Available online at www.sciencedirect.com
ScienceDirect
Thermochimica Acta 470 (2008) 77–82
thermochimica acta
www.elsevier.com/locate/tca

Effect of La^{3+} on heat production by mitochondria isolated from hybrid rice

Jie Dai [a,b], Chuan-Ling Li [a], Ye-Zhong Zhang [a], Qi Xiao [b], Ke-Lin Lei [b], Yi Liu [a,b,*]

[a] *College of Chemistry and Environmental Engineering, Yangtze University, Jingzhou, Hubei 434025, China*
[b] *State Key Laboratory of Virology, College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China*

Received 16 November 2007; received in revised form 2 February 2008; accepted 7 February 2008
Available online 10 March 2008

Abstract

The effect of lanthanum on mitochondria isolated from hybrid rice Fengyou 559 (*Oryza sativa* L.) was investigated. Through *in vivo* culture, low-dose La^{3+} promoted, but higher dose La^{3+}, restrained mitochondrial heat production. However, through *in vitro* incubation, La^{3+} manifested only inhibitory action on mitochondrial energy turnover, the concentration required for 50% and 100% inhibition being 50.9 and 230.2 μM (57.6 nmol/mg protein), respectively. In addition, La^{3+}, like Ca^{2+}, induced rice mitochondrial swelling and decreased membrane potential ($\Delta\psi$), which was inhibited by the specific permeability transition inhibitor cyclosporine A (CsA). The induction approached a constant limitation while mitochondrial metabolism was completely prevented by La^{3+}, and microscopy observation showed a high disruption of inner mitochondrial membrane in this state. These results demonstrated that lanthanum influenced rice mitochondria *in vivo* and *in vitro* via different action pathways, and the latter involved the opening of rice mitochondrial permeability.

Keywords: Mitochondria; Metabolism; La^{3+}; Mechanism; Permeability transition

1. 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 [2–7].

Early in 1940s, it was found that lanthanides could be used to facilitate plant growth, especially in enhancement of plant root and germination, increment of chlorophyll 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 [8,9]. Mitochondria play a crucial role in respiration and metabolism [10], however, to date, little is known about the mechanism by which lanthanides act at the mitochondria level in plant cells. On the other hand, lanthanides have been shown to promote apoptosis in mammal cells by inducing mitochondrial permeability [11,12]. For these reasons, we first determined the effects of La^{3+} on the heat production of hybrid rice Fengyou 559 mitochondria both *in vivo* and *in vitro*, and then we examined whether La^{3+} induced rice mitochondrial permeability *in vitro*. The selected hybrid rice (Fengyou 559) has been widely cultured in China because of its high yield, good grain quality, resistance to bacterial leaf blight and wide adaptability.

Many studies showed that rice grown in solution culture accumulated some concentrations of lanthanum in root and stem [13,14], and very low concentrations of lanthanum 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 [9,15,16]. Since lanthanide ions could replace Ca^{2+}, Cu^{2+} or Mg^{2+} in enzymes to facilitate enzymatic activity

Abbreviations: CsA, cyclosporine A; ADP, adenosine 5′-diphosphate sodium salt; DNP, 2,4-dinitrophenol; Rh 123, Rhodamine 123; AFM, atomic force microscopy.

* Corresponding author at: Department of Chemistry, State Key Laboratory of Virology, College of Chemistry and Molecular Sciences, Wuhan University, Wuhan 430072, China. Tel.: +86 27 87218284/87218304; fax: +86 27 68754067.
E-mail address: prof.liuyi@263.net (Y. Liu).

0040-6031/$ – see front matter © 2008 Elsevier B.V. All rights reserved.
doi:10.1016/j.tca.2008.02.011

[17], the mitochondrial metabolism of rice cultured with proper concentration of lanthanide would be promoted. Considering the vital role of mitochondria in plant respiration and metabolism, seed germination and rice growth would be accelerated by lanthanide. The present study offered a new pathway to understand action mechanism of La^{3+} on plant growth.

