

## NADH-Linked Electron Transfer Induces Cd<sup>2+</sup> Movement in Corn Root Plasma Membrane Vesicles

Shu-I Tu, Deidre Patterson, Siyuan Shen, David Brauer and An-Fei Hsu

USDA/ARS/NAA, Eastern Regional Research Center, 600 E. Mermaid Lane, Wyndmoor, PA 19118, U.S.A.

The rate of electron transfer from NADH to ferricyanide associated with corn root plasma membrane vesicles was not significantly affected by the presence of Ca<sup>2+</sup>, Cd<sup>2+</sup>, Zn<sup>2+</sup>, or Co<sup>2+</sup>. However, unlike Ca<sup>2+</sup>, other tested divalent cations induced an electron transfer-dependent increase in light scattering by the vesicles. The efficacy in inducing the light scattering increase followed the order of Cd<sup>2+</sup> > Zn<sup>2+</sup> > Co<sup>2+</sup>. The extent of the light scattering change increased as the concentration of added Cd<sup>2+</sup> increased. Using phase contrast microscopy, the increase in light scattering was related to an expansion of the vesicles. Accompanying the volume increase, a decrease of Cd<sup>2+</sup> concentration in the external space was observed by the use of Arsenazo III, a metallochromophore specific for divalent cations. Under a specified electron transfer condition, a similar percentage of decrease over a wide range of initial Cd<sup>2+</sup> concentration was detected. The presence of A23187 decreased the rate and extent of light scattering increase and its associated Cd<sup>2+</sup> concentration change. However, A23187 had no effect on scattering changes already established by the electron transfer and Cd<sup>2+</sup>. These results suggest that the NADH linked electron transfer reaction may induce a Cd<sup>2+</sup> uptake by the vesicles of corn root plasma membrane with inside-out orientation.

**Key words:** Cadmium transport — Corn (*Zea mays*) — Electron transfer — NADH-linked redox — Root plasma membrane.

The transport of metal ions by plant root cells has been, in general, considered as driven by the proton electrochemical potential associated with the plasma membrane H<sup>+</sup>-ATPase (Hanson 1978, Pitman 1982, Spanswick 1981). The energy components of the potential are then utilized to alter the properties of membranes to regulate the concentration of a specific ion in cells. For example, the outward proton pumping associated with the plasma membrane H<sup>+</sup>-ATPase and the inward proton pumping of tonoplast H<sup>+</sup>-ATPase are essential to maintain the physiological pH of cell cytosols (Sze 1985). To keep the cytosolic

[Ca<sup>2+</sup>] low, energy is needed to activate the operation of many efflux as well as influx Ca<sup>2+</sup> transporters (Bush 1993).

Based on a general concept of ion transport (Racker 1979), the transport of other divalent cations may share some of the molecular arrangements for the movement of Ca<sup>2+</sup>. The in vivo <sup>31</sup>P NMR results that the uptake of Mn<sup>2+</sup> is affected by Ca<sup>2+</sup>, are consistent with this concept (Pfeffer 1987). The inhibitory effects of Ca<sup>2+</sup> to the transport of Cd<sup>2+</sup> (Pawlik and Skowronski 1994) in plant cells provide additional examples. Different types of energy-dependent Ca<sup>2+</sup> efflux transporters in plant cells have been characterized (Evans et al. 1991, and refs. therein) and one of them is a P-type Ca-translocating ATPase associated with the plasma membrane. However, it remains to be established whether other energy releasing processes, such as NADH-linked electron transfer of the plasma membrane, are involved in the efflux of divalent cations from cell cytosols.

The plasma membrane of higher plant cells is known to contain a NADH-supported electron transfer system which expresses activities of NADH-cytochrome *c* reductase (Larsson 1985), flavin-NADH dehydrogenase (Ramirez et al. 1984) and b-type of cytochrome (Leong et al. 1981). Particles enriched with multiple NADH-linked redox activities have been obtained from corn root plasma membrane vesicles by detergent treatments (Luster and Buckout 1988). Both cytochrome *c* and ferricyanide have been extensively used to investigate the NADH-linked electron transfer. It has been previously determined (Askerlund et al. 1988) that the NADH oxidation and ferricyanide reduction occur mainly on the cytoplasmic face of the plasma membrane. Detailed kinetic analysis on the reduction of cytochrome *c* and ferricyanide (Tu et al. 1993) and the differential effects of poly(L-lysine) to the reduction of these two acceptors (Tu et al. 1994), suggest that the reduction of cytochrome *c* and ferricyanide should occur at different sites.

