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# Changes in lipid peroxidation, the redox system and ATPase activities in plasma membranes of rice seedling roots caused by lanthanum chloride

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**Abstract**

Highly purified plasma membranes were isolated by aqueous two-phase partitioning from rice (*Oryza sativa*) seedling roots. The effects of lanthanum chloride ($LaCl_3$) on the activities of lipid peroxidation, the redox system and $H^+$-ATPase, $Ca^{2+}$-ATPase of plasma membranes were studied. The lipid peroxidation of plasma membranes could be depressed by certain low concentrations of $LaCl_3$ and enhanced by high concentrations of $LaCl_3$, while the lipid peroxidation was also dependent on the plasma membrane protein and incubation time. The relative activity of $O_2$ uptake of plasma membranes was inhibited by all tested $LaCl_3$ concentrations. In contrast, the reduction rate of $Fe(CN)_6^{3-}$ by plasma membranes was stimulated below 40 $\mu$M of $LaCl_3$, but was reduced above 60 $\mu$M of $LaCl_3$. The relative activities of both $H^+$-ATPase and $Ca^{2+}$-ATPase increased constantly from control to $LaCl_3$ of concentration 60 $\mu$M where the activities of both enzymes were the maximum, but decreased remarkably at 80 $\mu$M $LaCl_3$ concentrations various $LaCl_3$ were added to culture solutions. In the other measurement case in which various $LaCl_3$ concentrations were added directly to reaction medium and the plasma membrane vesicles only came from the control cultured rice seedling roots, the response of $H^+$-ATPase activity to $La^{3+}$ was similar to the response in culture solution. However, the $La^{3+}$ concentration was only 20 $\mu$M when the activity of $H^+$-ATPase was the maximum. In contrast to the case of $LaCl_3$ addition to culture solution, $Ca^{2+}$-ATPase activity was inhibited by all concentrations of $La^{3+}$ which were added directly to the reaction medium. The above results revealed that REEs inhibited electron transfer from NADH to oxygen in plant plasma membranes, depressed the production of active oxygen radicals, and reduced the formation of lipid peroxides through plasma membrane lipid peroxidation. REEs ions also enhanced the $H^+$ extrusion by both standard redox system and $H^+$-ATPase in plasma membranes at certain concentrations. A possible role for the plant cell wall in REEs effects on plasma membranes was also suggested.

## Introduction

The rare earth elements (REEs) comprise a group of 17 trivalent metallic elements which have similar chemical properties. In the periodic table, these elements vary in relative atomic mass from 139 (Lanthanum) to 175 (Lutetium). Scandium (45) and Yttrium (89) are also commonly included in this group. Since the 1930s it has been known that the elements of this group have some physiological effects on organisms. Afterwards, there were lots of studies in the fields of agricultural and medical applications (Lao *et al.* 1995). Some results of Chinese research suggest that supplying these elements may have beneficial effects on plant growth, crop productivity formation and fruit or vegetable quality (Yu & Chen 1995). Unfortunately, their physiological mechanism of action is unknown.

Some researchers have demonstrated that REEs could not enter into the protoplast. These elements are

excluded by the plasma membrane (Gao *et al.* 1998). This phenomenon indicates that the plasma membrane might be the primary reaction site. Therefore, more attention should be paid to the relationship between REEs and plasma membrane.

The plasma membrane is involved in many physiological processes in plants, such as iron reduction, electron transfer and signal transduction. Recent research indicates that transmembrane electron transport may play an important role in plant cell growth (Misra 1991). During electron transfer, plant plasma membranes are capable of oxygen consumption with NAD(P)H as the electron donor (Moller & Berczi 1986). Meanwhile, some active oxygen radicals may be produced through the Fenton-reaction by the plasma membrane (Vianello *et al.* 1990). As a result, lipid peroxidation of membranes occurs, and finally leads to membrane damage (Osswald *et al.* 1992). It is attractive to examine the influence of REEs on the previously mentioned physiological processes for plant plasma membranes.

