Rat Hepatic Aldehyde Dehydrogenase in Normal Conditions and Thermal Injury: Partial Purification, Property Investigation

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Catalytical properties of aldehyde dehydrogenase were studied using preparations of this enzyme, obtained from control rats and rats with thermal injury. Aldehyde dehydrogenase was shown to participate in the metabolism of aromatic and aliphatic aldehydes. Kinetic characteristics of the enzyme with different substrates were studied under normal conditions and in thermal burn injury.

Key Words: *aldehyde dehydrogenase; thermal injury; substrate specificity*

Thermal injury is characterized by enhanced catabolic processes and lipid peroxidation with generation of toxic medium-chain aldehydes: alkanals, alkenals, and 4-hydroxy-alkenals [8]. An important role in the regulation of aldehyde content is played by aldehyde dehydrogenase (ALDH; aldehyde:NAD-oxidoreductase; EC 1.2.1.3), a member of biotransformation enzyme class oxidizing aldehyde or ketone groups of donors. ALDH is present in various mammalian tissues: liver, kidney, uterus, adrenal glands, small intestine, brain, heart, fat tissue, and lungs. Liver cells exhibit maximum enzyme activity [11].

Catalytic properties of the enzyme from animal [4] and human [11] tissues obtained under normal conditions have been reported. However, peculiarities of the enzyme functioning in thermal injury remain poorly studied.

The objective of the study was partial purification and analysis of catalytic and kinetic properties of ALDH from rat liver under normal conditions and in thermal burn injury.

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MATERIALS AND METHODS

Experiments were carried out on white Wistar rats of both sexes weighing 180-250 g, kept on a vivarium diet. The animals were divided into two groups: intact animals and animals with thermal injury (experimental). Experimental animals under ether anesthesia were exposed to flame burn (with a cottonwool swab soaked with ethanol and set in flame) on thoroughly shaved skin (10% body surface area) for 45 sec. One hour after thermal injury, the liver was removed under anesthesia, perfused with physiological saline, and used for further investigations.

ALDH activity was determined by the method [2], protein content was measured using modified Lowry method [9]. The following kinetic parameters of the enzyme were determined: K_t, time to $^1/_2V_{max}$ of enzymatic reaction (min), V_{max}, maximal reaction rate (μmol/min), V_{max}/K_t (K_a), catalytic efficiency coefficient for enzymatic reaction (μmol/min^2).

Purification of ALDH preparation from rat liver included several stages: protein fractionation with ammonium sulfate within 40-70% saturation, gel filtration on Sephadex G-25, and ion-exchange chromatography on DEAE-cellulose [5].

The experiments were repeated 3-4 times, analytic measurments were performed twice for each probe.

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TABLE 1. Results of Purification of Liver ALDH from Control Rats (Intact Animals) and Rats with Thermal Injury (*M*±*m*)

Purification stage	Total activity, nmol NADH/ min×ml	Protein amount, mg/ml	Specific activity, nmol NADH/min× mg protein	Yield, %	Pirification grade
Homogenate					
control	304.91±1.40	390.66±57.10	0.78±0.03	100.0	1.00
thermal injury	257.04±17.96*	400.97±23.69	0.64±0.01*	100.0	1.00
Fractionation $(NH_4)_2SO_4$					
control	247.99±8.76	69.09±4.58	3.59±0.17	81.3	4.60
thermal injury	208.09±5.51*	80.75±7.77	2.60±0.28*	80.9	4.10
Chromatography on Sephadex G-25					
control	217.79±5.79	28.00±0.08	7.73±0.98	71.4	9.97
thermal injury	156.73±12.98*	27.21±3.84	5.76±0.01*	60.9	9.00
Chromatography on DEAE-cellulose					
control	137.97±11.92	16.98±0.28	8.62±0.38	45.3	11.05
thermal injury	104.91±4.36*	15.40±1.48	6.81±0.19*	40.8	10.64

Note. Here and in Tables 2, 3: *$p<0.05$ in comparison with the control.

Results were processed using Student's *t* test.

RESULTS

Fractionation with ammonium sulfate and ion-exchanging chromatography on DEAE-cellulose yielded ALDH enzyme preparation with purification grade 11 (in intact rats) and 10.6 (in rats with burn). The yield of the enzyme in the control group was 45.3% *vs.* 40.8% in the experimental group (Table 1).

Baseline total ALDH (homogenate) activity was significantly higher in the control group (by 18.6%) compared to the experimental group. Specific activity in the control group also exceeded (by 21.9%) that in the experimental group, since there were no significant differences between the samples by protein content.

The decrease in total ALDH activity in rats with burn is probably associated with reduced proportion of catalytically active enzyme and accumulation of highly toxic compounds, *e.g.* medium-molecular-weight molecules. It can be hypothesized that these molecules binding to the protein and changing its conformation, thus reducing enzyme affinity to substrates and ALDH activity [3].