The exact physiological roles of this electron transfer process are yet to be fully explored. Proposed involvements include the reduction of Fe<sup>3+</sup> to Fe<sup>2+</sup> for uptake (Møller and Crane 1990), hormonal regulation of cell growth, and maintaining the -SH groups of membrane proteins in a reduced state (Crane et al. 1985, Møller and Crane 1990). In a previous study (Tu et al. 1995), we demonstrated that the electron transfer induced a light scattering increase of isolated corn root plasma membrane vesicles in the medium containing Cd<sup>2+</sup> but not Ca<sup>2+</sup>. In present work, we have

Reference to a brand or firm name does not constitute endorsement by the U.S. Department of Agriculture over others of a similar nature not mentioned.

further characterized this light-scattering increase in isolated corn root plasma membrane vesicles. The results suggest that the NADH-linked electron transfer may induce  $\text{Cd}^{2+}$  uptake by the plasma membrane vesicles with inside-out orientation.

## Materials and Methods

**Isolation of corn root plasma membrane**—The plasma membrane vesicles were isolated between 0 to 4°C from corn roots (*Zea mays* L. cv. W7551, Custom Farm Seed) as previously described (Brauer et al. 1988). The membrane had a protein content of 2 mg  $\text{ml}^{-1}$  determined by a modified Lowry method after precipitation by tri-chloroacetic acid in the presence of deoxycholate. We have shown in a previous study (Hsu et al. 1989) that the use of glycerol and discontinuous sucrose density centrifugation yielded a root plasma membrane preparation with about 80% of the total Mg-ATPase activity sensitive to vanadate. Thus, the isolated plasma membrane from the corn hybrid contained only a minimal contamination of mitochondria, golgi, and tonoplast. The obtained membrane fraction did contain significant molybdate-insensitive phosphatase activity (about 50% of the vanadate sensitive ATPase activity). Upon treating with 0.02% Triton X-100, the vanadate-sensitive ATPase activity increased by about two thirds indicating the presence of approximately 60% inside-out vesicles in the plasma membrane fraction.

**Measurement of NADH-linked electron transfer and light scattering**—The reduction of ferricyanide catalyzed by the membrane was monitored at 22°C by absorbance decrease at 420 nm. To quantify the reduction, difference in extinction coefficient between ferri- and ferrocyanides ( $-0.93 \text{ mM}^{-1} \text{ cm}^{-1}$ ) was used. The light scattering change of the membrane vesicles induced by the electron transfer from NADH to ferricyanide was monitored spectroscopically at 550 nm at 22°C. Typically 100  $\mu\text{l}$  of plasma membrane vesicles were diluted to 2 ml in a buffer containing 150 mM KCl, 20 mM HEPES, pH 7.5, 10  $\mu\text{g}$  of antimycin A, 50 or 100  $\mu\text{M}$   $\text{K}_3\text{Fe}(\text{CN})_6$ , and various concentrations of different divalent cations described in text. After acquiring a stable flat baseline, 200  $\mu\text{M}$  NADH was added to initiate the reduction of ferricyanide.

**Determination of  $\text{Cd}^{2+}$  concentrations by Arsenazo III**—The concentrations of free  $\text{Cd}^{2+}$  in the suspension media of the plasma membrane vesicles ( $\sim 100 \mu\text{g}$ ) were determined by the use of Arsenazo III. The reduction of ferricyanide by NADH, in the absence of added divalent cations, in 2.0 ml of the plasma membrane vesicle suspension described above was allowed to reach completion (about 2 to 3 min). Aliquots of  $\text{CdCl}_2$  solution were then added to the suspension to make the final concentration ranging from 0 to 100  $\mu\text{M}$ . Samples each of 1 ml of the suspension were then mixed with 1 ml of 35  $\mu\text{M}$  arsenazo III in 150 mM KCl and 20 mM HEPES, pH 7.5. Difference spectra were then recorded and the absorbance changes at 656 nm were used to construct a linear standard curve.