2. Experimental

2.1. Materials

Hybrid rice Fengyou 559 was supplied by College of Life Sciences, Wuhan University, and $LaCl_3$ (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.

2.2. Plant culture

Rice Fengyou 559 was grown in incubators without sunlight. First 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 $LaCl_3$ solution. The water or $LaCl_3$ 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.

2.3. 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 2000 × *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. [18] with modification. Briefly, the supernatant was layered onto a previously poured, 12% (v/v) Percoll, 26% Percoll, 40% Percoll density gradient 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 6300 × *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.

2.4. 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. Baselines were taken before each measurement and the calorimeter was calibrated electrically. Details of the instrument can be found in Ref. [19]. One sealed ampoule contained isolation buffer; the other contained the sample (4.0 mg/mL mitochondria suspension plus or minus $LaCl_3$). Each ampoule had 1.0 mL sample or reference and ~25.0 mL of air, which provided basically sufficient oxygen for mitochondria metabolism.

2.5. Measurement of mitochondrial swelling and membrane potential ($\Delta\psi$)

The swelling of mitochondria was monitored as the decrease in the absorbance at 540 nm in studies with La^{3+} and Ca^{2+}, in a spectrophotometer, Shimadzu UV-3000. 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). 8 mM pyruvate was used as the energizing substrate to induce swelling. The relative swelling rate was defined as: Swelling rate = ΔA of sample/ΔA of control [12]. 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 $\Delta\psi$ was assessed spectrophotometrically (Hitachi F-2500) 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 preincubated with 1 μM CsA.

2.6. Atomic force microscopy (AFM)

Atomic force microscopy imaging was conducted with a Picoscan atomic force microscope (Molecular Imaging, Tempe, AZ, USA) as reported previously [20]. About 10 μL of mito-

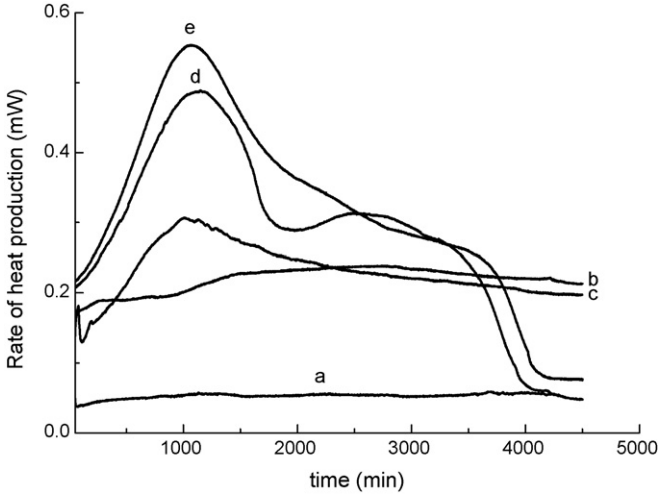

Fig. 1. The heat production rate of freshly prepared mitochondria from rice was promoted by mitochondrial substrate pyruvate, phosphate acceptor ADP or an uncoupling agent DNP. The heat production of 1.0 mL buffer B (a) and mitochondria (4.0 mg protein/mL) (b) were measured as the control, and 20 mM 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.

