

SELECTIVE INACTIVATION OF THE NADH-UBIQUINONE SEGMENT OF THE RESPIRATORY CHAIN OF SUBMITOCHONDRIAL PARTICLES BY ENDOGENOUS FREE FATTY ACIDS DURING HYPERTHERMIA

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Received 28 August 1978

1. Introduction

The respiration of reticulocytes and EMAC cells has been shown to be inactivated by incubation for 2 h at $> 41^{\circ}\text{C}$ [1]. Since preferably the NADH-ubiquinone (UQ) segment of the respiratory chain (complex I) is affected irreversibly, a denaturation of enzyme proteins has been suggested to occur in this region [1].

On the other hand, a slow and irreversible inhibition of the electron transport in complex I of the respiratory chain is also observed, if free fatty acids (FFA) are added to submitochondrial particles (ETP) [2,3]. This effect is strongly temperature dependent [3].

In this report endogenous FFA of ETP are shown to inhibit the NADH oxidase activity under hyperthermic conditions ($> 39^{\circ}\text{C}$). From the results it is evident that at least two different effects are involved in hyperthermic damage of the respiration of ETP:

1. A slow and irreversible inhibition by FFA in complex I;
2. A selective denaturation of certain proteins of the respiratory chain.

The effects observed with ETP may apply for intact cells in hyperthermia.

2. Materials and methods

Non-phosphorylating submitochondrial particles (ETP) were prepared from beef heart mitochondria by mild alkaline treatment by the method in [4] with the modifications in [5].

Human serum albumin (HSA) (Forschungsinstitut für Impfstoffe, Dessau) was defatted by lowering the pH and flotation of the released fatty acids by centrifugation as in [6].

Ovalbumin was isolated from hen's egg by ammonium sulphate fractionation.

The incubation mixture was as follows: 50 μl ETP (55–88 μg protein, Lowry method); 200 μl 0.03 M potassium phosphate buffer (pH 7.4).

After incubation for 2 h at the temperature indicated, 0.8 ml 0.05 M Tris- H_2SO_4 buffer (pH 8.0), containing 10 mg HSA/ml and 10 μl NADH (VEB Arzneimittelwerk, Dresden) solution containing 20 mg/ml were added. The NADH oxidase activity was monitored by a Unicam SP 800 spectrophotometer at 340 nm.

The measurement of the partial systems of the respiratory chain is detailed in [3].

The data are mean values of triplicate measurements. The inactivation was calculated according to the following equation:

Inactivation (%) =

$$\left[\frac{1 - \text{ETP activity after hyperthermia}}{\text{ETP activity after } 37^{\circ}\text{C incubation}} \right] \times 100$$

3. Results

The NADH oxidase activity of ETP is inactivated in a strongly temperature-dependent manner (fig.1).

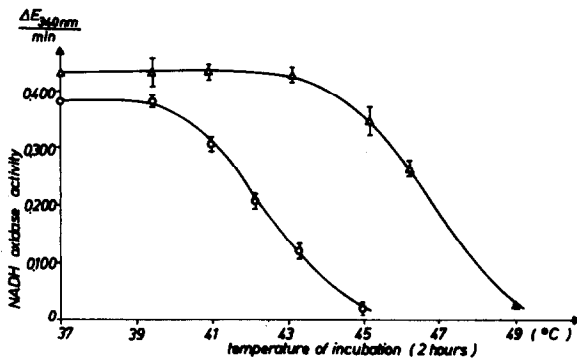


Fig. 1. Temperature dependence of the NADH oxidase activity of ETP during hyperthermia. (○—○) Incubation mixture without HSA; (△—△) incubation mixture with HSA (1%, w/v).

Incubation for 2 h at 39°C causes only slight inactivation which is complete at ~45°C. The extent of the inactivation is time dependent (fig.2) and cannot be abolished by subsequent addition of various concentrations of HSA (0.1–1%).

By contrast, the NADH oxidase activity of ETP is protected, if HSA is added before the heat treatment (fig.1). In the latter case the respiratory inactivation is just detectable at ~43°C.

The protective action of HSA depends on its concentration in the incubation mixture (fig.3). To decide whether the protection may be due to an unspecific protective protein effect, experiments were performed with ovalbumin and FFA-loaded HSA. Both failed to protect (tables 1, 2). Thus it

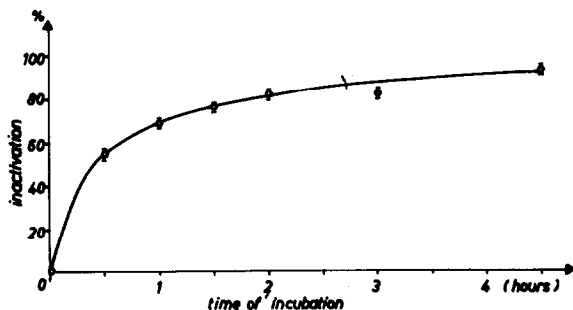


Fig. 2. Time dependence of the inactivation of the NADH oxidase activity of ETP during hyperthermia. Incubation for 2 h at 44.5°C.

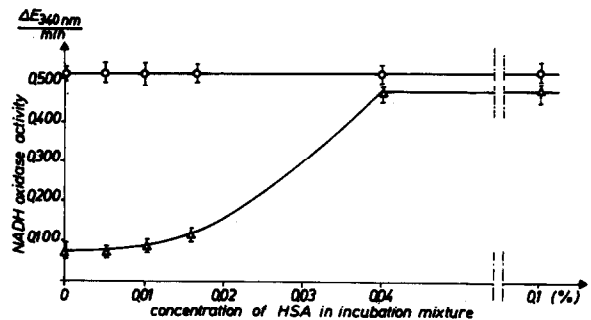


Fig. 3. Influence of the HSA concentration on the NADH oxidase activity of ETP during hyperthermia. (○—○) ETP incubated at 37°C; (△—△) ETP incubated at 45°C.

appeared that endogenous FFA of ETP are the cause of inactivation, which are bound by the added HSA [7,8].