**Microscopic determination on the size and population of the plasma membrane vesicles**—The samples used for measuring the electron transfer-induced,  $\text{Cd}^{2+}$ -related light scattering were removed from the reaction mixture as soon as the scattering increase reached a plateau along the time-course of scattering measurement. The withdrawn samples were mounted onto slides and examined by phase contrast microscopy at a total magnification of 800. The video frames which contained approximately 25 plasma membrane vesicles per frame, were captured and digitized using IPPLUS software. The total time lapse between withdrawing sam-

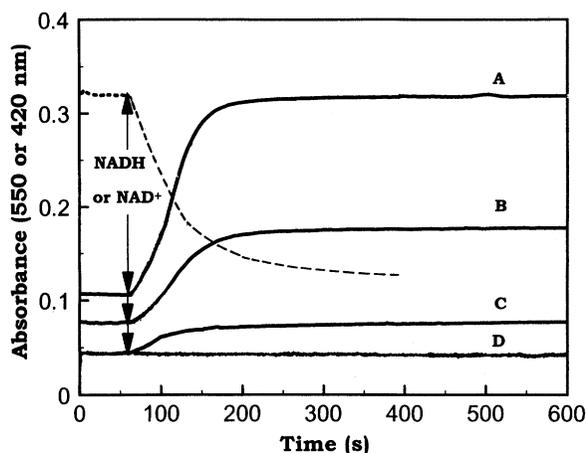
ples to completing image digitization was less than 5 s, a time period of no significant change of scattering levels. The cross sectional area of these vesicles was determined using NIH Image software. From the dimensions of the vesicles and the video frames, the population density of vesicle was also determined. Data reported were the median size of vesicles within an image, plus or minus the standard error determined from 5 replicates per sampling time.

## Results

**Effects of divalent cations on the light scattering of plasma membrane vesicle**—In our previous work (Tu et al. 1995), we reported that the presence of  $\text{Cd}^{2+}$  or  $\text{Ca}^{2+}$  did not significantly affect the rate of electron transfer from NADH to ferricyanide. However, the electron transfer induced an increase of the light scattering of the plasma membrane vesicles in the medium containing  $\text{Cd}^{2+}$  but not  $\text{Ca}^{2+}$ . To determine whether any other divalent cations can cause a similar increase in the light scattering,  $\text{Mg}^{2+}$ ,  $\text{Fe}^{2+}$ ,  $\text{Ba}^{2+}$ ,  $\text{Co}^{2+}$  and  $\text{Zn}^{2+}$  were used to replace  $\text{Cd}^{2+}$ . Like  $\text{Ca}^{2+}$ , the presence of  $\text{Mg}^{2+}$ ,  $\text{Ba}^{2+}$  and  $\text{Fe}^{2+}$  (up to 0.1 mM) did not cause any detectable light scattering change relating to NADH-linked electron transfer. The presence of  $\text{Co}^{2+}$ ,  $\text{Zn}^{2+}$  and  $\text{Mn}^{2+}$  induced a light scattering increase similar to that associated with  $\text{Cd}^{2+}$  (Fig. 1). In the same figure, we also found that a replacement of NADH by  $\text{NAD}^+$  or ferricyanide by ferrocyanide nullified the increase (Fig. 1, trace D). This result indicated that the electron transfer was essential for inducing the observed light scattering increase. Among the tested cations,  $\text{Cd}^{2+}$  appeared to be the most effective one to induce a light scattering increase related to the electron transfer. Thus, we have chosen this cation for further study. It should be mentioned that we have no intention to generalize the following findings associated with  $\text{Cd}^{2+}$  to other cations.

