BIOPHYSICS AND BIOCHEMISTRY

Effect of Delta Sleep-Inducing Peptide on Catalytic Properties of Mitochondrial Malate Dehydrogenase from Rat Brain

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Delta sleep-inducing peptide is shown to alter properties of malate dehydrogenase in brain mitochondria. The regulatory activity of the peptide is manifested in stabilization of catalytic properties of the enzyme at a higher level, which prevents their change during hypoxic stress. Regulation of malate dehydrogenase is presumed to occur through direct action of the peptide on mitochondrial membranes.

Key Words: *NAD-dependent malate dehydrogenase; delta sleep-inducing peptide; hypoxic stress; mitochondria; brain*

Delta sleep-inducing peptide (DSIP) is a regulatory neuropeptide influencing a wide range of functional reactions in the body [1,12]. Its physiological effects are particularly striking in stressful states [4,6,9]. However, the question of how DSIP affects metabolic processes in such states has not been adequately addressed; in particular, little is known about its effects on energy metabolism in the brain in hypoxia-induced stress.

One of the enzymes involved in energy metabolism is NAD-dependent malate dehydrogenase (MDH; EC 1.1.1.37). Our previous studies have shown that the mitochondrial isoenzyme NAD-MDH is characterized by lability of catalytic properties in hypoxia [7] and is capable of interacting with the inner mitochondrial membrane [8]. In the present study we tested DSIP for its effects on catalytic properties of NAD-MDH during hypoxic stress.

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MATERIALS AND METHODS

The study was carried out on male albino rats weighing 150-200 g. Catalytic properties of NAD-MDH were evaluated in four series of tests. In series I a group of intact rats was used (controls), in series II DSIP was incubated with a suspension of intact mitochondria, in series III rats were injected with DSIP intraperitoneally, while in series IV they were placed in a pressure chamber 20 min before being injected with DSIP by this route.

Hypoxic stress was simulated in a flow-through pressure chamber at 196 mm Hg, the exposure time being 15 min. A DSIP synthesized at the Institute of Bioorganic Chemistry, Moscow, was used. When mitochondria were added to the incubation medium, the final DSIP concentration was 10^{-6} M (incubation time was at least 20 min). For test series III and IV the peptide was dissolved in physiological saline and injected intraperitoneally in a dose of 120 μg/kg.

Mitochondria, obtained by differential centrifugation [11], were disrupted by hypotonic treatment. Kinetic characteristics of the reverse MDH reaction

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were determined in terms of oxaloacetate (OAA). The incubation medium contained 0.1 M phosphate buffer (pH 7.4), 0.1 mg of mitochondrial protein, a fixed NADH concentration (0.2 mM) and incremental OAA concentrations (from 0.16 to 10 mM). The reaction was evaluated spectrophotometrically by the decrease in NADH concentration. Initial rates (V_0) of the enzymatic reaction were calculated as described [10]. Kinetic constants were determined using Enzfitter software. The results were analyzed and treated statistically as described [3] and by Fisher's method. Protein concentration was measured by Lowry's method.

RESULTS

In the first test series, in which catalytic properties of NAD-MDH were studied using the mitochondrial fraction from brains of intact rats, the kinetics of NADH oxidation in this fraction was found not to obey the Michaelis-Menten law. When the results were depicted graphically in direct quantities (V_0/[OAA]), an attempt to describe the experimental data by a hyperbola revealed a considerable scatter of data points relative to the model (theoretical) curve (Fig. 1, *a*). To check the validity of the assumption that the errors were random, a secondary diagram of weighted differences between the observed and predicted V_0 values *vs.* the substrate concentration was utilized [3] (Fig. 1, *b*). The detection of systematic biases in the secondary diagram suggested a sigmoid shape of the curve describing enzyme saturation with the substrate.

The traditional methods used for determining kinetic constants are inapplicable if the shape of the enzyme saturation curve is not hyperbolic [2,3]. For this reason, the NAD-MDH kinetics was further analyzed using a mathematical model of the relationship between steady-state reaction rate and substrate concentration, based on Hill's equation as modified for bisubstrate reactions [2]:

$$V = V_{max} \frac{A^{Ha} \times B^{Hb}}{K_a \times K_b + K_a \times B^{Hb} + K_b \times A^{Ha} + A^{Ha} \times B^{Hb}}$$

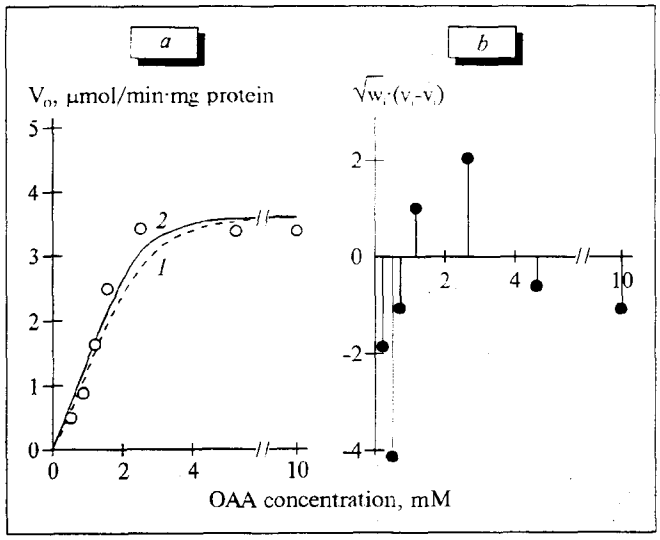

Fig. 1. Kinetics of the reverse MDH reaction in the brain mitochondrial fraction from intact rats. *a*) plots of initial reaction rate *vs.* OAA concentration: *1*) theoretical curve for the classical enzyme; *2*) curve for the model of allosteric interactions. *b*) secondary diagram for weighted differences between observed and predicted V_0 values for the hyperbolic model.

where V_{max} is the maximal reaction rate, A and B are substrate concentrations (OAA and NADH, respectively), K_a and K_b are equal to $K_{0.5}$ for both substrates, and H_a and H_b are Hill's cooperativity coefficients for the two substrates.