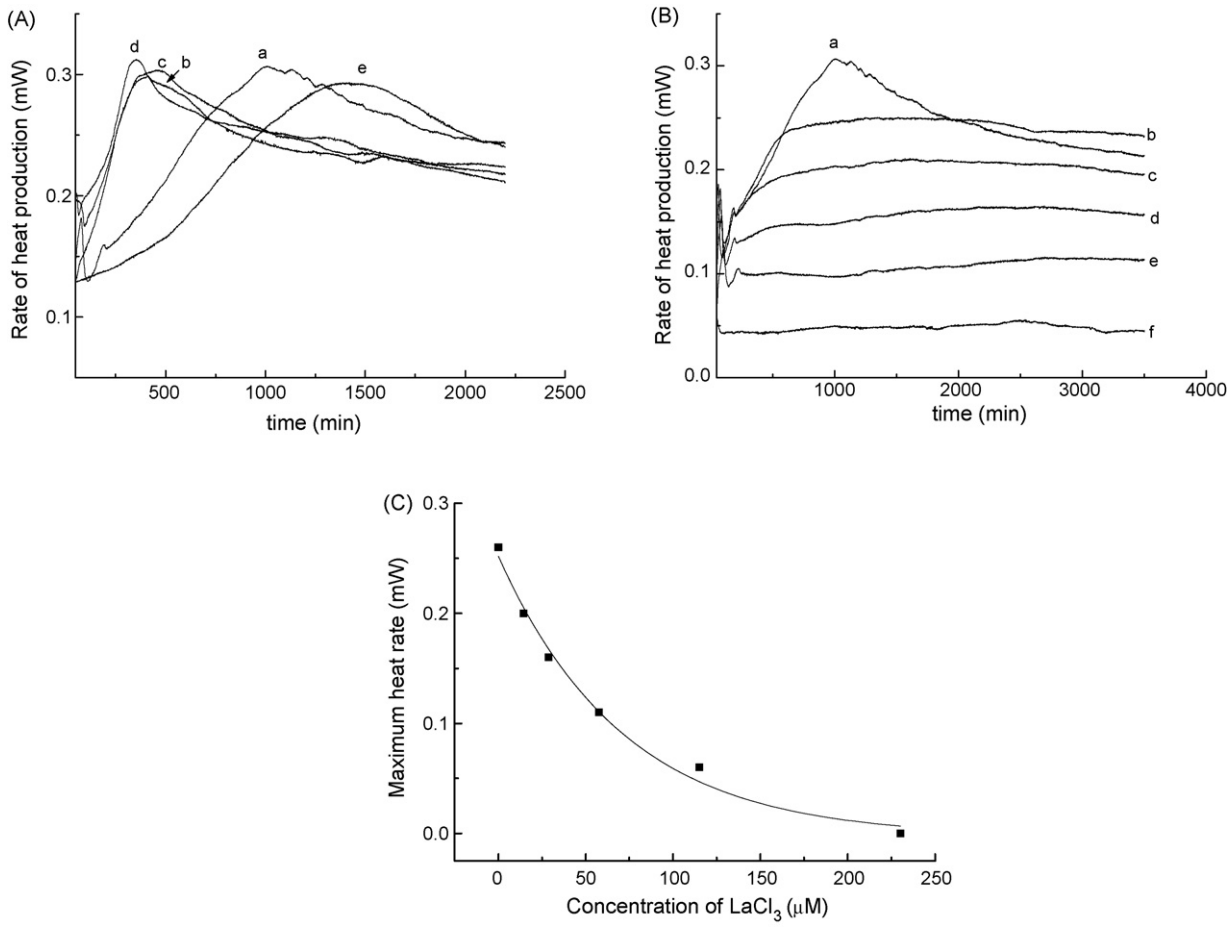

Fig. 2. Effect of La^{3+} on metabolism activity of rice mitochondria *in vivo* (A) and *in vitro* (B). *In vivo* treatment, the rice seeds were cultivated with $LaCl_3$ solution, and mitochondrial heat production of etiolated seedlings was measured as described in Section 2. The concentrations 0, 1.4, 7.0, 14.0, and 21.0 mg/L were corresponding to the curves signed with a, b, c, d, and e, respectively. *In vitro* treatment (B), 0, 14.4, 28.8, 57.6, 115.1, and 230.2 μM $LaCl_3$ were added to mitochondria suspension, and the microcalorimetric results were shown in curves a, b, c, d, e, and f. All results were respectively examples of three independent experiment. The plot (C) further elucidated the concentration-dependent inhibition of $LaCl_3$ on the maximum heat rate of rice mitochondria *in vitro* treatment.

chondria solution was dropped onto freshly cleaved ruby muscovite mica substrate and allowed incubation for 5 min. Then the mica surface was carefully rinsed with ultrapure water and gently blew dry with nitrogen. Freshly prepared samples were mounted on AFM stage and imaged under MAC mode in air using Type II MAC lever. Typical scan rate was 1 line/s. The images were registered at 256 × 256 pixels, unfiltered and flattened when needed.

