Effect of Ce(III) on Heat Production of Mitochondria Isolated from Hybrid Rice

**Jie Dai · Jing-Jing Liu · Gui-Qing Zhou ·
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Abstract The effect of cerium on mitochondria isolated from hybrid rice Shanyou 63 (*Oryza sativa* L) was investigated. Through in vivo culture, low dose Ce^{3+} promoted, but higher dose Ce^{3+}, restrained mitochondrial heat production. However, through vitro incubation, Ce^{3+} showed only inhibitory action on mitochondrial energy turnover, the concentration required for 50% inhibition being 46.7 μM. In addition, Ce^{3+}, like Ca^{2+}, induced rice mitochondrial swelling and decreased membrane potential ($\triangle \psi$), which was inhibited by the specific permeability transition inhibitor cyclosporine A (CsA). The induction approached a constant level while mitochondrial metabolism was fully prevented by Ce^{3+}. These results demonstrated that cerium influenced rice mitochondria in vivo and in vitro via different action pathways, and the latter involved the opening of rice mitochondrial permeability.

Keyword Mitochondria · Metabolism · Ce^{3+} · Mechanism · Permeability transition

Introduction

Mitochondria play a central role in energy metabolism within the cell. Isolated mitochondria still perform some metabolic processes, such as tricarboxylic acid oxidation and fatty acid β-oxidation in the presence of oxygen [1]. If the heat production of isolated mitochondria is monitored by calorimetry, much useful information, both qualitative and quantitative, may be obtained [2–6].

Early in 1940s, it was found that lanthanides can be used to facilitate plant growth, especially in enhancement of plant root and germination, increment of chlorophyll

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content and reinforcement of photosynthesis and nutrients absorption. Many studies demonstrated that because of their similarity to calcium regarding ionic radii, coordination chemistry, and preference for oxygen donor groups; lanthanides exerted similar biological and physiological effects on organisms, in particular enhancement of plant growth [7, 8]. Mitochondria play a crucial role in respiration and metabolism [9]; however, to date, little is known about the mechanism by which lanthanides act at the mitochondria level in plant cells. In addition, lanthanides have been shown to promote apoptosis in mammal cells by inducing mitochondrial permeability [10, 11]. For these reasons, we first determined the effects of Ce^{3+} on the heat production of hybrid rice Shanyou 63 mitochondria both in vivo and in vitro, and then we examined whether Ce^{3+} induced rice mitochondrial permeability in vitro. The selected hybrid rice (Shanyou 63) has been widely cultured in China because of its high yield, good grain quality, resistance to bacterial leaf blight, and wide adaptability.

Experiment

Materials

Hybrid rice Shanyou 63 was supplied by College of Life Sciences, Wuhan University, and $CeCl_3$ (A.R.) was purchased from Shanghai Reagent Co. Ltd. and dissolved in deionized water. CsA, rotenone, rhodamine 123 (Rh 123), Na_2ADP, and Na pyruvate were purchased from Sigma.

Plant Culture

Rice Shanyou 63 was grown in incubators without sunlight. Firstly, rice seeds were sterilized by H_2O_2 (10%) for 30 min and washed with deionized water. Then, they were germinated in deionized water overnight and transferred into plastic trays containing deionized water or designed concentration $CeCl_3$ solution. The water or $CeCl_3$ solution was changed twice daily, and the temperature was 25–28°C during growth. Once the etiolated seedlings grew to 6–7 cm long, they would be cut as experimental materials.