With the initial concentrations of both NADH and ferricyanide as 100  $\mu\text{M}$ , the extent and the initial rate of light scattering change increased as the concentration of  $\text{Cd}^{2+}$  increased (Fig. 2). At lower concentrations of  $\text{Cd}^{2+}$ , a lag in response time was noted. As described in our previous report (Tu et al. 1995), the light scattering ceased to increase when the electron transfer reaction reached completion. Under the experimental conditions of Figure 2, the electron transfer would stop once all added ferricyanide was reduced (NADH in excess). To eliminate the possibility of  $\text{Cd}^{2+}$  interacting with produced ferrocyanide to affect the scattering of the membrane vesicles, we have delayed the addition of  $\text{Cd}^{2+}$  until the redox reaction reached completion. No significant change in the light scattering of the vesicles was noted.

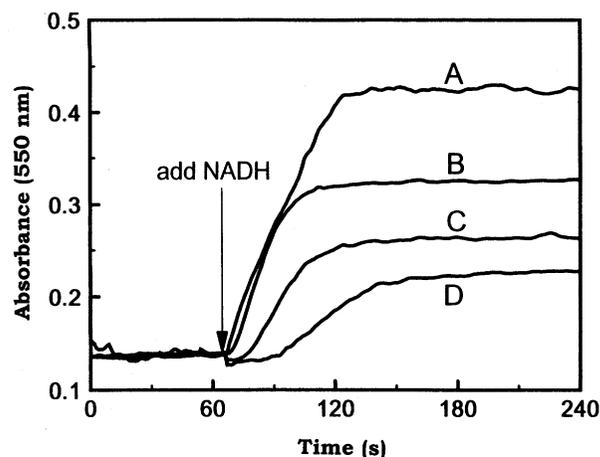
**Microscopic investigation on the origin of the light scattering increase**—A change in the light scattering usually reflects a change in the size/volume of the scattering entities. There are at least three possible mechanisms, namely membrane fusion, vesicle aggregation, and a movement of



**Fig. 1** Light scattering increase of the plasma membrane vesicles induced by the electron transfer and divalent cations. Plasma membrane vesicles were incubated with 0.1 mM of specified divalent cations in the electron transfer assay medium containing 100  $\mu$ M ferricyanide for 10 to 15 min at 22°C. Aliquots of NADH were then added (100  $\mu$ M) at the indicated time to initiate the reduction of ferricyanide. The light scattering of the vesicles was recorded at 550 nm. Traces A, B, and C represent the electron transfer-linked light scattering increase by  $\text{Cd}^{2+}$ ,  $\text{Co}^{2+}$ , and  $\text{Zn}^{2+}$ , respectively. In D, the membrane vesicles were treated as in A, with the exception that NADH was replaced by  $\text{NAD}^+$  (100  $\mu$ M). A replacement of ferricyanide by ferrocyanide in A also failed to induce any detectable light scattering increase after the addition of NADH. The presence of 0.1 mM  $\text{Ca}^{2+}$  or  $\text{Ba}^{2+}$  failed to induce the light scattering increase. The trace of dashed line is the time course of the reduction of ferricyanide by NADH measured at 420 nm in the same buffered media without the addition of divalent cations.

solvent into the internal aqueous space (swelling) to increase the light scattering of the plasma membrane vesicles. Membrane fusion should result in the formation of larger vesicles. Vesicle aggregation should lead to the formation of vesicle clusters in which the membrane separation of individual vesicle could be retained. If the electron transfer from NADH to ferricyanide can somehow activate a vectorial ion transport across the membrane, then the non-equilibrium distribution of solutes would induce a solvent movement to minimize the osmotic pressure difference across the vesicles.

To determine the origin of observed light scattering increase, we have examined the size/shape of the plasma membrane vesicles before the occurrence and after the completion of  $\text{Cd}^{2+}$ -related light scattering increase. As shown in Figures 3A, B, C and D, the scattering increase is accompanied by a volume increase of the vesicles. The population density determined by the same imaging technique, averaged about  $4 \pm 1 \times 10^9$  vesicles per ml independent of the presence of  $\text{Cd}^{2+}$  or the length exposure to  $\text{Cd}^{2+}$ . Thus, under the experimental conditions, there is no evidence for signifi-