ATPase is considered the most important constitutional enzyme in the plasma membrane (Hodges & Leonard 1974; Serrano 1985). $H^+$-ATPase is usually named the 'master enzyme' in the plasma membrane for plants (Serrano 1989). Its main physiological role is to generate an electrochemical gradient of protons, acidify the cell wall and maintain the relaxed state of the cell wall. This allows for plant cell expansion and division. To date there have been no detailed reports on the effects of lanthanide on the activity of the plasma membrane $H^+$-ATPase. $Ca^{2+}$-ATPase is another very special type of ATPase in the plasma membrane. It can drive the transmembrane transportation of calcium. Due to the similarity (Evans 1990) between calcium and lanthanum in ionic radius and chemical properties, calcium could be substituted by lanthanum. Therefore, lanthanum may interfere with the activity of $Ca^{2+}$-ATPase. Such effects of lanthanum have been demonstrated by many reports. Regretfully, most of the evidence comes from studies using animal materials (Hinghsmith & Head 1983).

In the present study, the effects of lanthanum chloride on lipid peroxidation, redox system and $H^+$-ATPase activities for plasma membranes from rice seedling roots were studied. To our knowledge, this is the first report to analys the possible relation between REEs and plasma membrane lipid peroxidation, the redox system and $H^+$-ATPase activities for crop plants. The changes of $Ca^{2+}$-ATPase activity caused by lanthanum chloride are also investigated.

## Materials and methods

### *Plant materials*

Rice (*Oryza sativa* cv. Longtezao) seeds (purchased from Tonan Seeds Co., Xiamen, P.R. China) were sterilized by 0.5% sodium hyper-chlorite (NaOCl) solution for 20 min, washed with flow water, then soaked in water for 24 h and germinated in the dark at 25 °C for 24 h. The germinated seeds were transferred to quartz sand and irrigated with 0, 20, 40, 60, 80 $\mu$M $LaCl_3$ solution, and grown under illumination for 12 h every day with light intensity of 120 $\mu$mol photons $m^{-2}$ $s^{-1}$ for 7 days at 25 °C. The roots were harvested for the experiments.

### *Plasma membrane isolation and purification*

Plasma membranes were prepared by the two-phase partitioning method following the procedure of Sandelius & Morre (1990). Roots were washed by water and were cut into pieces, and then immediately homogenized in isolation buffer (1:3, w/v) containing 0.25 M sucrose, 3 mM EDTA, 2.5 mM DTT, 0.6% PVP, 2.5 mM PMSF and 25 mM Tris-Mes (pH 7.5). The homogenate was filtered through 240 $\mu$M nylon cloth and centrifuged at 10 000 $\times$ $g$ for 15 min. The supernatant was then centrifuged for 30 min at 50 000 $\times$ $g$ to obtain a microsomal pellet that was resuspended in a buffer containing 0.25 M sucrose, 0.1 mM DTT and 5 mM potassium phosphate (pH 6.8). The suspension was then added to a 27 g phase mixture to obtain a phase system consisting of 6.2% Dextran T-500, 6.2% PEG 3350 in 5 mM potassium phosphate (pH 7.8), with 0.25 M sucrose. The final upper phases were collected, diluted at least twice with 5 mM potassium phosphate (pH 6.8) and 0.25 M sucrose, and centrifuged for 30 min at 100 000 $\times$ $g$. The resulting microsome was resuspended in 5 mM potassium phosphate buffer (pH 6.5) containing 0.25 M sucrose. All the above operations were carried out at 4 °C.

The purity of the plasma membrane was estimated using the method described by Widell & Larsson (1990). Vanadate inhibition of ATPase activity was over 69%. The inhibitions of ATPase activity by nitrate, azide and molybdate were less than 1%, 1.2% and 3.2%, respectively.

### *Measurements of lipid peroxidation*

The lipid peroxidation produced by the plasma membrane was evaluated as thiobarbituric acid reaction

(Fodor & Marx 1988). One mL plasma membrane suspension containing 0.5 mg membrane protein and 2 mM NADH were combined in a 5 mM potassium phosphate buffer (pH 6.0); then the mixture was incubated at 37 °C. After 60 min, 2 mL thiobarbituric acid (in 5% trichloride actate) was added thoroughly. After heating at 100 °C for 30 min in a boiling water bath, the tube was cooled with flow water, and the reaction product was extracted with 4 mL of *n*-butanol-pyridine mixture (15:1, v/v). The absorbance at $\lambda = 532$ nm was determined, and the values ($A_{532}$) were used to express the concentration of lipid peroxides.