Isolation of Mitochondria

The etiolated seedlings were rinsed in cold sterilized isolation medium A consisting of 400 mM sucrose, 50 mM Tris, 1 mM EDTA, 5 mM KCl, 0.1% (w/v) BSA, pH 7.4, and minced, homogenized, and centrifuged at 900×g for 10 min. The supernatant was centrifuged at 2,000×g for 8 min and then 10,000×g for 15 min in a new tube. The pellets were suspended in isolation buffer B consisting of 400 mM sucrose, 20 mM Tris, 1 mM HEPES, pH 7.4. The later gradient centrifugation was implemented as described by Luo et al. [12] with modification. Briefly, the supernatant was layered onto a previously poured, 12% (v/v) Percoll, 26% Percoll, 40% Percoll density gradient which consisted of 250 mM sucrose, 5 mM HEPES, and 0.1% BSA, pH 7.2. The suspension/gradient was centrifuged at 40,000×g for 40 min. The mitochondria were removed from the brownish band at 1.10 g/mL with a transfer pipette. Mitochondrial pellets were washed with buffer B by centrifuging for 10 min at 6,300×g. The purified mitochondrion were resuspended in buffer B to a given protein concentration. All the above operations were performed aseptically at 0–4°C. Mitochondria protein concentration was determined by biuret method.

Calorimetry Determination

The heat flux of mitochondria metabolism was determined with a 3114/3236 TAM air isothermal calorimeter (Thermometric AB, Sweden) using the ampoule method at 28°C. The sensitivity (precision) of this calorimeter is ±10 μW. Baselines were taken before each measurement, and the calorimeter was calibrated electrically. Details of the instrument can be found in Ref. [13]. One sealed ampoule contained isolation buffer; the other contained the sample (4.0 mg/mL mitochondria suspension plus or minus $CeCl_3$). Each ampoule had 1.0 mL sample or reference and ~25.0 mL of air, which provided basically sufficient oxygen for mitochondria metabolism.

Measurement of Mitochondrial Swelling and Membrane Potential ($\triangle \psi$)

The swelling of mitochondria was monitored as the decrease in the absorbance at 540 nm in studies with Ce^{3+} and Ca^{2+}, in a spectrophotometer, Shimadzu UV-3,000. Mitochondria (1.0 mg/mL) was suspended in 3 mL buffer (300 mM sucrose, 10 mM HEPES, 5 mM KH_2PO_4, pH 7.2). Pyruvate (8 mM) was used as the energizing substrate to induce swelling. The relative swelling rate was defined as: swelling rate = ΔA of sample/ΔA of control [10]. For membrane potential measurement experiments, mitochondria (1.0 mg/mL) was incubated at 25°C in buffer C containing 300 mM sucrose, 10 mM HEPES, 5 mM KH_2PO_4, 8 mM pyruvate, and 1 μg/mL rotenone. The $\triangle \psi$ was assessed spectrophotometrically (Hitachi F-2,500) by Rh 123 uptake with excitation at 505 nm and recording at 530 nm after addition of 1 μM Rh 123.

Depending on these experiments, mitochondria were pre-incubated with 1 μM CsA.

Results

Heat Rate from Mitochondrial Metabolism Isolated from Hybrid Rice Shanyou 63

The heat production rate from freshly isolated rice mitochondria is shown in Fig. 1. Pyruvate, the substrate of tricarboxylic acid cycle, accelerated the mitochondrial heat production and increased the maximum heat rate, suggesting that tricarboxylic acid cycle was promoted by the substrate. This station is defined as mitochondrial metabolism baseline [2]. Addition of phosphate acceptor ADP or uncoupling agent DNP to the mitochondria also accelerated their energy expenditure and greatly increased heat rate, which further demonstrated that respiration of isolated mitochondria was coupled with phosphorylation to carry out ADP/ATP translation.