3. Results

3.1. Heat rate from mitochondrial metabolism isolated from hybrid rice Fengyou 559

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 state is defined as mitochondrial 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.

3.2. Effect of La^{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 $LaCl_3$ solution agreed with that. $LaCl_3$ solution ranging from 1.4 to 14.0 mg/L remarkably increased biomass of the rice etiolated seedlings, but 21.0 mg/L $LaCl_3$ had a negative effect (data not shown). To further elucidate action mechanism of La^{3+} on plant growth, the effect of $LaCl_3$ on heat production of rice mitochondria (mitochondria suspension plus 20 mM pyruvate) *in vivo* was determined (Fig. 2(A)). Application of $LaCl_3$ did not induce significant change in mitochondrial maximum heat rate, but the heat rate was accelerated by 1.4–14.0 mg/L $LaCl_3$ and delayed by 21.0 mg/L $LaCl_3$. This result, combined with our preliminary experiment, indicated that the beneficial effect of La^{3+} on rice growth may be due to its stimulating action on mitochondrial metabolism via *vivo* pathway.

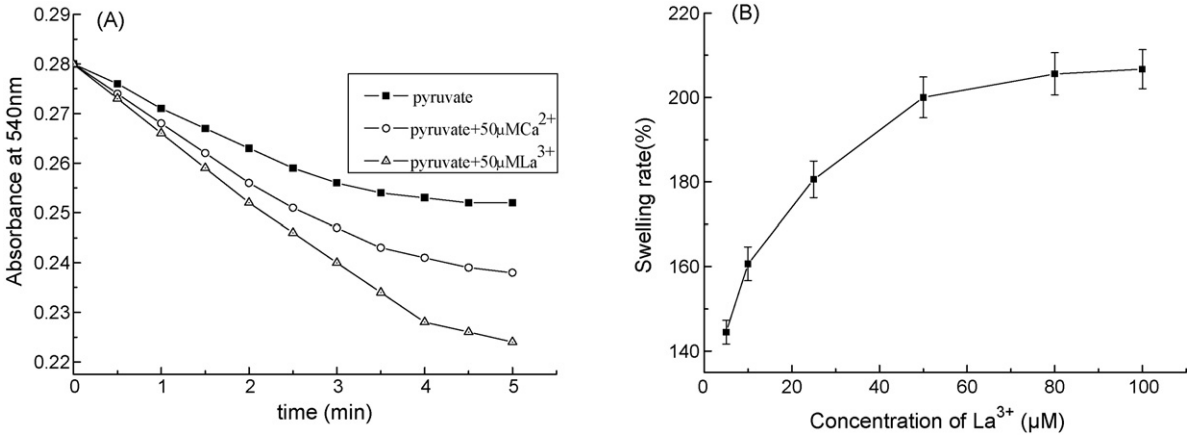

Fig. 3. Induction of rice mitochondrial (1.0 mg/mL) swelling by La^{3+} and Ca^{2+}. Change in the optical absorbance at 540 nm was recorded as described in Section 2. The time-dependent decrease of the absorption of mitochondria upon incubation with 50.0 μM La^{3+} and Ca^{2+} in the presence of 8 mM pyruvate was shown in (A). The dose-dependent effect of La^{3+} on mitochondrial swelling rate was shown in (B).

In contrast, addition of $LaCl_3$ to rice mitochondria *in vitro* lead to inhibitory effect on mitochondrial heat rate, the concentration required for 50% and 100% inhibition being 50.9 and 230.2 μM (57.6 nmol/mg protein) (Fig. 2(B) and (C)).

