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MICROCALORIMETRIC STUDY OF MITOCHONDRIAL METABOLISM

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ABSTRACT

The thermograms and heat output of the metabolism of mitochondria isolated from fish liver tissue have been determined using an LKB-2277 bioactivity monitor. The thermograms are similar to those obtained from bacteria, and can be divided into four parts: the lag phase, activity recovery phase, stationary phase and decline phase. In the activity recovery and decline phases the thermogensis curves are logarithmic and can be described by 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), where k_1 and k_2 are designated the recovery rate constant and decline rate constant, respectively. The values of these constants have been calculated.

INTRODUCTION

Mitochondria are important cell organs involved in ATP production and even after isolation remain capable of active metabolism with release of much heat. They are often referred to as the "power-house" of the cell. Since ATP may be produced (by the oxidation of a variety of elementary substrates) within the mitochondria present in virtually all plant and animal cells, the study of mitochondria is of great importance. Mitochondria contain large amounts of enzymes, and enzymes which catalyse oxidation cellular reactions are the specific loci of the oxidation. These enzymes still are active after the isolation of mitochondria and there remains large amounts of nutrients in the mitochondria, hence enzymic reactions can occur and release much heat. The heat effects of the metabolic process can be monitored using sufficiently sensitive calorimeters and their thermograms can be obtained.

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In the present work, the energy metabolism of mitochondria isolated from fish liver tissue was studied using an LKB 2277 bioactivity monitor. The thermograms of mitochondria from three kinds of fish were recorded in two groups of experiments.

INSTRUMENT AND MATERIALS

Instrument

An LKB 2277 bioactivity monitor, the performance of which, and the details of its construction, have been described previously [1,2], was used.

Materials

The fish used in the experiments are mirror carp from Germany; hybrid fish from the nucleus of common carp and the cytoplasm of crucian carp, and hybrid progeny from these two, with the mirror carp as male parent. All fish were provided by the Aquatic Product Institute of Changjing River.

EXPERIMENTAL METHODS

Isolation of mitochondria

Mitochondria were isolated by first removing the liver from the fish and washing it with sterile isolating medium (sucrose, 0.25 M; EDTA, 1 mM; Tris–HCl 10 mM; pH = 7.5). The liver was then weighed, homogenised and centrifuged at 900 G for 10 min. The clear supernatant was centrifuged three times, for 20 min at 900 g each time, with the sediment 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 done aseptically at 273–277 K.

Experimental determination

The metabolic thermograms of the mitochondria were recorded using sealed ampoules, one containing a reference solution such as distilled water, and the other the sample (suspension of mitochondria). The sample normally occupied position A in the monitor and the reference occupies position B. Each ampoule contained 1 ml sample or reference and 2 ml of air. Samples from both parent fish were examined in the first channel and samples from the hybrid progeny in the second channel.

The temperature of all experiments was 298.00 K and the amplifiers of the monitor were set at 300 μW.

RESULTS AND DISCUSSION

The thermogenesis curves for two groups of thermograms are shown in Figs. 1 and 2.

These results indicate that thermogensis curves for various kinds of mitochondrial metabolism can be determined. The thermograms are similar to those obtained from bacteria. Similarities between bacteria and mitochondria have long been recognised.

Analysis of the thermograms reveals four regions: the lag phase, activity recovery phase, stationary phase and decline phase. Sometimes the stationary phase is not very obvious, without any clear boundary. During isolation of the mitochondria, the enzymatic system is damaged (and is in a resting condition at low temperature) so time is necessary for activity to be recovered and for adaptation to the new conditions of the isolating medium. During this period, the lag phase, the thermogenesis curve is a horizontal straight line. Once adequate adaptation has taken place, enzymatic activity will gradually recover and both substance metabolism and energy metabolism will start, producing a logarithmic curve, i.e. the activity recovery phase. When all enzymes have become active, a stationary phase without much change in the curve is reached. When nutrients and oxygen are exhausted the curve enters the logarithmic decline phase. Feedback inhibition of enzymes by metabolites also contributes to this decline.