Effect of Ce^{3+} on Heat Production of Rice Mitochondria In Vivo and In Vitro

Lanthanides are known to facilitate plant growth; our preliminary experiment for rice culture with $CeCl_3$ solution agreed with that. $CeCl_3$ solution ranging from 1.4 to 14.0 mg/L remarkably increased biomass of the rice-etiolated seedlings, but 21.0 mg/L $CeCl_3$ showed a negative effect (data not shown). To further elucidate action mechanism of Ce^{3+} on plant growth, the effect of $CeCl_3$ on heat production of rice mitochondria (mitochondria suspension plus 20 mM pyruvate) in vivo was determined (Fig. 2a). Application of $CeCl_3$ did not induce significant change in mitochondrial maximum heat rate, but the heat production was accelerated by 1.4–14.0 mg/L $CeCl_3$ with the curtailment of mitochondrial
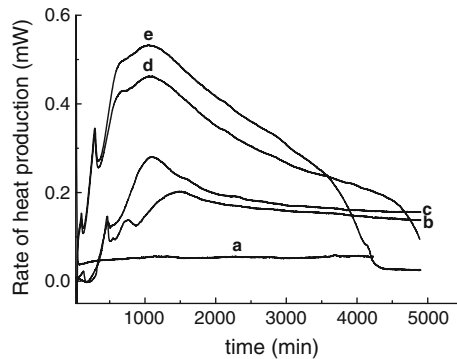
Fig. 1 The heat production rate of freshly prepared mitochondria from rice was promoted by mitochondrial substrate pyruvate, phosphate acceptor ADP, or an uncoupling agent DNP. The heat production of 1.0 mL buffer B (*a*) and mitochondria (4.0 mg protein/mL) (*b*) were measured as the control, and 20 mM pyruvate (*c*), 2 mM ADP (*d*), or 50 μM DNP (*e*) were respectively added to mitochondria to test mitochondrial metabolic activity. The results were typical of three independent experiments

activity recovery phase and restrained by 21.0 mg/L $CeCl_3$ with the delay of activity recovery phase. This result, combined with our preliminary experiment, indicated that the beneficial effect of Ce^{3+} on rice growth may be due to its stimulating action on mitochondrial metabolism via vivo pathway.

In contrast, application of $CeCl_3$ to rice mitochondria in vitro lead to inhibitory effect on mitochondrial heat production (Fig. 2b, c). The plot (c) elucidated linear dose-dependent inhibition of $CeCl_3$ on the maximum heat rate of rice mitochondria. The correlation coefficients of plot (c) reached 0.9986, and IC50 (inhibition concentration for 50% of maximum heat rate of rice mitochondria) of Ce^{3+} was 46.7 μM.

Effects of Ce^{3+} on Mitochondrial Swelling and Membrane Potential ($\triangle\psi$)

Liu et al. reported that lanthanide, like Ca^{2+}, induced mitochondrial permeability transition in mammal cells, which triggered the apoptotic procedure [10]. So, we investigated the effects of Ce^{3+} and Ca^{2+} on rice mitochondrial swelling (Fig. 3) and membrane potential ($\triangle\psi$) (Fig. 4). Ce^{3+} (50.0 μM) and 50.0 μM Ca^{2+} induced rice mitochondrial swelling (Fig. 3a). The same concentrations of Ce^{3+} and Ca^{2+} induced $\triangle\psi$ loss. The induction of Ce^{3+} on $\triangle\psi$ loss was blocked by 1 μM CsA completely (Fig. 4). These results indicated that Ce^{3+} increased mitochondrial permeability.

Mitochondrial swelling and membrane potential $\triangle\psi$ loss caused by Ce^{3+} increased with increase in Ce^{3+} from 5.0 to 50.0 μM, but it tended to level when Ce^{3+} exceeded 50.0 μM. The fluorescent intensity of mitochondria treated with 50.0 μM Ce^{3+} approached Rh 123 intensity of isolated medium (Fig. 4), indicating that mitochondrial membrane permeability in this state was nearly whole open. So, further increase in concentration of the cation did not induce any change in mitochondrial permeability.