3.3. Effects of La^{3+} on mitochondrial swelling and membrane potential ($\Delta\psi$)

Liu et al. reported that La^{3+}, like Ca^{2+}, induced mitochondrial permeability transition in mammal cells, which triggered the apoptotic procedure [12]. So we investigated the effects of La^{3+} and Ca^{2+} on rice mitochondrial swelling (Fig. 3) and membrane potential ($\Delta\psi$) (Fig. 4). 50.0 μM La^{3+} and 50.0 μM Ca^{2+} induced rice mitochondrial swelling (Fig. 3(A)). The same concentrations of La^{3+} and Ca^{2+} induced $\Delta\psi$ loss. The induction of La^{3+} on $\Delta\psi$ loss was blocked by 1 μM CsA completely (Fig. 4). These results indicated that La^{3+} increased mitochondrial permeability.

Mitochondrial swelling and membrane potential $\Delta\psi$ loss caused by La^{3+} increased with increase in La^{3+} from 5.0 to 50.0 μM, but it tended to level when La^{3+} exceeded 50.0 μM (50.0 nmol/mg protein). The fluorescent intensity of mitochondria treated with 50.0 μM La^{3+} approached Rh 123 intensity of isolated medium (Fig. 4), indicating that mitochondrial membrane permeability in this state was completely open. To verify this assumption, the morphology change of mitochondria treated with 50.0 nmol/mg protein La^{3+} was observed by AFM.

3.4. Atomic force microscopy analysis of mitochondrial appearance

When permeability transition is induced by Ca^{2+}, the structure of the mitochondrial inner membrane is disrupted [21–23]. Fig. 5(A) shows two representative mitochondria visualized by AFM. Mitochondria appeared ellipsoidal with an average diameter of 400–600 nm (determined from ~20 mitochondria). In addition, AFM revealed mitochondria as unexpectedly flattened,

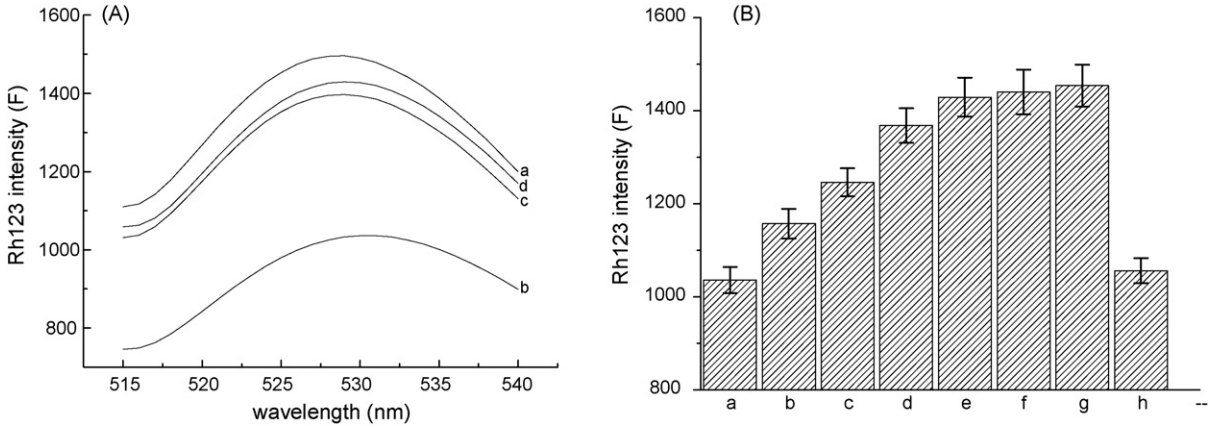

Fig. 4. Rice mitochondria (1.0 mg/mL) were incubated with La^{3+}, Ca^{2+} or 1 μM cyclosporine A, and induction of $\Delta\psi$ was assessed by measuring the $\Delta\psi$-dependent uptake of Rh 123 as described in Section 2. The Rh 123 intensity of buffer C (a) and mitochondria without further treatment (b), treated with 50.0 μM Ca^{2+} (c) and treated with 50.0 μM La^{3+} (d) were shown in (A). The dose-dependent effect of La^{3+} and CyA inhibition on $\Delta\psi$ loss were shown in (B). The Rh 123 intensity of mitochondria (a), mitochondria treated with La^{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 CyA before treatment with 50.0 μM La^{3+} (h) were shown.

