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MICROCALORIMETRIC INVESTIGATION OF THE EFFECT OF La^{3+} ON MITOCHONDRIA ISOLATED FROM AVIAN CHICKEN LIVER TISSUE CELLS

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Abstract

A microcalorimetric technique was used to evaluate the influence of La^{3+} on mitochondria isolated from the liver tissue of Avian chicken. By means of LKB-2277 bioactivity monitor, ampoule method at 37°C, we obtained the thermogenic curves of the metabolism of mitochondria. After isolation from the chicken liver tissue, mitochondria still have metabolic activity and can live for a long time depending on the stored nutrients. In order to analyze the results, the maximum power (P_m) and the decline rate constants (k_d) were obtained. The addition of La^{3+} results in an increase of the maximum heat production and decline rate constants. Furthermore, values of P_m and k_d are linked to the concentration of La^{3+}. According to the thermogenic curves under different conditions, it is clear that metabolic mechanism of mitochondria has been changed with the addition of La^{3+}.

Keywords: La^{3+}, microcalorimetry, mitochondria, thermochemistry, thermogenic curves

Introduction

Mitochondria play an important role in vital activity. Because mitochondria are involved in ATP production which provided energy for organism, they are often viewed as 'power station' of cell. ATP may be produced (by the oxidation of variety of elementary substrates) within the mitochondria present in virtually all plant and animal cells. Therefore, study on mitochondria is very significant and interesting.

It is well known that there are rich rare-earth element resources in China. Because trace rare-earth can promote the growth of plant [1], they are added into fertilizer which is used in agricultural production widely. As a result, rare-earth almost reach every corner of Chinese mainland. The rare-earth elements were characterized with their physiological functions and biological effects on some organisms [2]. After they get into living body, however, what effect they have on organism and how they take part in metabolism remain unknown. In recent years, growing concern has been

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expressed about rare-earth element, because they are of potential threat to human health and possible danger to environment. In the long run, rare-earth may be a new kind of pollution [3].

In this paper, the thermogenic curves of the metabolism of mitochondria isolated from chicken Avian chicken liver tissue and the effect of lanthanum (La) on it were studied using LKB-2277 bioactivity monitor. Obviously, there are differences among their metabolic thermogenic curves and kinetics. Comparing these metabolic thermogenic curves with each other, we obtained a lot of interesting results, which will made a contribution to understanding the process of metabolism. All the experimental results are very important and significant to the study of metabolism of mitochondria and the biological effect of rare-earth element.

Materials and methods

Materials

The Animal Room, College of Life Science, Wuhan University, Wuhan 430072, P.R. China, provided chicken Avian.

Isolating medium was: sucrose 0.25 mol dm^{-3}; EDTA $1 \cdot 10^{-3}$ mol dm^{-3}; Tris-HCl $1 \cdot 10^{-2}$ mol dm^{-3}; pH=7.4.

The La_2O_3 was roasted, and massed 1 g. Then changed it into $LaCl_3$ with HCl, and made it into 1 L solution with sterile deionized water. So, the concentration of La^{3+} is $3.07 \cdot 10^{-3}$ mol dm^{-3}.

Isolation of mitochondria

Mitochondria were isolated by first removing the liver tissue from the chicken and washing with sterilized isolating medium. The liver was then weighted 2 g, cut into small pieces, homogenized 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 (12000 g) 20 min each time, to deposit the mitochondria as sediment. This was resuspended in the isolating medium 1 mL for calorimetric measurements. All the above operations were performed aseptically at 273–277 K.

Equipment

The LKB 2277 bioactivity monitor, which is a type of heat conduction microcalorimeter, was used to determine the metabolism of cells. It is designed to monitor continuously a wide variety of processes and complex systems over the temperature range 20–80°C. A schematic representation of the calorimetric system is shown in Fig. 1. Each measuring cylinder normally contains a sample and a reference in separate measuring cups (twin system). The heat output from the sample flows from the thermoelectric detector to the large heat sink (in close contact with the water bath). In response, the detector produces a voltage which is proportional to the power output

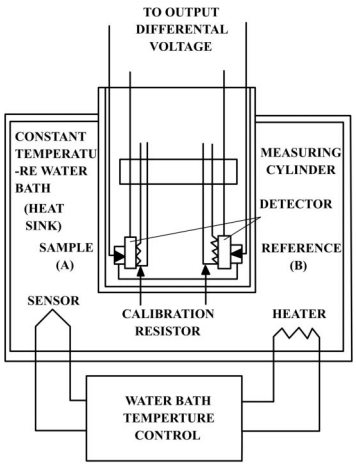

Fig. 1 Simplified operation diagram

from the sample. In order to minimize the systematic error and disturbance effect, a differential or twin detector system is used. This system is very sensitive, the detection limit is 0.15 μW, and the baseline stability (over a period of 24 h) is 0.2 μW. There are three operating modes for the LKB 2277 bioactivity monitor: ampoule mode, flow-through mode and flow-mix mode.