Discussion

We investigated the effects of cerium, a beneficial element to plants, on rice mitochondrial heat production in vivo and in vitro. The in vivo results demonstrated that Ce^{3+} at low dose

Fig. 2 Effect of Ce³⁺ on metabolism activity of rice mitochondria in vivo (a) and in vitro (b). In vivo treatment, the rice seeds were cultivated with CeCl₃ solution, and mitochondrial heat production of etiolated seedlings was measured as described in the "Experiment" section. The concentrations 0, 1.4, 7.0, 14.0, and 21.0 mg/L were corresponding to the curves signed with a, b, c, d, e, respectively. In vitro treatment (b), 0, 3.6, 14.4, 28.8, 57.6 μM CeCl₃ were added to mitochondria suspension, and the calorimetric results were shown in curves a, b, c, d, and e. All results were respectively examples of three independent experiments. The plot (c) further elucidated the concentration-dependent inhibition of CeCl₃ on the maximum heat rate of rice mitochondria in vitro treatment
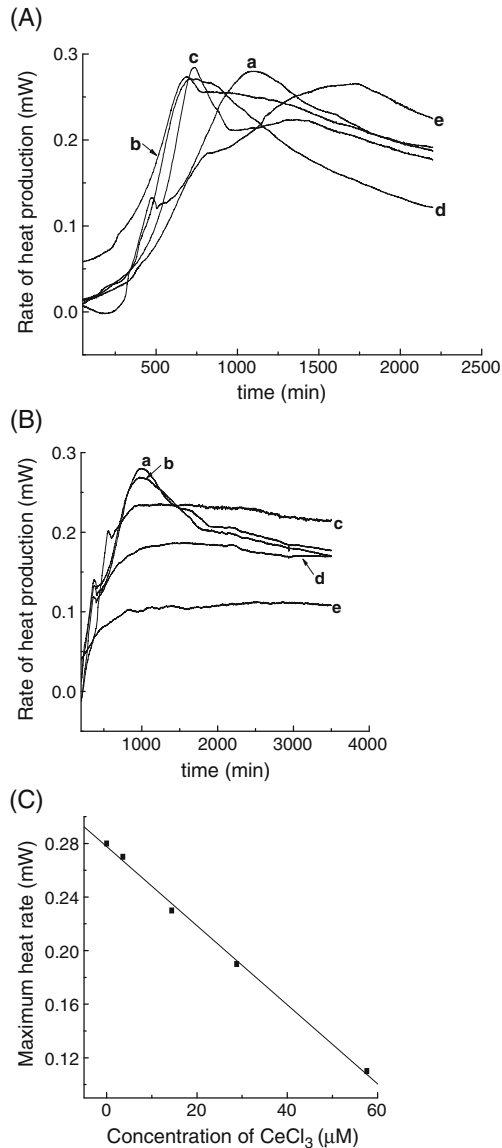
accelerated mitochondrial metabolism, but high dose restrained it. Many studies showed that rice grown in solution culture accumulated some concentrations of lanthanides in root and stem [14], and very low concentrations of lanthanides could pass through plant cell wall with the help of carriers such as protein, hormone, etc., and even enter into cell organelles via certain cation channels [15, 16]. Since lanthanides could replace Ca^{2+}, Cu^{2+}, or Mg^{2+} in enzymes to facilitate enzymatic activity [7, 17], the mitochondrial metabolism of rice cultured with proper concentration of lanthanides would be promoted. Considering the vital role of mitochondria in plant respiration and metabolism, seed germination and rice growth would be enhanced by lanthanides. The present study offered a new pathway to understand action mechanism of lanthanides on plant growth.
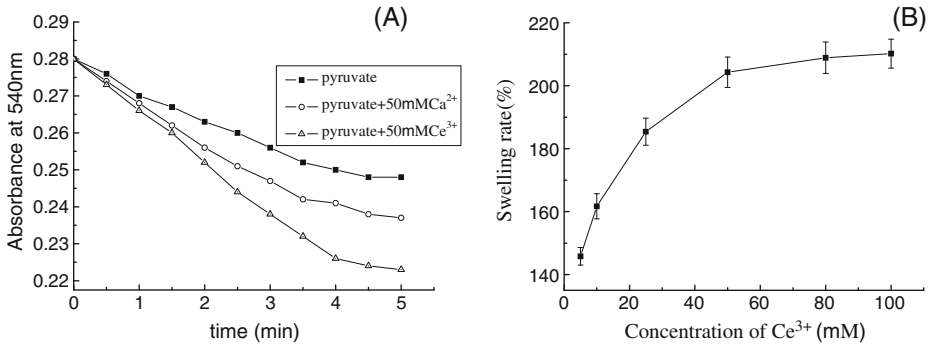
Fig. 3 Induction of rice mitochondrial (1.0 mg/mL) swelling by Ce^{3+} and Ca^{2+}. Change in the optical absorbance at 540 nm was recorded as described in the "Experiment" section. The time-dependent decrease of the absorption of mitochondria upon incubation with 50.0 μM Ce^{3+} and Ca^{2+} in the presence of 8 mM pyruvate was shown in **a**. The dose-dependent effect of Ce^{3+} on mitochondrial swelling rate was shown in **b**

