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Microcalorimetric study on mitochondrial metabolism inhibited by toxicant

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Abstract

The metabolic thermograms and heat output of mitochondria isolated from musculus liver tissue have been determined by using an LKB 2277 bioactivity monitor; the recovery rate constant k_1 (of the activity recovery phase) and the decline rate constant k_2 (of the activity decline phase) have been calculated. The metabolism activity of mitochondria inhibited by toxicant (humic acid) has been determined. The experimental results indicate that mitochondria influence the heat output and lag time of metabolism in an isolating medium containing toxicant.

INTRODUCTION

Mitochondria are important organelle involved in ATP production, and even after isolation they remain capable of active metabolism with release of much heat. They are often referred to as the "power house" of the cell. ATP may be produced (by the oxidation of a variety of elementary substrates) within the mitochondria present in virtually all plant and animal cells, so the study of mitochondria is very important.

Some toxicant damage to organisms is also involved in the structural damage and metabolic change of mitochondria. Some research has indicated that humic acid can extensively damage the liver; the main damage-causing cell organelle are mitochondria, which become cavitas (marrow-like) structures [1].

In this paper, the thermograms and heat output of the metabolism of mitochondria isolated from young rat liver tissue was studied using an LKB 2277 bioactivity monitor. The thermograms of mitochondria were obtained

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by various isolating methods. The first sample was isolated in the normal isolating medium (A); the second sample was isolated in a toxicant-containing (humic acid) isolating medium (B). The experimental results indicate that there were some differences in the thermograms: the metabolic heat output is greater than normal for the isolating medium containing humic acid; in contrast, the lag plase time is less than for the normal isolating medium. These facts, combined with electron microscopic observations, are significant for toxicant physiology.

INSTRUMENT AND MATERIALS

Instrument

An LKB 2277 bioactivity monitor was used; the performance of this and the details of its construction have been described previously [2, 3].

Materials

The young rats (musculus) were provided by the animal farm of the Department of Biology, Wuhan University.

The humic acid was produced by Shanghai Second Chemical Reagent Factory, Shanghai, China.

Isolating medium A was sucrose 0.25 M; EDTA 1 mM, Tris-HCl 10 mM; pH = 7.5.

Isolating medium B was sucrose 0.25 M; EDTA 1 mM, Tris-HCl 10 mM; humic acid 0.1 wt.%; pH = 7.5.

EXPERIMENTAL METHODS

Isolation of mitochondria

Mitochondria were isolated by first removing the liver from the young rat and washing it with sterile isolating medium (one part was treated with isolating medium A; the other part was treated with isolating medium B). The liver was then weighed (2.0 g for each one), homogenised and centrifuged at 900 *g* for 10 min; the clear supernatant was centrifuged twice for 20 min at 900 *g* each time, the sediment being discarded after each step. The clear liquid was then centrifuged twice at high speed (12 000 *g*) 10 min each time, to deposit the mitochondria as sediment. This was resuspended in the isolating medium for measurements. All the above operations were performed aseptically at 273–227 K.

Experimental determination

The metabolism thermograms of mitochondria were recorded using sealed ampoules, one containing a reference solution such as the isolating medium, the other containing the sample (suspension of mitochondria). The sample normally occupied position A in the monitor and the reference occupied position B. Each ampoule contained a 1 ml sample (contained mitochondria of 0.5 g liver) or reference and 2 ml of air. Sample A (treated with isolating medium A) was examined in the first channel and sample B (treated with isolating medium B) in the second channel.

The temperature of all experiments was 30.00°C and the amplifiers of the monitor were set at 300 μW.

RESULTS AND DISCUSSION

The thermogenesis curves for two samples are shown in Fig. 1. Curve a is the thermogram of mitochondria metabolism of sample A; curve b is the thermogram of sample B.

As previously reported [3, 4], analysis of the thermograms reveals four regions: lag phase; activity recovery phase; stationary phase; decline phase. The activity recovery rate constant (k_1) and decline rate constants (k_2) have been calculated from the equations $P_t = P_0 e^{k_1 t}$ (activity recovery phase) and $P_t = P_0 e^{-k_2 t}$ (activity decline phase). The results are shown in Table 1. The total heat output and the maximum heat power were calculated and are shown in Table 2.

From these data we can see that the amount of heat (ΔH) released, the maximum heat output and the activity recovery rate constant of sample B are greater than those for sample A. Contrarily, the lag phase time of the former is less. At first appearance the metabolic activity of mitochondria is increased in the isolating medium containing toxicant (humic acid). However, essentially, the reason for this is that the toxicant (humic acid) damaged the structure of mitochondria and influenced their metabolism:

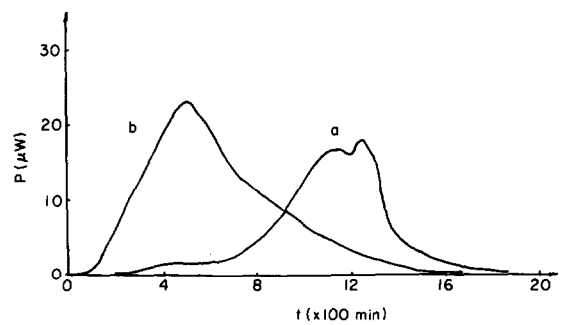

Fig. 1. Thermograms of mitochondrial metabolism at 30°C: curve a, sample A; curve b, sample B.

TABLE 1

Lag time, heat production, liver weight and maximum thermal power of mitochondrial metabolism

Sample	Lag time t (min)	Heat ΔH (J)	Liver weight W (g)	$\Delta H/W$ ($J\,W^{-1}$)	Max. power (μW)
A	700	0.54	0.50	1.08	18.0
B	100	0.79	0.50	1.58	23.0

TABLE 2

Values of mitochondrial recovery rate constant k_1 and decline rate constant k_2

Sample	k_1 (min^{-1})	k_2 (min^{-1})
A	0.00547	−0.00619
B	0.00707	−0.00310

some researchers have indicated that humic acid can inhibit ATP synthesis in mitochondria [5], so the energy metabolism of nutrients was for the most part released with the heat output ΔH. This result is significant for studies of the relationship between some local diseases (e.g. Kashin–Beck disease and Keshan disease) and toxicant (humic acid).

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