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MICROCALORIMETRY STUDY ON THE EFFECT OF Nd(III) ION ON METABOLISM OF MITOCHONDRIA ISOLATED FROM FISH LIVER TISSUE

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Abstract

Metabolic thermogenic curves of mitochondria isolated from fish liver tissue and the effect of Nd(III) on it were determined by LKB-2277 Bioactivity Monitor, ampoule method, at 28.00°C. From these thermogenic curves, rate constant of the activity recovery phase (k_1), rate constant of the stationary increase phase (k_2), rate constant of the decline phase (k_3), the maximum heat production rate (P_m) are obtained. These results show that Nd^{3+} has changed the metabolism of mitochondria completely.

Keywords: metabolism, microcalorimetry, mitochondria, rare earth

Introduction

Lanthanoid ions (Ln^{3+}) are trace metal ions. Their similarity to Ca^{2+} with respect to ionic radii, coordination chemistry and preference for the oxygen donor groups provides the basis for their strong interaction with Ca^{2+} binding sites on biomembranes, proteins, nucleic acids, carbohydrates and cells. The bioactivity of Ln^{3+} has been investigated for plants [1], nerve tissue [2], enzyme [3], cells [4, 5], protein [6] and biomembrane [7]. However, influence of Ln^{3+} on the metabolism has not been sufficiently investigated. Since mitochondria produce most of the cell's energy, investigation of the influences of lanthanides on the function of mitochondria is considered to be very important to understand the role of lanthanides in the body.

Microcalorimetry can directly determine the 'biological activity' of a living system and provide a continuous measurement of the heat production, thereby is much useful in measuring the effects of various substances and culture conditions on metabolism in both qualitative and quantitative way [8]. Microcalorimetry has a great

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advantage over many conventional bioassay procedures. First, it is a completely non-specific method which is often a valuable property when the method is used for monitoring the complex and poorly characterized biological systems. Secondly, the experimental record (thermogenic curve) reveals not only thermal data but also kinetic data.

The purpose of this paper is to investigate the bioactivity of the Nd^{3+} acted on mitochondria. In this study, the thermogenic curves of the metabolism of mitochondria isolated from fish liver tissue and the effect of Nd^{3+} on it were determined by using LKB 2277 Bioactivity Monitor. The experiments showed that the metabolic activity of mitochondria was significantly promoted by Nd^{3+} added.

Experimental

Materials

Carassius auratus gibelio was supplied by the College of Life Science, Wuhan University.

Isolating medium was: 0.25 mol L^{-1} sucrose , 1 mmol L^{-1} EDTA, 10 mmol L^{-1} Tris with pH buffered to 7.4 using HCl. The medium was sterilized at 120°C for 30 min.

$Nd(NO_3)_3$ was obtained as oxides (at least 99.9% purity) and dissolved in deionized water with small amounts of HNO_3.

Chemicals used throughout were analyzed grade without further purification.

Equipment

A microcalorimeter, LKB 2277 Bioactivity Monitor was used to obtain the thermogenic curves of the mitochondria. The microcalorimeter was thermostated at 28.00°C. The signal was recorded by means of an LKB-2210 recorder (1000 mV range). More details about the performance and construction of the instrument can be found in [9, 10]

Isolation of mitochondria

Mitochondria were isolated by first removing the liver from the Crucian and washed with sterile isolating medium. The liver was then weighed, homogenized and centrifuged at 4000 rpm for 15 min. The clear supernatant was centrifuged again for 15 min at 4000 rpm. The sediment was discarded after each step. The clear liquid was then centrifuged twice at high speed (10 000 rpm) for 15 min each time to deposit the mitochondria as sediment. This was resuspended in the isolating medium to give a protein concentration of about 2.8 mg mL^{-1}. Protein was determined by the biuret method [11]. All the above operations were performed aseptically at 273–277 K.

The isolated particles we obtained can exhibit characteristic green color of mitochondria under phase contrast microscope after being stained with Janus green. This indicate the mitochondria still had metabolic activity.

Experimental determination

The thermogenic curves of mitochondria were recorded using the ampoule method. One sealed ampoule contained a reference solution such as the isolating medium, the other ampoule contained the sample (suspension of mitochondria). Each ampoule contained a 1 mL sample or reference and 2 mL of air.

$Nd(NO_3)_3$ was added to the sample at the beginning of the experiment. The final concentration of $Nd(NO_3)_3$ is 0, 20, 40, 120, 200 μg mL^{-1} in different experiments. The temperature of all calorimetric experiments was 28.00°C, the amplifier of the monitor was set at 100 or 300 μW.

