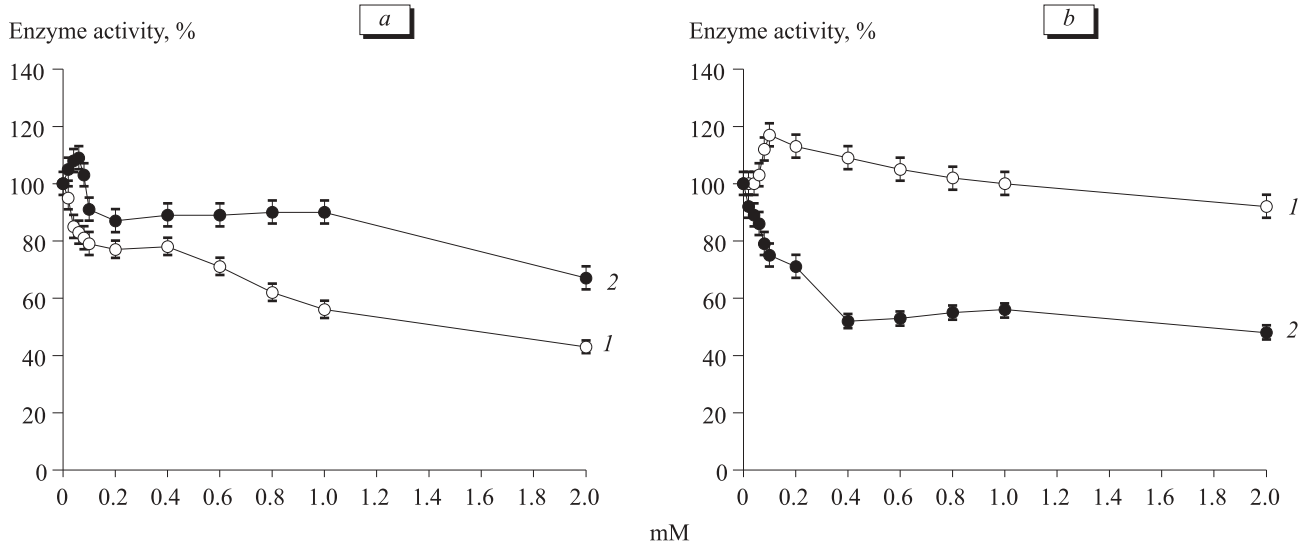

Fig. 2. Effect of oxidized (*a*) and reduced glutathione (*b*) on activity of cytoplasmic NAD-MDH from the intact (*1*) and ischemic rat myocardium (*2*).

accompanied by activation of NAD-MDH from the intact myocardium. However, enzyme activity in the ischemic heart remained low under these conditions (Fig. 1, *c*).

No differences were revealed between NAD-MDH activity in the ischemic and intact tissue treated with hydrogen peroxide.

The glutathione peroxidase/glutathione reductase system is an important antioxidants systems involving glutathione as the substrate. Oxidized and reduced glutathione pools play a role in the regulation of enzyme activity under normal and ischemic conditions (Fig. 2, *a*, *b*). Oxidized glutathione decreases cytoplasmic NAD-MDH activity in the intact rat myocardium. MDH isolated from ischemic cardiomyocytes and applied in a concentration <0.08 mM insignificantly increased enzyme activity. Increasing the concentration of this compound was accompanied by an inhibitory effect. Reduced glutathione in a concentration below 0.8 mM increased cytoplasmic NAD-MDH activity under normal conditions, but inhibited MDH from the ischemic heart.

Our results indicate that ischemic tissue damage is accompanied by changes in activity and kinetic and regulatory characteristics of cytoplasmic NAD-MDH from rat heart. It probably results from conformational changes in the enzyme molecule during ROS generation and activation of free radical processes. These changes can play a role in the protective mechanisms suppressing FRO under pathological conditions.

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