Although the detected anomalous kinetics of the NADH oxidation reaction does not yet permit NAD-MDH to be regarded as an allosteric enzyme, this model describes the experimental data in a satisfactory manner (Fig. 1, *a*). Enzyme saturation with the substrate occurs at an OAA concentration of 2.5 mM. Estimated values of the major kinetic parameters are presented in Table 1.

Preincubation of DSIP with a suspension of intact mitochondria (test series II) modified catalytic properties of NAD-MDH (Fig. 2, *a*). V_{max} changed significantly to exceed the control value by 25% (Table 1). The sigmoid shape of the curve in direct quantities, the substrate concentration at which the enzyme was saturated, and K_m values did not differ from those recorded for intact animals.

TABLE 1. Effect of DSIP on Kinetic Characteristics of the Reverse MDH Reaction in Brain Mitochondria of Intact Rats and Rats with Hypoxic Stress ($M \pm m$; $n = 3-5$)

Experimental conditions	Control	DSIP + isolated mitochondria	DSIP intraperitoneally	
			control	hypoxic stress
$K_{0.5}$ for OAA, mM	0.59±0.09	0.89±0.18	1.28±0.77	1.29±0.73
Hill's coefficient for OAA	1.73±0.16	1.52±0.16	1.28±0.29	1.69±0.41
V_{max}, nmol/min×mg protein	3659.8±119.5	4548.2±681.6*	4431.5±533.3*	4672.2±617.1*

Note. The asterisk denotes a significant difference from control samples at $p<0.01$.

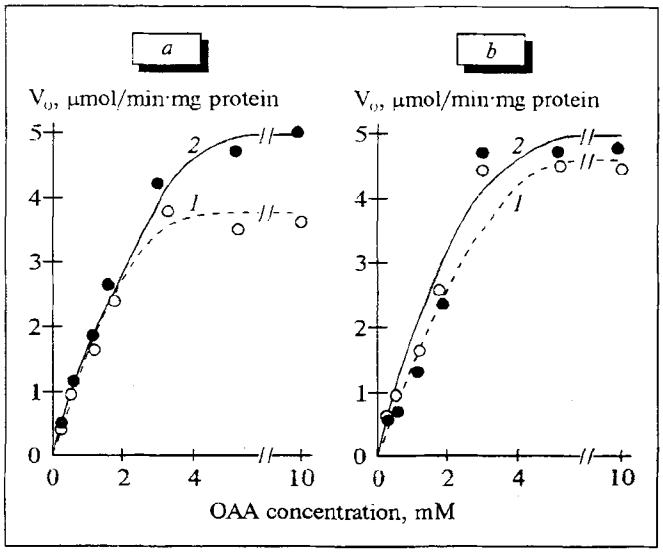

Fig. 2. Plots of initial rate of the reverse MDH reaction *vs.* OAA concentration. *a*) isolated mitochondria: *1*) intact; *2*) preincubated with DSIP. *b*) after intraperitoneal DSIP injection: *1*) into control rats; *2*) into rats under hypoxic stress.

After the intraperitoneal injection of DSIP (test series III), the catalytic properties of the enzyme were essentially the same as those observed when the peptide was added to the incubation medium containing intact mitochondria (Fig. 2, *b* and Table 1).

In test series IV, where DSIP was injected into rats that were then exposed to hypoxic stress in the pressure chamber, the kinetic features of the MDH reaction again did not differ from those observed in the two previous test series (Fig. 2, *b* and Table 1).

Therefore, as this study has shown, DSIP increases the V_{max} of the NAD-MDH-catalyzed reaction in rat brain mitochondria without affecting other kinetic constants of the reaction, and this effect does not depend on the experimental conditions, i.e., on whether DSIP is added to the incubation medium of isolated mitochondria or injected into animals by the intraperitoneal route. In the latter case the effects recorded for control and hypoxia-exposed animals were the same. This finding indicates that DSIP prevents inhibition of the enzyme under conditions of severe oxygen deficiency [7] and stabilizes NAD-MDH in the state of heightened activity. Moreover, DSIP was found to alter the catalytic properties of NAD-MDH in isolated mitochondria to the same extent as it did following intraperitoneal administration. These results, in conjunction with those obtained using a radioimmunoassay [5], suggest that DSIP acts on mitochondria directly and alters the kinetic characteristics of the labile membrane-bound NAD-MDH by the processes of the enzyme's association/dissociation with their membrane. We have documented similar effects of the peptide for Type A monoamine oxidase and brain hexokinase.

In sum, DSIP alters the catalytic properties of NAD-MDH in brain mitochondria. Its regulatory influence is manifested in the stabilization of these properties at a higher level, with the result that the enzyme's activity remains unchanged during hypoxic stress. The end result of its action is the same regardless of whether DSIP is added to the incubation medium of mitochondria or injected into animals by the intraperitoneal route.

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