The heat output and average heat power in each phase were determined, and are shown in Tables 1–4.

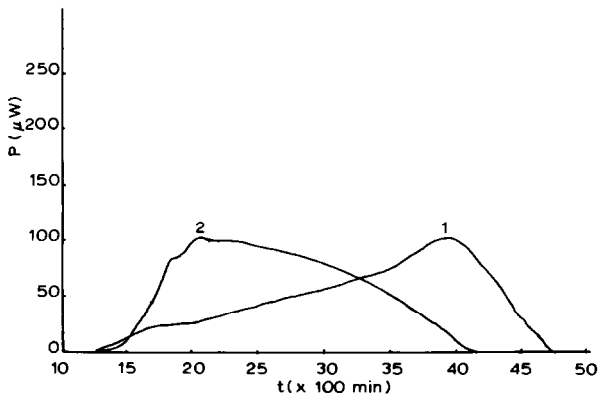

Fig. 1. Thermograms of mitochondrial metabolism of the female parent (curve 1) and the hybrid progeny (curve 2). Mitochondria isolated from 3.3 g liver.

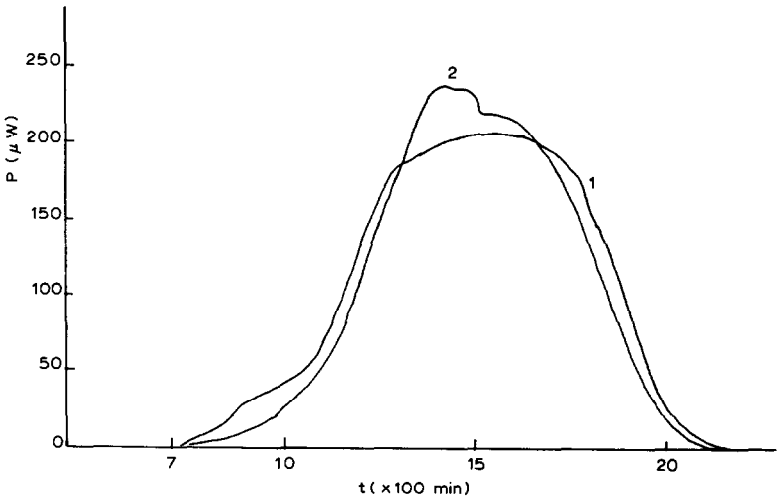

Fig. 2. Thermograms of mitochondrial metabolism of the male parent (curve 1) and the hybrid progeny (curve 2). Mitochondria isolated from 3.7 g liver.

TABLE 1

The amount of heat (ΔH) released and average thermal power ($\bar{P}$) in each phase of mitochondrial metabolism for the hybrid progeny in experiment 1

	Phase				Total
	Lag	Recovery	Stationary	Decline	
t (min)	1100	600	800	1600	4100
$\bar{P}$ (μW)	0	7.83	89.78	61.36	58.23
ΔH (J)	0	0.2821	4.3094	5.8905	10.4820

TABLE 2

The amount of heat (ΔH) released and average thermal power ($\bar{P}$) in each phase of mitochondrial metabolism for the female parent in experiment 1

	Phase				Total
	Lag	Recovery	Stationary	Decline	
t (min)	1300	2600	100	800	4850
$\bar{P}$ (μW)	0	41.05	68.33	57.54	45.60
ΔH (J)	0	6.404	0.410	2.726	9.576

The weight of the liver sample used determines the mitochondrial concentration and thus has an effect on the thermograms, which are records of aerobic respiration. The mono-substrate enzymatic reaction is

$$S + E \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P$$

TABLE 3

The amount of heat (ΔH) released and average thermal power ($\bar{P}$) in each phase of mitochondrial metabolism for the hybrid progeny in experiment 2