*Determination of oxygen uptake*

Oxygen uptake was monitored by a platinum electrode of the Clark-type (Qiu *et al.* 1995). The assay medium contained 10 mM Tris-Mes (pH 6.0), 0.25 M sucrose, 10 mM NaCl, 5 mM $MgCl_2$ and 0.25 mM NADH in a final volume of 2.0 mL. The reaction was started by purified plasma membrane containing 100 $\mu$g protein.

*Measurement of reduction rate of Fe(CN)$_6^{3-}$*

According to the procedures of Rubinstein *et al.* (1990) for measurements of $Fe(CN)_6^{3-}$ reduction rate, a medium which consisted of 50 mM KCl, 5 mM $MgSO_4$, 4 mM $CaCl_2$, 50 mM Tris-HCl (pH 7.5), 0.25 mM NADH and 1 mM $Fe(CN)_6^{3-}$ was prepared. The reaction was started by adding purified plasma membrane containing 10 $\mu$g protein. The reduction rate of $Fe(CN)_6^{3-}$ was calculated from the extinction coefficient of ferricyanide ($\varepsilon = 1.0\ mM^{-1}\ cm^{-1}$) and the value of absorbance for mixture at 420 nm.

*Assays of plasma membrane ATPase activity*

The activities of plasma membrane ATPase were measured by the release of Pi according to the method of Onishi *et al.* (1975). The values are presented as mmol Pi released (mg membrane protein)$^{-1}$ min$^{-1}$. Membrane protein contents were measured by the method of Bradford (1976) with a modification. Triton X-100 in 0.01% was added in order to solubilize membrane protein during the measurement.

Two kinds of ATPase, i.e., $H^+$-ATPase and $Ca^{2+}$-ATPase activities were determined. The reaction medium contained 3 mM $MgSO_4$, 25 mM $K_2SO_4$, 0.02% Triton X-100, 50 mM Tris-Mes (pH 6.5) and 3 mM ATP-$Na_2$ in final volume of 0.5 mL for $H^+$-ATPase activity measurement (Wang & Sze 1985). As for the measurement of $Ca^{2+}$-ATPase activity, the reaction medium consisted by 10 mM imidazole, 5 mM $MgCl_2$, 50 $\mu$M $CaCl_2$, 0.02% Triton X-100, 50 mM Tris-Mes (pH 7.0) and 3 mM ATP-$Na_2$ in final volume of 1.0 mL (Li *et al.* 1995).

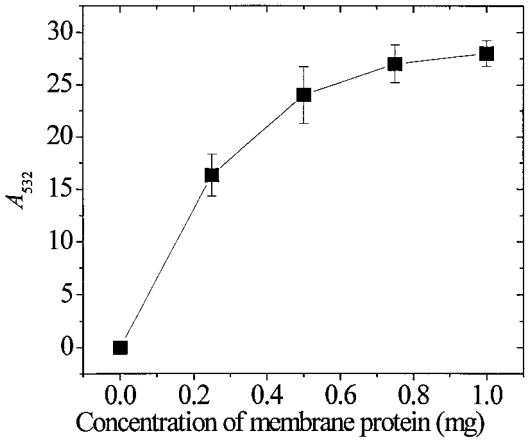


*Fig. 1.* Effects of protein concentration of plasma membrane on vesicles lipid peroxidation.

## Results

*Effects of membrane protein contents, incubation time and $La^{3+}$ concentration on plasma membrane lipid peroxidation*

The lipid peroxidation of plasma membranes was dependent on the protein concentration of vesicle membranes (Figure 1). There were no products of lipid peroxidation ($A_{532} = 0$) when no membrane protein was added into the reaction medium. The contents of lipid peroxides increased markedly when the plasma membrane protein was increased by 0.25 mg. After that, the increase of lipid peroxides content became relatively small with an increase of membrane protein from 0.5 mg to 1 mg. According to the curve in Figure 1, the protein concentration of 0.5 mg was used in later experiments.