After being determined by microcalorimetry, the samples containing mitochondria were inoculated into two tubes of trypticase soy broth and two tubes of nutrient broth with yeast extract separately. Incubated these two broths as follows: one tube at 37°C and other at 26°C under aerobic conditions. The examining results with an inverted microscope periodically for 21 days indicated no contamination.

Results

Thermogenesis curves

The thermogenic curves for mitochondria isolated from crucian liver tissue with $Nd(NO_3)_3$ at concentrations of 0, 20, 40, 120, 200 μg mL^{-1} are shown in Fig. 1.

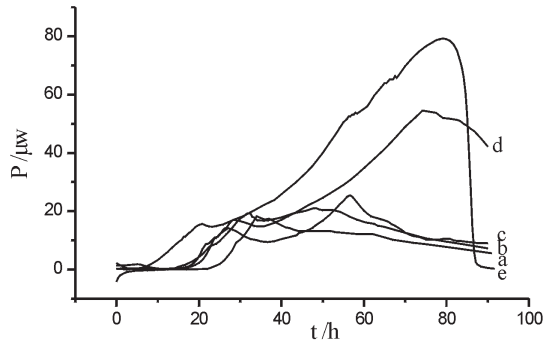

Fig. 1 Thermogenic curves of mitochondria metabolism with different concentration of $Nd(NO_3)_3$; a – control, b – 20 μg mL^{-1}, c – 40 μg mL^{-1}, d – 120 μg mL^{-1}, e – 200 μg mL^{-1}

Thermokinetics

Analysis of the thermogenic curves in Fig. 1 reveals four phases: lag phase, activity recover phase, stationary increase phase, and decline phase, except for the curve of the control, the stationary increase phase of which is not very obvious and without any clear boundary.

In the activity recover phase and the decline phase, the power-time curves obey the exponential equation:

$$P_t=P_0e^{kt} \quad (1)$$

According to this equation, the activity recovery rate constants (k_1) and the decline rate constants (k_2) of all experiments were calculated. Corresponding k_1 and k_2 are shown in Table 1.

Table 1 Rate constants of *Carassius auratus gibelio* liver mitochondria at 28.00°C

$C_{Nd(NO_3)_3}$/μg mL^{-1}	$k_1/10^{-5}$ s^{-1}	R_1	$k_2/10^{-5}$ s^{-1}	R_2
0	4.44	0.989	–0.458	0.995
20	11.2	0.995	–0.771	0.993
40	13.2	0.991	–1.32	0.993
120	3.32	0.992	–0.816	0.990
200	2.98	0.990	–44.2	0.992

k_1 – rate constants of activity recover phase, k_2 – rate constants of decline phase, R_1, R_2 – related coefficients for k_1 and k_2, respectively

The shapes of the thermogenic curves obtained at the stationary increase phase appear to be '*S*'. To describe this type of curves, we used the 'logistic equation':

$$dP_t/dt=kP_t(1–SP_t) \quad (2)$$

k – rate constants of stationary increase phase or decline phase, P_t – heat production rate at time t, S – factor of limitation.

The integral form of Eq. (2) is:

$$\ln[P_t/(1–SP_t)]=\ln[P_0/(1–SP_0)]+kt \ (t=0, P=P_0) \quad (3)$$

P_0 – heat production rate when t equals 0.

As $[P_t/(1–SP_t)]$ should greater than 0, so $0\leq S\leq 1/P_{max}$ is essential.

The rate constants of stationary increase phase (k_3) of all experiments were calculated by Eq. (3). The values are shown in Table 2.

Table 2 Values of metabolism parameters of *Carassius auratus gibelio* liver mitochondria at 28.00°C

$C_{Nd(NO_3)_3}$/μg mL^{-1}	$k_3/10^{-5}$ s^{-1}	$S/10^{-2}$ μW^{-1}	R	P_m/μW
0	–	–	–	18.25
20	1.25	0.0211	0.991	21.10
40	1.51	0.000	0.991	25.35
120	1.32	0.00980	0.991	54.50
200	1.23	0.00625	0.993	79.25

k_3 – rate constants of stationary increase phase, S – factor of limitation, R – related coefficients, P_m – maximum heat production rate

Relationship between maximum heat production rate (P_m) and the concentration of Nd^{3+}

The addition of $Nd(NO_3)_3$ caused an increase of the maximum heat production rate (P_m). And the dose-maximum heat production rate relationship is linear for $Nd(NO_3)_3$

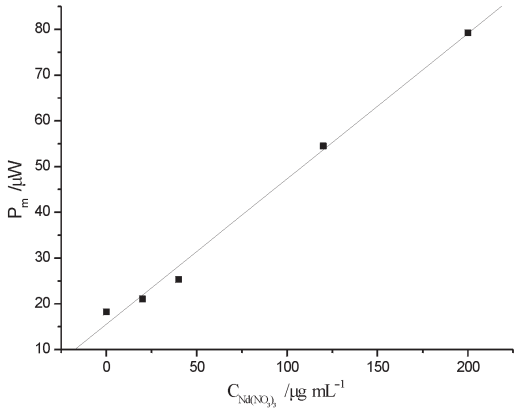

Fig. 2 Plot of P_m for the growth *vs. c* for $Nd(NO_3)_3$

over the concentration range of 0~200 μg mL^{-1}, as shown in Fig. 2.