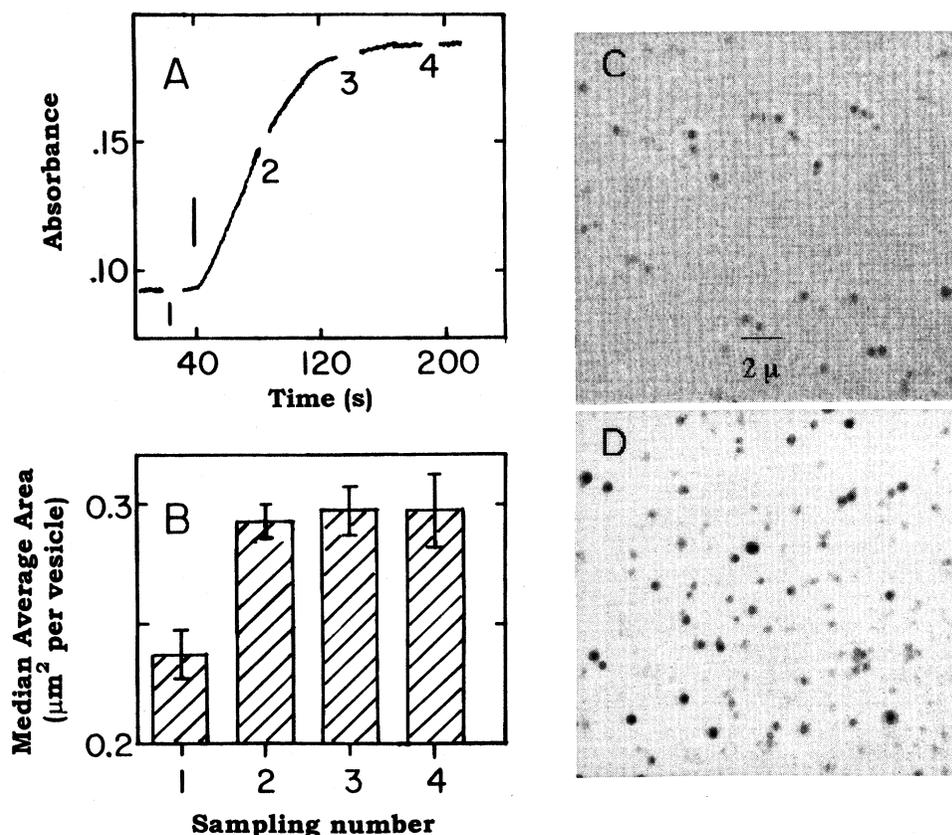
**Fig. 2** Dependence of the light scattering increase on  $[\text{Cd}^{2+}]$ . The plasma membrane vesicles were incubated with various  $[\text{Cd}^{2+}]$  in the same electron transfer assay medium containing 100  $\mu$ M ferricyanide as described in Fig. 1. The light scattering increases after the addition of NADH (100  $\mu$ M), in the presence of 0.2, 0.12, 0.08 and 0.04 mM of  $\text{Cd}^{2+}$  are shown as traces A, B, C and D, respectively.

cant fusion or aggregation of the vesicles. It appears that the volume increase may be, to a great extent, due to swelling of the vesicles.

*Change of the  $\text{Cd}^{2+}$  concentration in the media*—As shown in Figure 2, the light scattering increase is closely related to the volume increase of individual vesicles (swelling). If the electron transfer induces an uptake of  $\text{Cd}^{2+}$ , then to decrease the osmotic effects, solvent should be transported into the internal space of the vesicles. The solvent movement would then result in a swelling of vesicles.

To test this possibility, we measured the effects of the electron transfer on the  $\text{Cd}^{2+}$  concentration in the media. As described in Material and Methods, we have chosen arsenazo III, a divalent cation metallochromophore, to measure the concentration of  $\text{Cd}^{2+}$ . In a control experiment, we found that arsenazo III inhibits the electron transfer from NADH to ferricyanide. Thus, it is not possible to follow the time course of the change of  $\text{Cd}^{2+}$  in the suspending media, if any, by this indicator.