Isolation of partially purified enzyme allowed us to study substrate specificity of ALDH. ALDH is responsible not only for acetaldehyde metabolism, but also for oxidation of other aldehyde groups present in biogenic monoamines, diamines, and polyamines, for retinoic acid metabolism and even for oxidation of aldehydes, originated in the processes of lipid peroxidation processes in the membrane. It remains unknown which aldehydes are the main ALDH substrates *in vivo* [6].

The use of substrate series (aliphatic with various carbon skeleton lengths and aromatic) showed that in both intact and injured rats the highest oxidation rate was typical for salicylic aldehyde (SA), a cyclic aldehyde, somewhat lower rate was observed for glutaric aldehyde (GA), and aldehyde with spacious aliphatic

TABLE 2. ALDH Activity (nmol NADH/min×mg protein) with Various Substrates under Normal Conditions and after Thermal Injury (*M*±*m*)

Group	ALDH substrate				
	GA	acetaldehyde	SA	benzaldehyde	formaldehyde
Control	7.22±0.22	0.072±0.001	7.92±1.28	0.35±0.01	0.13±0.01
Thermal injury	4.62±0.69*	0.036±0.001*	5.70±0.02*	0.25±0.01*	0.03±0.01*

TABLE 3. Kinetic Characteristics of Liver ALDH from Intact Rats and after Thermal Injury (*M*±*m*)

Group		ALDH substrate				
		GA	acetaldehyde	SA	benzaldehyde	formaldehyde
Control	K_t	0.61±0.01	3.33±0.01	2.09±0.12	0.41±0.03	0.14±0.01
	V_{max}	34.86±0.05	0.31±0.01	58.73±1.48	0.43±0.01	1.68±0.05
	K_a	45.38±0.03	0.91±0.02	14.80±0.38	5.00±0.04	2.75±0.07
Thermal Injury	K_t	0.56±0.03	0.95±0.13*	1.34±0.12*	2.44±0.09*	1.75±0.17*
	V_{max}	21.73±1.02*	0.35±0.04	2.76±0.50*	1.59±0.19*	0.12±0.03*
	K_a	39.88±3.02*	0.62±0.15*	2.45±0.78*	7.61±1.29*	0.07±0.01*

residual. Rates for transformation of benzaldehyde (aromatic aldehyde), formaldehyde, and acetaldehyde were significantly lower (Table 2).

ALDH exists at least in two kinetic forms characterized by high and low affinity to the substrates [7]. Enzyme with highest affinity to acetaldehyde (Km 3 μM) was localized predominantly in mitochondrial fraction. Therefore it is considered to be the main enzyme responsible for acetaldehyde oxidation [10]. This finding may explain observed low ALDH activity in partially purified enzyme sample, with acetaldehyde used as substrate.

In thermal injury, ALDH activity with various aldehydes as the substrates in partially purified enzyme preparation was reduced in comparison with the control (Table 2). Thus, ALDH activity for GA after burn decreased by 36%, for acetaldehyde by 50%, for SA by 28%, for benzaldehyde by 28.6%, and for formaldehyde by 77%. Reduced activity for all substrates is apparently associated with a decrease in total enzyme activity after burn.

Basing on kinetic parameters (Table 3) one may claim that the time to $^1/_2V_{max}$ for enzymatic reaction after thermal injury was reduced for following substrates: GA (by 8.2%), acetaldehyde (by 71.5%), and SA (by 35.9%). These findings suggest that enzyme affinity to them increased after the burn.

Rats with thermal injury are characterized by increased (in comparison with control animals) time to $^1/_2V_{max}$ of enzymatic reaction for benzaldehyde and formaldehyde (by 6.0 and 12.5 times, respectively), which is indicative of reduced affinity to these aldehydes.

The highest catalytic activity of ALDH was observed when GA was used as the substrate. Thus, in intact rats and rats with thermal injury V_{max}/K_t was 45.38±0.03 μM/min^2 and 39.88±3.02 μM/min^2, respectively.

In thermal injury, ALDH exhibits its highest affinity to GA. Half-time for GA transformation was 0.56±0.03. Animals from experimental group had decreased coefficient of catalytic activity in comparison with control animals for majority of substrates: GA by 121%, acetaldehyde by 31.9%, SA by 83.5%, formaldehyde by 97.5%.

Thus, ALDH is involved in metabolism of GA, acetaldehyde, SA, benzaldehyde, and formaldehyde. In both intact and thermally injured animals the highest rate of ALDH-mediated oxidation was observed for SA and GA. The highest catalytic efficiency of ALDH was observed when GA was used as the substrate in both control and experimental animals.

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