In the monitoring system, two precision resistors for electrical calibration are built into each measuring cylinder and one for each detector. When a known current is passed through the appropriate resistor, the detector can be calibrated easily. The performance of this instrument and the details of its construction have been previously described [4–6].

Experimental determination

The metabolic thermogenic curves of mitochondria were recorded using ampoule method. One sealed ampoule contained a reference solution such as the isolating medium, the other ampoule contained the sample (suspension of mitochondria). The sample normally occupied position A in the monitor and reference occupied B. Each ampoule contained a 1 mL sample (contained mitochondria of 2.0 g liver) or reference and 2 mL of air.

The temperature of all calorimetric experiments was 37.00°C, the amplifier of the monitor were set at 300 μW.

Results

Thermogenic curves

The metabolic thermogenic curve of chicken Avian liver mitochondria is shown in Fig. 2.

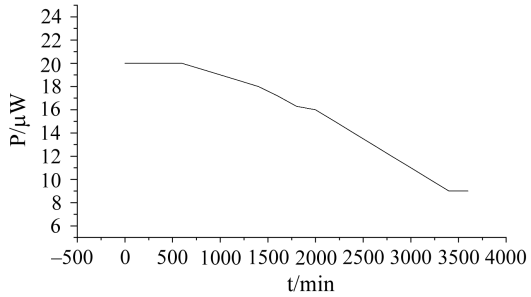

Fig. 2 The metabolic thermogenic curve of Avian liver mitochondria. P_m is the maximum heat production rate

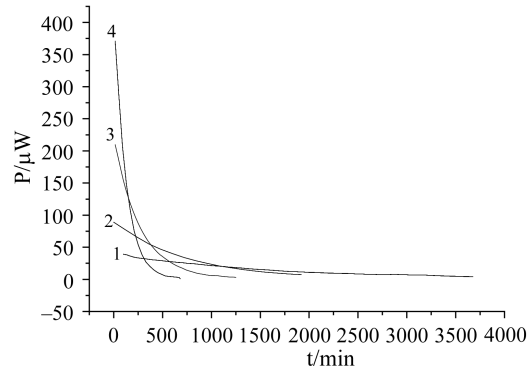

Fig. 3 The metabolic thermogenic curves of Avian liver mitochondria effected by La^{3+}

When the mitochondria were isolated, we added different amounts of La^{3+} solution into the suspension of mitochondria, and then determined the metabolic thermogenic curve respectively. The metabolic thermogenic curves are shown in Fig. 3.

Thermokinetics

In accordance with Fig. 2, at the beginning, heat output of mitochondria keep constant. After this section, heat output of mitochondria decline almost linearly. In the decline part, we can obtain that:

$$P_t=23.5213–0.004111t \quad R=–0.99278 \qquad (t=800–3400 \text{ min}) \tag{1}$$

So, we can obtain the thermokinetic equation

$$\mathrm{d}P/\mathrm{d}t=–k_dP^0 \tag{2}$$

and the order of metabolism is 0.

From Fig. 3, obviously, the heat power declined exponentially. we can calculate the decline rate constant of metabolism, k_d, from the equation:

$$P_t=P_0 \exp(–k_dt) \tag{3}$$

$$\ln P_t = \ln P_0 - k_d t \quad (4)$$

According to Eq. (4), we can obtain values of k_d and the correlation coefficient R when different amount of La^{3+} was added.

From Eq. (4), we can obtain that the thermokinetic equation is

$$dP/dt = -k_d P^1 \quad (5)$$

and the order of the metabolism is 1.

Relationship between P_m and the concentration of La^{3+}

From the thermogenic curves in Figs 2 and 3, we can see that the effect of La^{3+} on mitochondria metabolism was concentration-dependent. With the addition of La^{3+}, the maximum heat production power, P_m, increased, the dose *vs*. P_m relationship is very linear (as shown in Fig. 4b). We can obtain the P_m *vs*. C (8.53–68.21 μg mL^{-1}) equation:

$$P_m = -1.03519 + 5.57342C, \text{ and } R = 0.99562 \quad (6)$$

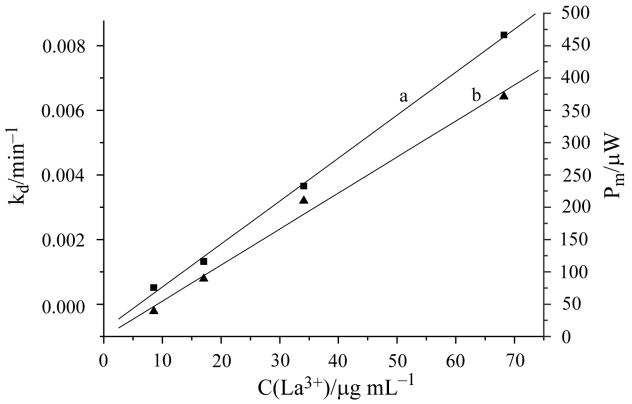

Fig. 4 a – Relationship between k_d and the concentration of La^{3+}; b – Relationship between P_m and the concentration of La^{3+}

Relationship between k_d and the concentration of La^{3+}

All powertime curves changed with an increase in the mass of La^{3+}, resulting in an increase of k_d. Values of k_d are correlated to the concentration of La^{3+}, C (8.53–68.21 μg mL^{-1}), as

$$k_d = -7.2569 \cdot 10^{-4} + 1.31609 \cdot 10^{-4} C, \text{ and } R = 0.99841 \quad (7)$$

The linear relationship was shown in Fig. 4a.