Using the arsenazo III method, we determined the concentration of  $\text{Cd}^{2+}$  in the media when the electron transfer ceased or the light scattering increase reached a plateau stage. We found that the  $\text{Cd}^{2+}$  concentration in the media substantially decreased at the end of the electron transfer (Table 1). The decrease indicated that  $\text{Cd}^{2+}$  was tightly adsorbed by and/or transported into the plasma membrane vesicles. Both the adsorption and the transport of  $\text{Cd}^{2+}$  must be activated by the electron transfer process. To account for the swelling of the vesicles (Fig. 3), the decrease in free  $\text{Cd}^{2+}$  concentration must involve transporting the cation from external to internal aqueous space of the vesicles.



**Fig. 3** Light microscopic determination on the size/shape of the plasma membrane vesicles. The light scattering of the plasma membrane induced by the electron transfer and  $50 \mu\text{M Cd}^{2+}$  was followed as described in Fig. 2 except that the wavelength is different (656 nm). Aliquots ( $10 \mu\text{l}$ ) of the vesicle suspension were rapidly withdrawn from the sample at indicated time intervals as shown in (A). The vesicles were placed on microscopic slides and viewed by a phase-contrast microscope and the images digitally recorded. The images were then processed as described in Materials and Methods. The size distribution of the vesicles at specified time intervals were shown in (B). The actual images of the vesicles obtained at time intervals 1 and 4 were shown in (C) and (D), respectively.

**Table 1** Electron transfer induced  $[\text{Cd}^{2+}]$  change in the external media

[Ferricyanide], mM	Initial $[\text{Cd}^{2+}]$ , $\mu\text{M}$	Final $[\text{Cd}^{2+}]$ , $\mu\text{M}$	% of $\text{Cd}^{2+}$ taken <sup>a</sup>
0.1	15.0	9.0	40
0.1	25.0	14.3	43
0.1	37.5	19.3	49
0.1	50.0	21.5	57
0.1	75.0	31.5	58
0.05	15.0	9.9	34
0.05	50.0	25.0	50
0.05	75.0	32.3	56

The plasma membrane vesicles were incubated in the electron transfer media containing different concentrations of  $\text{Cd}^{2+}$  and potassium ferricyanide for 15 min before the addition of NADH. Arsenazo III was added when the light scattering increase reached the maximum to determine the residual  $[\text{Cd}^{2+}]$  in the external aqueous media.

<sup>a</sup> The readings of arsenazo- $\text{Cd}^{2+}$  complex obtained from samples without the addition of NADH were used to represent the initial  $[\text{Cd}^{2+}]$  in the external media. The percentage of change was the average of three independent measurements with a relative error of  $\pm 5\%$ .

**Table 2** Effects of A23187 on the electron transfer induced  $[Cd^{2+}]$  change

A23187 added, $\mu g (\mu g \text{ of protein})^{-1}$	% of external $[Cd^{2+}]$ decrease, $\pm 2\%$ <sup>a</sup>
0.000	100.0
0.033	95.1
0.067	88.3
0.100	84.1

The plasma membrane vesicles in the electron transfer assaying medium were incubated with 0.1 mM  $Cd^{2+}$  and various concentrations of A23187 for 10 minutes at 22°C before the addition of NADH. The  $[Cd^{2+}]$  in the external media at the maximum of light scattering was determined by the use of Arsenazo III.

<sup>a</sup> The extent of  $[Cd^{2+}]$  decrease in the absence of A23187 was assigned as 100%. The values represent the average of 3 independent measurements.

It should be emphasized that tightly bound  $Cd^{2+}$  would not contribute to the osmotic pressure and thus would not induce swelling of the membrane vesicles.