Further study showed that the production of lipid peroxidation was strongly proportional to the incubation time (Figure 2). When the production of lipid peroxidation was determined immediately after plasma membranes were added to the reaction medium, the content of lipid peroxides ($A_{532}$) was rather small. With the increase of incubation time (from 10 to 50 min) the values of $A_{532}$ increased constantly. However, the changes of $A_{532}$ were very small when

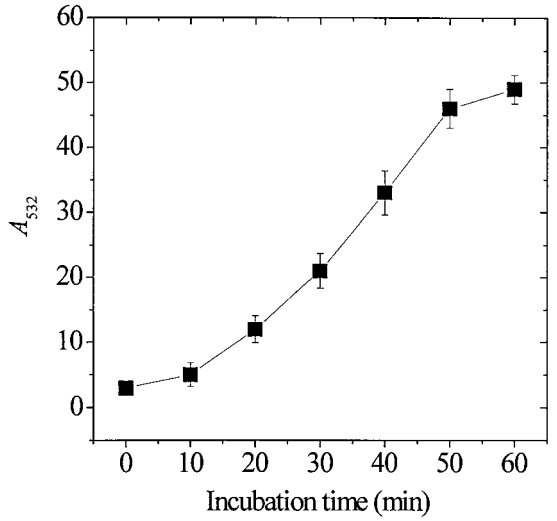


*Fig. 2.* Time course of lipid peroxidation for plasma membrane.

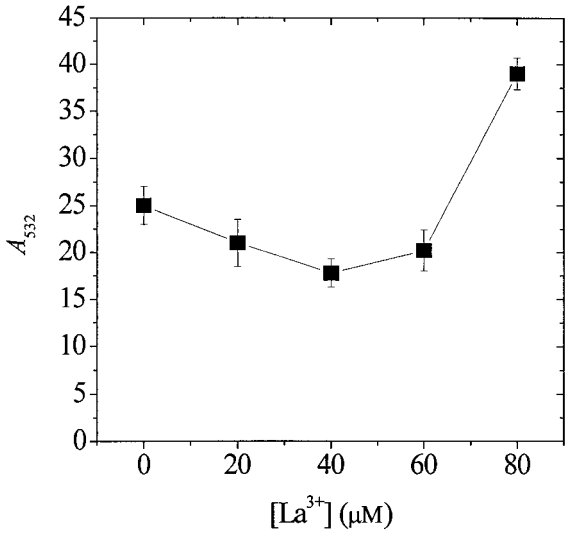


*Fig. 3.* Effects of different $La^{3+}$ concentrations on lipid peroxidation of plasma membrane from rice roots.

incubation time varied from 50 min to 60 min. Therefore, the incubation time of 50 min was employed in later measurements.

The absorbance of lipid peroxidation products was around 25 for the control treatment, in which there was no addition of lanthanum chloride. After increasing $La^{3+}$ from 20 to 40 $\mu$M, the content of lipid peroxides reduced obviously (Figure 3). In contrast, the absorbance of lipid peroxidation products increased with the increase of $La^{3+}$ concentration within the range of 40 to 80 $\mu$M. Particularly after adding 80 $\mu$M of $La^{3+}$, the value of $A_{532}$ went dramatically up.