The FFA-mediated inactivation is, however, not due to lipid peroxidation [9], since it also appears under anaerobic conditions (table 3).

The investigation of partial systems of the respiratory chain revealed, that the HSA-prevented inactivation is located only in the iron-sulphur protein region of the complex I (table 4). On the other hand there was no prevention by HSA of the inactivations of other components of the respiratory chain such as NADH-ferricyanide reductase, succinate-cytochrome *c* reductase and cytochrome *c* oxidase. These systems are inactivated at somewhat higher temperatures (table 4). These effects cannot be related to FFA and may represent direct denaturation of proteins. The inactivation of the succinate dehydrogenase is counteracted by succinate, whereas the absence of oxygen or an inhibition of the electron

Table 1
Influence of ovalbumin on NADH oxidase activity during hyperthermia

Incubation	Activity ($\Delta E_{340} \text{ nm/min}$)		Inactivation (%)
	37°C	44°C	
ETP	0.265	0.085	68
ETP + HSA	0.275	0.250	9
ETP + ovalbumin	0.260	0.080	69

Table 2
Influence of FFA-loaded HSA on NADH oxidase activity during hyperthermia

Incubation	Activity (ΔE_{340} nm/min)		Inactivation (%)
	37°C	45°C	
ETP	0.492	0.080	84
ETP + HSA	0.595	0.550	8
ETP + FFA-loaded HSA ^a	0.520	0.050	90
ETP + FFA ^b	0.000	0.000	100

^a 155 nmol myristic acid/31 nmol HSA; (5:1)/incubation

^b 155 nmol myristic acid (620 μ M/incubation) as control for the formation of FFA-HSA complex

Table 3
Influence of oxygen on NADH oxidase activity during hyperthermia

Incubation	Gas phase	Activity (ΔE_{340} nm/min)		Inactivation (%)
		37°C	44.5°C	
ETP	oxygen	0.515	0.120	77
ETP ^a	nitrogen	0.495	0.110	78
ETP + succinate ^b	nitrogen	0.620	0.100	84

^a All samples were carefully deoxygenated

^b Potassium succinate (13 mM/incubation)

Table 4
Hyperthermic inactivation in partial systems of the respiratory chain

System	Activity ^a - HSA		Inactivation (%)	Activity ^a + HSA		Inactivation (%)
	37°C	45°C		37°C	45°C	
NADH-O ₂	0.465	0.068	85	0.518	0.505	2
NADH-UQ	0.522	0.060	89	0.560	0.440	21
NADH-ferricyanide	0.150	0.080	47	0.210	0.110	47
Succinate-cytochrome <i>c</i>	0.120	0.050	58	0.110	0.020	82
Cytochrome <i>c</i> - O ₂	0.150	0.105	30	0.160	0.105	34

^a ΔE_{340} nm/min or ΔE_{550} nm/min

Table 5
Hyperthermic inactivation of succinate-cytochrome *c* reductase

Incubation	Gas phase	Activity (ΔE_{550} nm/min)		Inactivation (%)
		37°C	44°C	
ETP	oxygen	0.050	0.018	64
ETP	nitrogen	0.048	0.024	50
ETP + succinate ^a	oxygen	0.070	0.050	29
ETP + succinate	nitrogen	0.085	0.072	15
ETP + rotenone ^b	oxygen	0.042	0.015	65

^a Potassium succinate, 0.33 M

^b Rotenone, 2.5×10^{-4} M (in methanol)

flow through complex I do not exert substantial effects (table 5).

From the extent of the inactivation of the various respiratory systems it is evident that the FFA-mediated (HSA-prevented) inactivation of the NADH-UQ reductase segment predominates in the 39–43°C range.

4. Discussion

The features of the hyperthermic inactivation of complex I of the respiratory chain such as prevention by HSA (fig.1,3), irreversibility, time lag (fig.2), the specific action site (table 4) and the marked temperature dependence (fig.1) are in accordance with the inhibition of the oxygen uptake of ETP by added FFA [3]. Furthermore, only the FFA fraction of the lipid extract of ETP, separated by thin-layer chromatography, inhibited the respiratory chain in a similar manner (unpublished results).

Obviously the small amount of FFA present in ETP is not sufficient to affect respiration at < 39°C (fig.1), presumably owing to binding of ETP to other proteins. Increased temperatures may give rise to both release of the unspecifically bound endogenous FFA and an enhancement of their inhibitory effect [3].

The results presented here suggest that the thermic inactivation of the components of the respiratory chain proceeds in two different ways:

1. A direct protein denaturation, which attacks preferably the succinate dehydrogenase (table 4);
2. An indirect denaturation of one or several com-

ponents of the iron-sulphur region of the NADH-UQ reductase segment mediated by endogenous FFA.

The latter effect is more pronounced, both with ETP here and with intact cells in [1]. The FFA may cause conformational change of the proteins with which it interacts and thus increase their tendency to denaturation [3]. It should be emphasised that the inactivation of cell respiration mediated by endogenous FFA appears at a temperature close to the physiological range. Therefore the effect may be of biological importance under some pathological conditions, such as fever or with increased FFA levels (e.g., in hypoxia).

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