Relationship between P_m and decline rate constants k_d

The addition of La^{3+} caused an increase of the maximum heat production rate, P_m , and the decline rate constants, k_d. In the range of concentration of La^{3+} (8.53–68.21 μg mL^{-1}), values of P_m are correlated to the decline rate constants, k_d, as

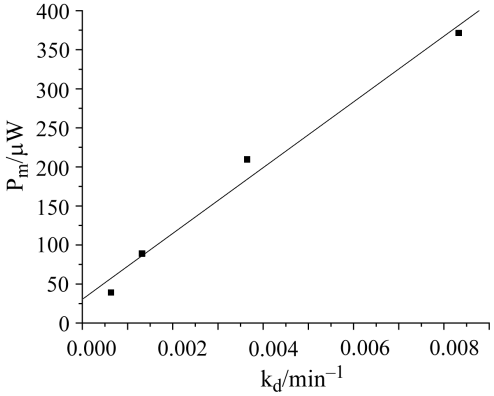

Fig. 5 Relationship between P_m and decline rate constants k_d

$$P_m=30.53506+4.2108\cdot10^4\, k_d\,, \text{ and } R=0.99154 \quad (8)$$

It is a linear relationship, which was shown in Fig. 5.

Discussion

Comparing Fig. 2 with Fig. 3, we can find that addition of La^{3+} has changed the mechanism of metabolism completely. Without ion of La^{3+}, the heat output of mitochondria decline linearly, however, the heat output decline exponentially in the presence of La^{3+}. The similar result can be drawn from Eqs (2) and (5). Without La^{3+}, the metabolic thermokinetic equation is $dP/dt = -k_dP^0$. When La^{3+} was added, metabolic thermokinetic equation is $dP/dt = -k_dP^1$. The order of metabolism has been changed from 0 to 1.

From the thermogenic curves in Figs 2 and 3, we can see that when La^{3+} was added, the heat power was larger than that without La^{3+} at the beginning. What's more, the higher the concentration of La^{3+} was, the larger the maximum heat power was. Judith [7] reported that low concentration of La^{3+} had promoter action on enzyme activity. Since mitochondria produce most of the cell's energy, investigation of the influences of lanthanide on the function of mitochondria is considered to be very important to understand the role of lanthanide in the living body [8].

Ion of rare-earths has influence on organelle and microorganism, which result from complexation of rare-earths and biological macromolecule. It is reported that coordination compound of rare-earths with amino acids can be easily formed [9]. For instance, ion of rare-earths have inhibitive effect on resilience of muscular and bone. The reason is that ion of rare-earths take place of Ca^{2+} in the tissue of muscular and bone [10].

Nowadays, people pay more and more attention to environmental problems, because toxic chemicals are contaminating the land, air, and water. Some of them can lead to discomforts directly, but others cause possible life-threatening ailments that may not manifest themselves for years [11], such as rare earths. It is remarkable that pollution can result in increasing of rare earth elements in environment [12]. Our study may reveal some useful information as to applicability of rare-earth in agriculture and industry.

In our experiments, the isolating medium was used to supply the osmotic pressure, without added nutrients that can be used by mitochondria. Consequently, we can come to conclusion that the metabolism of mitochondria is a kind of endogenous metabolism. But for different kinds of mitochondria, or under different conditions, there will be different kinds of thermogenic curves and their thermokinetic equation will be different. Accordingly, their metabolic mechanism are also different.

Microcalorimetry is a non-destructive and non-invasive technique. Therefore, it is valuable for monitoring a variety of processes, such as metabolism of microorganism. Due to the high sensitivity of the monitor and the fact that the whole metabolism of samples may be examined automatically and continuously, microcalorimetric method may reveal more and newer details about the metabolism than the existing methods do [13]. The microcalorimetric method requires only an observable difference between the power production in the treated and controlled incubations. Unlike many other procedures, transparent solution is not required. Colored or turbid solutions, even suspensions can be put into the calorimeter [14]. That approximates more closely the vivo state than many other techniques do. We think that microcalorimetric method can be used in many areas of biological sciences. Through the technique, we can study the kinetics and thermodynamics of biological sciences further, and all of these are very significant to understanding biological processes.

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