*Effects of $La^{3+}$ concentration on redox system activity of plasma membrane*

In our studies, the relative activity of $O_2$ uptake and the reduction rate of $Fe(CN)_6^{3-}$ by plasma membranes were used to measure the activity of the redox system. Table 1 gives the effects of various concentrations of $La^{3+}$ on the relative activity of $O_2$ uptake and the reduction rate of $Fe(CN)_6^{3-}$ by purified vesicles from rice seedling roots. When $La^{3+}$ concentrations varied from 20 to 40 $\mu$M, the reduction rate of $Fe(CN)_6^{3-}$ by the isolated membrane increased in contrast to the control treatment. The highest activity appeared at 40 $\mu$M of $La^{3+}$ concentration. Afterward, the value of reduction rate of $Fe(CN)_6^{3-}$ decreased at higher $La^{3+}$ concentrations (60 to 80 $\mu$M). In the result, the reduction rate of $Fe(CN)_6^{3-}$ at 80 $\mu$M $La^{3+}$ concentration was much lower than that of the control treatment. In contrast to the changes of the reduction rate of $Fe(CN)_6^{3-}$, the responses of relative activity of $O_2$ uptake to $La^{3+}$performed a simple tendency (Table 1). The relative activity of $O_2$ uptake decreases constantly from 100% to 61.6% with the increase in $La^{3+}$ concentration.

*Effects of $La^{3+}$ concentration on $H^+$-ATPase and $Ca^{2+}$-ATPase activities of plasma membrane*

The activities of the plasma membrane ATPase, purified from rice seedling root, were dependent on the concentration of lanthanum chloride in culture solution (Table 2). The relative activity of $H^+$-ATPase changed from 100% to 140.5% when the solution concentration of $La^{3+}$ increased from 0 up to 60 $\mu$M. At same time, the relative activity of $Ca^{2+}$-ATPase also varied from 100% to 137%. With higher $La^{3+}$ concentrations (80 $\mu$M), the relative activities of $H^+$-ATPase and $Ca^{2+}$-ATPase declined rapidly to 75.7% and 74.1%, respectively. In this experiment, the maximal relative activities of both $H^+$-ATPase and $Ca^{2+}$-ATPase appeared at 60 $\mu$M of $La^{3+}$ concentration.

Another activity measurement was carried out in which the purified vesicles from control grown rice seedling roots of the control treatment were used and various concentrations of $La^{3+}$ were added directly into the reaction medium. The results showed that both the relative activities of $H^+$-ATPase and $Ca^{2+}$-ATPase with a series of $La^{3+}$ additions were lower than that of the control treatment, except at the condition of $La^{3+}$ concentration of 20 $\mu$M for $H^+$-ATPase (Table 3). This implied that the activity of $H^+$-ATPase could be

*Table 1.* Effects of various concentrations of $La^{3+}$ on relative activity of $O_2$ uptake and reduction rate of $Fe(CN)_6^{3-}$ by purified plasma membranes from rice seedling roots. The values are the mean±S.E.

| $[La^{3+}]$ ($\mu$M) | Relative activity of $O_2$ uptake (%) | Reduction rate of $Fe(CN)_6{}^{3-}$ ($\mu$mol $Mg^{-1}$ prot. $min^{-1}$) |
|---|---|---|
| 0 | 100 | $0.80 \pm 0.03$ |
| 20 | $93.1 \pm 4$ | $0.98 \pm 0.02$ |
| 40 | $82.6 \pm 6$ | $1.13 \pm 0.02$ |
| 60 | $70.2 \pm 2$ | $0.85 \pm 0.04$ |
| 80 | $61.6 \pm 5$ | $0.62 \pm 0.01$ |

*Table 2.* Effects of various concentrations ($\mu$M) of $La^{3+}$ in culture solution on specific activities (expressed as mmol Pi $mg^{-1}$Protein $min^{-1}$) and relative activities (expressed as %) of $H^+$-ATPase and $Ca^{2+}$-ATPase by purified plasma membranes from rice seedling roots.

| $[La^{3+}]$ | $H^+$-ATPase | | $Ca^{2+}$-ATPase | |
|---|---|---|---|---|
| | Specific activity* | Relative activity | Specific activity* | Relative activity |
| 0 | $3.7 \pm 0.2$ | 100.0 | $2.7 \pm 0.2$ | 100.0 |
| 20 | $4.5 \pm 0.2$ | 121.6 | $3.2 \pm 0.1$ | 118.5 |
| 40 | $4.3 \pm 0.1$ | 116.2 | $3.3 \pm 0.2$ | 122.2 |
| 60 | $5.2 \pm 0.2$ | 140.5 | $3.7 \pm 0.2$ | 137.0 |
| 80 | $2.8 \pm 0.3$ | 75.7 | $2.0 \pm 0.3$ | 74.1 |

* The values are the mean±S.E.

stimulated by low concentrations of $La^{3+}$, but could be depressed by high concentrations of $La^{3+}$($\geq$ 40 $\mu$M). The activity variation ranges were 32.4% and 17.2% for $H^+$-ATPase and $Ca^{2+}$-ATPase, respectively.