From Table 2 and Fig. 2, we can obtain P_m~c equations as follows:

$$P_m=15.54+0.3177c, \text{ and } R=0.9969 \quad (4)$$

Relationship between rate constants (k_1, k_2, k_3) and the concentration

The effect on the rate constants was concentration-dependent but the dose-rate constant relationship is not linear over the concentration range of 0~200 μg mL^{-1}. Both the rate constant of activity recovery phase (k_1) and rate constant of stationary increase phase (k_2) reach maximum value with the concentration of $Nd(NO_3)_3$ increasing from 0 to 40 μg mL^{-1}. Then the values of k_1 and k_2 decreased when the concentration of $Nd(NO_3)_3$ continued to increase.

Discussion

This experiment revealed the action of $Nd(NO_3)_3$ on the mitochondria isolated from *Carassius auratus gibelio* liver tissue. As we can see from the thermogenic curves, the shapes of the thermogenic curves with different concentrations of Nd^{3+} are very similar. They all have a distinct stationary increase phase, and two apparent peaks of each curve. As to the thermogenesis curve of the control, there is only one apparent peak, and the boundary between the stationary increase phase and decline phase is not clear. The changes of rate constants of different phases and the maximum heat pro-

duction rate outputs of each experiment indicate that Nd^{3+} has changed the metabolic mechanism of mitochondria greatly.

Reports about the bioeffect of rare earth elements show that at low concentrations, they can increase growth rates of plant, or has no obvious effect on the proliferation of cells, while under certain high concentrations, growth rates are lower than that of control [1, 4]. The rate constants of activity recovery phase and stationary increase phase we obtained were consistent with these reports. The rate constant of activity recovery phase and stationary increase phase increased while the concentration of $Nd(NO_3)_3$ increased over the range of 0~40 μg mL^{-1} and decreased while the concentration of $Nd(NO_3)_3$ continued to increase over the range of 40~200 μg mL^{-1}. The bioeffects of rare earth elements on organism may be caused mainly by changes of the metabolism mechanism of mitochondria, for it is the power-houses of cells which are the structural and functional unit of organism.

It seems strange that the relation between the maximum heat production rate and the concentration of Nd^{3+} is linear. We believe the metabolism mechanism of isolated mitochondria in vitro include two processes. It is well known that mitochondria have some of their own DNA, ribosomes, and can make many of their own proteins, some of which are enzymes involved in both substance metabolism and energy metabolism. After isolation there remains large amounts of nutrients in mitochondria that can be used to synthesize proteins. One exothermic process, we think, is synthesis proteins or enzymes necessary for oxidative phosphorylation. The other exothermic process is oxidative phosphorylation. In the activity recovery phase, the first process is predominant. And in the stationary increase phase, the second process contributes more. Kohn and Tatsuo have studied the effect of rare earth elements on enzyme and proteins. Their results showed that rare earth elements can increase the stability of protein [6] or the complex between enzyme and substrate [3]. We believe Nd^{3+} can lead to the result that the catalytic activity of enzyme in mitochondria maintains longer. Though the synthesis rate of enzymes is a little slower under certain high concentration of rare element, the length of time that the enzymes maintain its activity is longer with the concentration of Nd^{3+} increases. So the neat effect is total catalytic activity of enzymes involve in metabolism increases, so the maximum heat production rate is linear with the concentration of Nd^{3+}.

These results demonstrate that microcalorimetry is a good method for the studies of the kinetics of the metabolism of mitochondria under the actions of various substances. Currently used biological reaction microcalorimetry systems have reached the resolution of 0.2 μW mL^{-1}. With such a high sensitivity and the fact that the samples can be examined automatically and continuously, microcalorimetric method may reveal more and even new details about the metabolism than the existing methods do [12]. Unlike many other methods, the microcalorimetric method does not require a transparent solution or any pretreatment (for example, adding additional reagents), all it requires is only an observable difference between the heat production rate of the sample and the reference. Thus coloured, turbid solutions or even suspensions can be determined by microcalorimetry [13]. With these advantages, microcalorimetry is believed to be of wide applicability in the biological science, especially

in the fields of comparative physiology, ecology, microbiology, agronomy, medicine and pharmacology.

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