	Phase				Total
	Lag	Recovery	Stationary	Decline	
t (min)	750	800	200	600	2350
$\bar{P}$ (μW)	0	39.25	207.37	125.96	92.78
ΔH (J)	0	1.8842	2.4884	4.5347	8.9073

TABLE 4

The amount of heat (ΔH) released and average thermal power ($\bar{P}$) in each phase of mitochondrial metabolism for the male parent in experiment 2

	Phase				Total
	Lag	Recovery	Stationary	Decline	
t (min)	700	600	200	800	2500
$\bar{P}$ (μW)	0	25.00	169.92	76.71	82.96
ΔH (J)	0	1.200	4.070	3.682	8.9600

where S is the substrate, E is the enzyme and P denotes products. The reaction rate, V, of the enzyme reaction is given by

$$V = -\mathrm{d}[\mathrm{S}]/\mathrm{d}t = V_s/(1 + K_s/[\mathrm{S}])$$

where $V_s = k_2\ [\mathrm{E}]_0$ ($[\mathrm{E}]_0$ is the concentration of the enzyme), $K_s = (k_{-1} + k_2)/k_1$ [S] and is the concentration of the substrate.

The rate, V, may be expressed in terms of the thermal power, Q, as $V = \mathrm{d}Q/\mathrm{d}t$ from which $Q = \int V\,\mathrm{d}t$.

The greater the concentration of mitochondria, and thus of substrate and enzyme, the greater is V, and more heat is released per unit time. So in experiment 2 with the larger liver sample, the heat-release rate is greater than in experiment 1 because of the greater number of mitochondria. However, the amount of oxygen is limited to that in 2 ml of air, and once

TABLE 5

Variation of metabolic heat production (ΔH) with liver weight (W) in experiment 1 (Fig. 1) and experiment 2 (Fig. 2)

		ΔH (J)	W (g)	$\Delta H/W$ (J g^{-1})	ΔH per ml O_2 [a]
Experiment 1	Curve 1	9.5760	3.3	2.90	22.80
	Curve 2	10.482	3.3	3.15	24.96
Experiment 2	Curve 1	8.9602	3.7	2.42	21.33
	Curve 2	8.9073	3.7	2.40	21.20

[a] Volume of oxygen 0.42 ml in all cases.

TABLE 6

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

		k_1 (s^{-1})	k_2 (s^{-1})
Experiment 1	Curve 1	1.0991×10^{-5}	7.220×10^{-5}
	Curve 2	1.1455×10^{-4}	2.182×10^{-5}
Experiment 2	Curve 1	7.8878×10^{-5}	1.476×10^{-4}
	Curve 2	1.6188×10^{-4}	1.404×10^{-4}

this oxygen is used up the respiration and enzymatic reactions must stop. Also metabolic products can inhibit the enzymatic reactions and the mitochondria's activity, so the system enters the decline phase. Table 5 shows the variation of metabolic heat generated for liver samples of different weight.

We believe that mutual interactions between mitochondria accelerate their adaptation to new conditions, resulting in a more rapid recovery of activity at high mitochondrial concentrations. So in experiment 2 where mitochondrial concentration was higher, the lag phase lasted about 700 min, compared with over 1000 min in experiment 1.

In experiment 2 each phase of the thermogram is clearly resolved. In experiment 1, the thermogram for the hybrid progeny has no obvious boundary between stationary and decline phases, probably because an adequate oxygen supply slowed down the rate of decline. For the female parent, the recovery phase is very long, during which time most of the oxygen was consumed. This shortened the stationary phase because insufficient oxygen is now present to maintain vigorous metabolism for very long.

The thermograms, which are plots of power, P_t, against time, t, are logarithmic in the recovery and decline phases, being described by

$P_t = P_0 \, e^{k_1 t}$ (the activity recovery phase)

and

$P_t = P_0 \, e^{-k_2 t}$ (the activity decline phase)

where k_1 and k_2 are referred to as recovery rate and decline rate constants, respectively. The values of k_1 and k_2 for both experiments are shown in Table 6.

REFERENCES

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