## Discussion

Our experimental results showed that the products of lipid peroxidation of plasma membranes were dependent on the protein content of plasma membranes and the incubation time when the reaction medium contained NADH, which is the most common electron donor in a cell (Figures 1 and 2). These results agreed very well with the conclusion of Qiu & Liang(1995). Our data suggested that the redox system of plasma membranes could cause the peroxidation of membrane lipid. It is attractive to elucidate the roles of REEs in these lipid peroxidation processes. Although Wang *et al.* (1998) and Feng *et al.* (1995) proved that $Ce^{3+}$ could scavenge superoxide radicals and hydroxyl radicals in chemical solution systems, no further direct information is available on the effects of REEs on lipid peroxidation in biological systems. Our further studies revealed that the existence of $La^{3+}$ could obviously depress such lipid peroxidation of purified plasma membrane vesicles at below 60 $\mu$M of $La^{3+}$ (Figure 3). This implies that, within a certain concentration, $La^{3+}$ has some physiological activities and functions as a free radical scavenger, such as superoxide dismutase and peroxidase, for example.

Oxygen is the natural acceptor of electrons in redox systems (Rubinstein & Luster 1993). If there are no exogenous electron acceptors the electron of NADH would transfer along the redox component to oxygen, and active oxygen free radicals could be generated. This process was observed directly by the research of Vianello *et al.* (1990) and Qiu *et al.* (1995) using ESR methods. Therefore, the relative activity of $O_2$ uptake reflects the activity of the redox system and the formation capacity of radicals. The results in Table 1 indicate that the presence of $La^{3+}$ inhibited the relative activity of $O_2$ uptake. Compared to the results showing in figure 3 that a certain concentration of $La^{3+}$ depressed the formation of lipid peroxides, it could be concluded that REEs inhibited the electron transfer from NADH to oxygen, then depressed the production of active oxygen radicals,

*Table 3.* Effects of various concentrations ($\mu$M) of $La^{3+}$ in reaction medium on specific activities (expressed as mmol Pi $mg^{-1}$Protein $min^{-1}$) and relative activities (expressed as %) of $H^+$-ATPase and $Ca^{2+}$-ATPase by purified plasma membranes from rice seedling roots.

| $[La^{3+}]$ | $H^+$-ATPase | | $Ca^{2+}$-ATPase | |
|---|---|---|---|---|
| | Specific activity* | Relative activity | Specific activity* | Relative activity |
| 0 | $3.7 \pm 0.1$ | 100.0 | $2.9 \pm 0.2$ | 100.0 |
| 20 | $3.8 \pm 0.1$ | 102.7 | $2.8 \pm 0.2$ | 96.6 |
| 40 | $3.0 \pm 0.2$ | 81.1 | $2.8 \pm 0.1$ | 96.6 |
| 60 | $2.8 \pm 0.2$ | 75.7 | $2.7 \pm 0.2$ | 93.1 |
| 80 | $2.9 \pm 0.1$ | 78.4 | $2.5 \pm 0.1$ | 86.6 |
| 100 | $2.6 \pm 0.2$ | 70.3 | $2.4 \pm 0.1$ | 82.8 |

* The values are the mean±S.E.

and reduced the formation of lipid peroxides through plasma membrane lipid peroxidation.