*Effects of calcimycin (A23187) on  $Cd^{2+}$  movement*—Calcimycin (A23187) is a carboxylic ionophore specific for electric neutral exchange of divalent cations or with protons (Pressman 1976). The ionophore has been used to study ATPase-related  $Ca^{2+}$  transport in plant membrane systems (Brauer et al. 1990, Giannini et al. 1987). We have investigated the effects of this ionophore on NADH-linked electron transfer and its associated light scattering increase by  $Cd^{2+}$  and the concentration changes of  $Cd^{2+}$ . Although the presence of calcimycin did not affect the electron transfer from NADH to ferricyanide (data not shown), it did slow down the rate and the extent of light scattering increase (Table 2). Furthermore, the presence of the ionophore also lowered the extent of  $[Cd^{2+}]$  decrease associated with the electron transfer. Thus, the data indicate that the electron transfer process must induce a transport of  $Cd^{2+}$  from the external to the internal space of the vesicles. However, the obtained results do not exclude the possible involvement of tight-binding of  $Cd^{2+}$ , induced by the electron transfer, to either the interior or exterior or both of the vesicle membrane surfaces.

### Discussion

The data reported in this study showed that the presence of  $Cd^{2+}$ ,  $Mn^{2+}$ ,  $Co^{2+}$  and  $Zn^{2+}$  caused a light scattering increase of corn root plasma membrane vesicles under the conditions of active electron transfer from NADH to ferricyanide. Detailed study indicated that the increase caused by  $Cd^{2+}$  was a result of size increase of individual vesicles without detectable involvements of vesicle aggregation and/or membrane fusion. Measurements on the con-

centrations of  $Cd^{2+}$  in the external aqueous space showed a decrease in  $[Cd^{2+}]$  was accompanied by an increase in light scattering. These results suggested a movement of  $Cd^{2+}$  from the external to the internal aqueous spaces of the vesicles. The entry of  $Cd^{2+}$  would increase the concentration of osmolites in the internal aqueous space. To minimize the osmotic difference, the permeable solvent (water) would then move in and thus, increased the size of the vesicles. Possibility of  $Cd^{2+}$  movement was further supported by the inhibitory effects of calcimycin (A23187) to the light scattering increase of the vesicles and the concentration changes of  $Cd^{2+}$ .

It should be emphasized that NADH-linked electron transfer reaction, an energy releasing process, is essential to induce the observed effects associated with  $Cd^{2+}$ . Thus, detected movement of  $Cd^{2+}$  should be at least an energy facilitated process. Based on vanadate-sensitive ATPase activity measurement, the isolated plasma membrane vesicles contained both inside-out (~60%) and right-side-out orientations. Because both NADH and ferricyanide are considered as membrane impermeable, only the inside-out vesicles may express the electron transfer activity under the experimental conditions. Transmembrane electron transfer could be observed only in vesicles pre-loaded with NADH (Askerlund and Larsson 1991). The uptake of  $Cd^{2+}$  observed in this report would then be equivalent to an extrusion of the cation in intact root cells. In an in vivo NMR study (Pfeffer et al. 1987), we have described that the entry of  $Cd^{2+}$  to excised roots may be slowed down by  $Ca^{2+}$ . The observation suggests that the divalent cations may share the same mechanisms for entering intact root cells. Since the cation movement described in current study was not shared by  $Ca^{2+}$ , the NADH-linked electron transfer process could be a mean for root cells to specifically export certain divalent cations such as  $Cd^{2+}$ . Whether such a process operating in intact cells remains to be determined.

### References

- Askerlund, P. and Larsson, C. (1991) Transmembrane electron transfer in plasma membrane vesicles loaded with an NADH-generating system or ascorbate. *Plant Physiol.* 96: 1178–1184.
- Askerlund, P., Larsson, C. and Widell, S. (1988) Localization of donor and acceptor sites of NADH dehydrogenase activities using inside-out and right-side-out plasma membrane vesicles from plants. *FEBS Lett.* 239: 23–28.
- Brauer, D., Hsu, A-F. and Tu, S. (1988) Factors associated with the instability of nitrate-insensitive proton transport by maize root microsomes. *Plant Physiol.* 87: 598–602.
- Brauer, D., Schubert, C. and Tu, S. (1990) Characterization of a  $Ca^{2+}$ -translocating ATPase from corn root microsomes. *Physiol. Plant.* 78: 335–344.
- Bush, D.C. (1993) Regulation of cytosolic calcium in plants. *Plant Physiol.* 103: 7–13.
- Crane, F.L., Low, H. and Clark, M.G. (1985) Plasma membrane redox enzymes. *In* The Enzymes of Biological Membranes. Edited by Martonosi, A.N. pp. 465–510. Plenum Press, New York.
- Evans, D.E., Briars, S.A. and Williams, L.E. (1991) Active calcium trans-