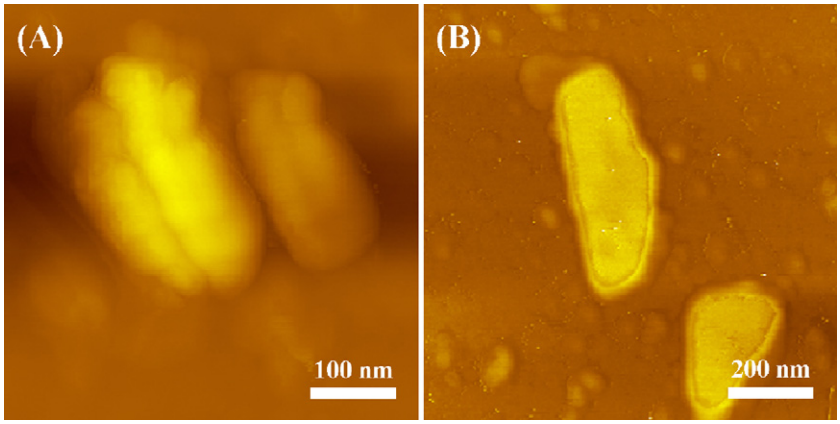

Fig. 5. AFM images of rice mitochondria were obtained under condition as described in Section 2. Mitochondria (4.0 mg/mL) without further treatment (A) and mitochondria pre-incubated with 200.0 μM $LaCl_3$ for 15 min (B) were analyzed. Of approximately 10 randomly selected sections for each condition, typical images are shown.

presumably caused by adsorption to the mica surface. On incubation with 200.0 μM (50.0 nmol/mg protein) $LaCl_3$ (Fig. 5(B)), mitochondria obviously became elongated and more flattened, moreover some intermembrane proteins were released from mitochondria matrix, suggesting a high disruption of inner mitochondrial membrane. The disruption was significantly inhibited by 1 μM CsA (data not shown), which indicated that La^{3+} increased mitochondrial permeability.

4. Discussion

We investigated the effects of lanthanum, a beneficial element to plants, on rice mitochondrial heat production *in vivo* and *in vitro*. The *in vivo* results demonstrated that La^{3+} at low dose accelerated mitochondrial metabolism, but high dose restrained it. In contrast, the *in vitro* incubation of La^{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 [12,24–26], we further examined whether La^{3+} increased permeability of rice mitochondria. The results showed that La^{3+} and Ca^{2+} did induce mitochondrial swelling and decreased mitochondrial potential ($\Delta\psi$), and the induction ability of La^{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 [27]. Lanthanide ions, as analogy to calcium, were reported to produce ROS in mitochondria [12], which may be the reason why La^{3+} induced mitochondrial permeability. As La^{3+} had similar ion radii to Ca^{2+}, its relevant high covalence may make it have greater induction ability than Ca^{2+}.

It was noteworthy that when La^{3+} exceeded 50.0 μM (50.0 nmol/mg protein), its action approached a constant (Figs. 3 and 4). Mitochondrial metabolism was completely inhibited by 57.6 nmol/mg protein La^{3+} (Fig. 2), and application of 50.0 nmol/mg protein La^{3+} resulted in a high disruption in inner mitochondrial membrane (Fig. 5). Mitochondrial function in this situation was completely damaged, further increase in concentration of the cation did not induce any change in mitochondrial permeability.

Acknowledgements

We gratefully acknowledge financial support of project supported by the National Natural Science Foundation of China (nos. 30570015, 20621502); Natural Science Foundation of Hubei Province (2005ABC002); Science Research Foundation of Chinese Ministry of Education (no. [2006]8IRT0543), the Doctoral Program from the State Education Ministry of China, and the Research Program of Hubei Provincial Department of Education, China (no. Q200812006). We also gratefully acknowledge Dr. Yi Lin gave us some kindly help on AFM experiments.