Another important redox system is the standard system which exists widely in plant plasma membranes (Rubinstein & Stern 1986). In this system, the electron donor is NAD(P)H, and the electron acceptor is $Fe(CN)_6^{3-}$. In our experiment, when $Fe(CN)_6^{3-}$ was provided in a reaction medium the reduction rate of $Fe(CN)_6^{3-}$ by purified plasma membrane vesicles performed a very different change in comparison to the relative activity of $O_2$ uptake (Table 1). Since the main role of the standard system is the reduction of $Fe^{3+}$ to $Fe^{2+}$, such differences may reflect the influences of REEs on the uptake of iron by plant roots. From the results of our studies, low concentrations of $La^{3+}(\leq 60\ \mu M)$ can stimulate the reduction of $Fe(CN)_6^{3-}$, but high concentration of $La^{3+}(80\ \mu M)$ can depress the reduction of $Fe(CN)_6^{3-}$. Thus, REEs are beneficial to plant uptake of iron. Further detailed mechanistic processes are awaiting elucidating.

$H^+$ extrusion is one of the most important functions of the plasma membrane. It establishes an electrochemical gradient across the membrane, which provides energy for the transport of ions and metabolites in and out of the plant cells (Reinhold & Kaplan 1984; Sze 1985). And furthermore, the acidification of the plant cell wall provides for the possibility of cell expansion and plant growth. There are two opinions on the $H^+$ extrusion by plasma membranes. Some researchers considered that the $H^+$ extrusion was the result of electron transport along the redox system to $Fe(CN)_6^{3-}$ (Rubinstein & Stern 1986). Many studies support another opinion that the $H^+$ extrusion was driven by $H^+$-ATPase (proton pump) on the plasma membrane (Barr 1988). The results of our experiments showed that the effect of $La^{3+}$ on the activity of $H^+$-ATPase is similar to the effect of $La^{3+}$ on the reduction of $Fe(CN)_6^{3-}$ in rice seedling root (Tables 1 and 2). That is, both the activities of $H^+$-ATPase and the standard system of redox were stimulated by low concentrations of $La^{3+}$ and were inhibited by high concentrations of $La^{3+}$. We can conclude from our experimental results that REEs ions could enhance the $H^+$ extrusion by plasma membranes at certain concentrations. This conclusion agrees well with the results of Yu & Chen (1995), in which low $La^{3+}$ concentrations improved the growth of plant and yield of crops as well, but high $La^{3+}$ concentrations depressed both those processes.

Because there exists a cell wall in plant cells, the response processes of plasma membranes to REEs ions may be more complicated. In order to eliminate the influences of cell wall a further study was carried out, in which only the purified plasma membrane vesicles from the control treatment were used for $H^+$-ATPase activity measurement, while various concentrations of $La^{3+}$ were added directly into the reaction medium (Table 3). Although the responses of $H^+$-ATPase activity to $La^{3+}$ in this case are similar to the responses in culture solution case, the $La^{3+}$ concentration was smaller when the activity of $H^+$-ATPase was the maximum. These $La^{3+}$ concentrations are 20 $\mu$M and 60 $\mu$M for the reaction medium case and culture solution case respectively. This difference may be due to most of the $La^{3+}$ being absorbed by the cell wall.

It is well known that $Ca^{2+}$ is the second messenger in plant cells. The $Ca^{2+}$ channel and $Ca^{2+}$-ATPase in plasma membranes are the most important way to control the $Ca^{2+}$ in and out of plant cells. It has been proven that the $Ca^{2+}$ channel could be blocked by $La^{3+}$ (Rengel 1994; Lewis & Spalding 1998).

Although some researchers have studied the relation between $La^{3+}$ and $Ca^{2+}$-ATPase activity in animals, it is not clear whether $La^{3+}$ inhibits the activity of $Ca^{2+}$-ATPase in plant plasma membranes. The results listed in Table 2 and Table 3 reveal that the effects of REEs ions on $Ca^{2+}$-ATPase activity are different *in vivo* and *in vitro* conditions of $La^{3+}$ treatments. The activity of $Ca^{2+}$-ATPase was inhibited by all treatments of $La^{3+}$ where REEs ions were added directly into the reaction medium. In contrast, the activity of $Ca^{2+}$-ATPase was stimulated at certain $La^{3+}$ concentrations where $La^{3+}$ was varied in the culture solution. Such differences are interesting, and also imply that the physiological mechanism of $La^{3+}$ *in vivo* is sophisticated and that some unclear factors might be involved.

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