In contrast, the in vitro incubation of Ce^{3+} resulted in progressive decrease in heat rate of rice mitochondria. As many transition metals including lanthanides, like Ca^{2+}, increased mitochondrial permeability and promoted apoptosis in mammal cells [10, 18–20], we further examined whether Ce^{3+} increased permeability of rice mitochondria. The results demonstrated that Ce^{3+} and Ca^{2+} did induce mitochondrial swelling and decreased mitochondrial potential ($\triangle\psi$), and the induction ability of Ce^{3+} was stronger than that of Ca^{2+} (Figs. 3 and 4). The mechanism by which excess Ca^{2+} induced mitochondrial permeability was much less clear. The conventional hypothesis was that calcium overload leads to the generation of reactive oxygen species (ROS), and calcium overload resulted from excess stimulation of NMDA receptors by glutamine [21]. Lanthanide ions, as analogy to calcium, were reported to produce ROS in mitochondria [10, 20], which may be the reason why Ce^{3+} induced mitochondrial permeability. As Ce^{3+} had similar ion radii to Ca^{2+}, its relevant high covalence may make it have greater induction ability than Ca^{2+}.
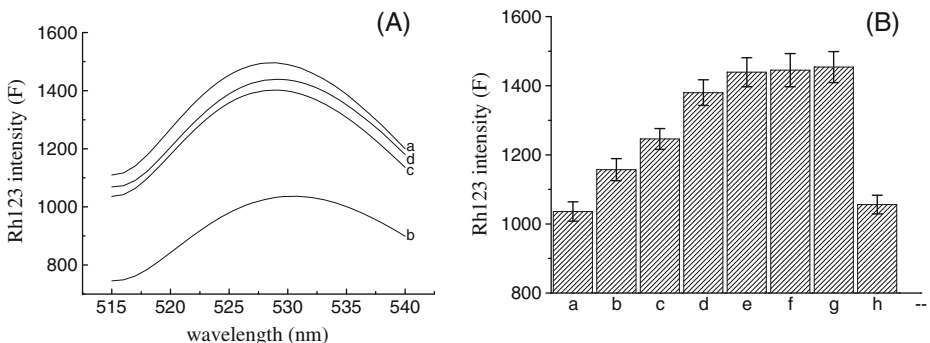
Fig. 4 Rice mitochondria (1.0 mg/mL) were incubated with Ce^{3+}, Ca^{2+}, or 1 μM cyclosporin A, and induction of $\triangle\psi$ was assessed by measuring the $\triangle\psi$-dependent uptake of Rh 123 as described in the "Experiment" section. The Rh 123 intensity of buffer C (*a*) and mitochondria without further treatment (*b*), treated with 50.0 μM Ca^{2+} (*c*) and treated with 50.0 μM Ce^{3+} (*d*) were shown in **a**. The dose-dependent effect of Ce^{3+} and CsA inhibition on $\triangle\psi$ loss were shown in **b**. The Rh 123 intensity of mitochondria (*a*), mitochondria treated with Ce^{3+} of 5.0 μM (*b*), 10.0 μM (*c*), 25.0 μM (*d*), 50.0 μM (*e*), 80.0 μM (*f*), 100.0 μM (*g*), and mitochondria treated with CsA before treatment with 50.0 μM Ce^{3+} (*h*) were shown

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