References

[1] D.K. Myers, E.C. Slater, Biochem. J. 67 (1957) 572–579.
[2] D. Köhnke, M. Schramm, J. Daut, Mol. Cell Biochem. 74 (1997) 101–113.
[3] J. Nedergaard, B. Canno, O. Lindberg, Nature 267 (1977) 518–520.
[4] X.Q. Wang, C.L. Xie, S.S. Qu, Thermochim. Acta 176 (1991) 69–74.
[5] Y. Liu, F.J. Deng, R.M. Zhao, X.S. Shen, C.X. Wang, Chemosphere 40 (2000) 851–854.
[6] P.J. Zhou, H.T. Zhou, Y. Liu, S.S. Qu, Y.G. Zhu, J. Therm. Anal. Calorim. 76 (2004) 1003–1013.
[7] J. Dai, Y. Liu, J.C. Zhu, Y.Z. Zhang, Chin. J. Chem. 24 (2006) 997–1000.
[8] Z. Hu, H. Richter, G. Sparovek, E. Schnug, J. Plant Nutr. 27 (2004) 183–220.
[9] X.Q. Shan, H.O. Wang, S.Z. Zhang, H.F. Zhou, Y. Zheng, H. Yu, B. Wen, Plant Sci. 165 (2003) 1343–1353.
[10] D.C. Logan, Biochim. Biophys. Acta: Mol. Cell Res. 1763 (2006) 430–441.
[11] J.K. Greisberg, J.M. Wolf, J. Wyman, L. Zou, R.M. Terek, J. Orthop. Res. 19 (2001) 797–801.
[12] H.X. Liu, L. Yuan, X.D. Yang, K. Wang, Chem. Biol. Interact. 146 (2003) 27–37.
[13] X.D. Cao, Y. Chen, Z.M. Gu, X.R. Wang, Int. J. Environ. Anal. Chem. 76 (2000) 295–309.

[14] B. Wen, D.A. Yuan, X.Q. Shan, F.L. Li, S.Z. Zhang, Chem. Spec. Bioavailab. 13 (2001) 39–48.
[15] Q.Q. Wang, Y. Lai, L.M. Yang, B.L. Huang, Anal. Sci. 17 (2001) 789–791.
[16] X.P. Wang, X.Q. Shan, S.Z. Zhang, B. Wen, Anal. Bioanal. Chem. 376 (2003) 913–917.
[17] G. Tyler, Plant Soil 267 (2004) 191–206.
[18] X. Luo, I. Budihardjo, H. Zou, C. Slaughter, X.D. Wang, Cell 94 (1998) 481–490.
[19] I. Wadsö, Thermochim. Acta 394 (2002) 305–311.
[20] Z.X. Lu, Z.L. Zhang, M.X. Zhang, H.Y. Xie, Z.Q. Tian, P. Chen, H. Huang, D.W. Pang, J. Phys. Chem. B 109 (2005) 22663–22666.
[21] D.W. Jung, P.C. Bradshaw, D.R. Pfeiffer, J. Biol. Chem. 272 (1997) 21104–21112.
[22] Y. Shinohara, S. Bandou, S. Kora, S. Kitamura, S. Inazumi, H. Terada, FEBS Lett. 428 (1998) 89–92.
[23] Y. Shinohara, M.R. Almofti, T. Yamamoto, T. Ishida, F. Kita, H. Kanzaki, M. Ohnishi, K. Yamashita, S. Shimizu, H. Terada, Eur. J. Biochem. 269 (2002) 5224–5230.
[24] D.J. McConkey, S. Orrenius, Biochem. Biophys. Res. Commun. 239 (1997) 357–366.
[25] K.B. Wallace, A.A. Starkov, Annu. Rev. Pharmacol. Toxicol. 40 (2000) 353–388.
[26] A. Szewczyk, L. Wojtczak, Pharmacol. Rev. 54 (2002) 101–127.
[27] D.G. Nicholls, L. Johnson-Cadwell, S. Vesce, M. Jekabsons, N. Yadava, J. Neurosci. Res. 85 (2007) 3206–3212.