- port by plant cell membranes. *J. Exp. Bot.* 42: 285–303.
- Giannini, J.L., Ruiz-Cristin, J. and Briskin, D.P. (1987) Calcium transport in sealed vesicles from red beet (*Beta vulgaris* L.) storage tissue. II. Characterization of  $^{45}\text{Ca}^{2+}$  uptake into plasma membrane vesicles. *Plant Physiol.* 85: 1137–1142.
- Hanson, J.B. (1978) Application of the chemiosmotic hypothesis to ion transport across the root. *Plant Physiol.* 62: 402–405.
- Hsu, A-F., Brauer, D. and Tu, S. (1989) Characterization of reconstituted plasma membrane  $\text{H}^{+}$ -ATPase from maize roots. *Physiol. Plant.* 76: 544–550.
- Larsson, C. (1985) Plasma membranes. In *Methods of Plant Analysis* (NS). Edited by Linekens, H.F. and Jackson, J.F. pp. 85–104. Springer-Verlag, Berlin.
- Leong, T.Y., Vierstra, R.D. and Briggs, W.R. (1981) A blue light-sensitive cytochrome-flavin complex from corn coleoptiles. Further characterization. *Photochem. Photobiol.* 34: 697–703.
- Luster, D.G. and Buckhout, T.J. (1988) Characterization and partial purification of multiple electron transport activities in plasma membrane from maize (*Zea mays*) roots. *Physiol. Plant.* 73: 339–347.
- Møller, I.M. and Crane, F.L. (1990) Redox process in plant plasma membrane. In *The Plant Plasma Membrane: Structure, Function, and Molecular Biology*. Edited by Larsson, C. and Møller, I.M. pp. 93–126. Springer, Berlin.
- Pawlik, B. and Skowronski, T. (1994) Transport and toxicity of cadmium—Its regulation in the cyanobacterium *synechocystis-aquatilis*. *Environ. Exp. Bot.* 34: 225–233.
- Pfeffer, P.E., Tu, S., Gerasimowicz, W.V. and Boswell, R.T. (1987) Role of the vacuole in metal ion trapping as studied by in vivo  $^{31}\text{P}$ -NMR spectroscopy. In *Plant Vacuoles*. Edited by Martins, B. pp. 349–359. Plenum, New York.
- Pitman, M.G. (1982) Transport across plant roots. *Quart. Rev. Biophys.* 15: 481–554.
- Pressman, B.C. (1976) Biological applications of ionophores. *Annu. Rev. Biochem.* 45: 501–530.
- Racker, E. (1979) Transport of ions. *Act. Chem. Res.* 12: 338–344.
- Ramirez, J.M., Gallego, G.G. and Serrano, R. (1984) Electron transfer constituents in plasma membrane fractions of *Avena sativa* and *Saccharomyces cerevisiae*. *Plant Sci. Lett.* 34: 103–110.
- Spanswick, R.M. (1981) Electrogenic ion pumps. *Annu. Rev. Plant Physiol.* 32: 267–289.
- Sze, H. (1985)  $\text{H}^{+}$ -translocating ATPase: advances using membrane vesicles. *Annu. Rev. Plant Physiol.* 36: 175–208.
- Tu, S., Brauer, D. and Hsu, I. (1994) Differential effects of polylysine on NADH-linked electron transfer in corn root plasma membrane vesicles. *J. Plant Nutri.* 17: 97–108.
- Tu, S., Patterson, D. and Brauer, D. (1993) NADH-linked ferricyanide and cytochrome *c* reduction activities in corn root plasma membrane. *Plant Cell Physiol.* 34: 1213–1218.
- Tu, S., Patterson, D., Brauer, D. and Hsu, A-F. (1995) Effects of divalent cations on NADH-linked electron transfer in corn root plasma membrane. *J. Plant Nutri.* 18: 923–936.

(Received September 25, 1995; Accepted December 19, 1995)