식물학회지 제36권 제4호 (1993)
Korean J. Bot. 36(4) : 383~389

Influence of the Mesophyll on the Change of Electrical Potential Difference of Guard Cells Induced by Red-light and CO_2 in *Commelina communis* L. and *Tradescantia virginiana* L.

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닭의장풀과 자주달개비에서 적색광과 이산화탄소에 의해 유도된 공변세포의 전위차 변화에 미치는 엽육세포의 영향

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ABSTRACT

The effects of light and CO_2 on the electrophysiological characteristics of guard cells in the intact leaf and in the detached epidermis have been investigated. Guard cells in intact leaves showed the membrane hyperpolarization in response to light. The biggest induced change of the membrane potential difference (PD) in the guard cells of the intact leaf was 13 mV by light and 42 mV by CO_2 *in Commelina communis*. Similar results were obtained with *Tradescantia virginiana*. However, there were no changes of membrane PD in detached epidermis. In order to determine the influence of the mesophyll on the changes of membrane PD, infiltration of the mesophyll cells with photosynthetic inhibitors was performed. In CCCP infiltrated leaf discs the guard cell membrane was depolarized slightly by red-light and hyperpolarized by CO_2, but in leaf discs infiltrated with DCCD and DCMU the guard cell membrane was hyperpolrized by both red-light and CO_2 as the control leaf discs. In azide infiltrated leaf discs the guard cell membrane showed no response to light and there was a much reduced membrane hyperpolarization by CO_2 compared to other responses. It was likely that azide caused leaf damage and the activity of cell metabolism was decreased greatly, resulting in small membrane PD changes by CO_2 and no changes by red-light. Therefore, it can be suggested that red light was sensed by the mesophyll and the light induced guard cell membrane hyperpolarization was related to energy produced by cyclic-photophosphorylation, but CO_2-induced guard cell membrane hyperpolarization was not related to photosynthesis. Alkalisation of the vacuole was observed when the intact leaf was exposed to CO_2, indicating that membrane hyperpolarization was mainly the result of proton efflux.

INTRODUCTION

Stomata open in the light and close in the dark. Such a movement is driven by the turgor changes in guard cells. The increase in turgor of guard cells is the result of ions accumulated by the generation of a primary electrochemical potential gradient across the plasmalemma. This gradient is assumed to originate through the activity of a proton efflux pump, driven by ATP, which is electrogenic and therefore creates both a pH and a potential difference (PD) gradient across the membrane (Spanswick, 1981). The energy required for such ion uptake can be driven from photosynthesis and respiration (Zeiger, 1983; Assmann and Zeiger, 1987). Zeiger (1983) has proposed that the accumulation of potassium and chloride ions by guard cells during stomatal opening occurs by

such a chemi-osmotic mechanism. He has postulated that potassium moves into the cell down the electrical gradient generated by the proton pump whilst chloride is accumulated as a result of the proton gradient set up. Therefore, stomatal opening is due to uptake of K^+ and other ions (Cl^- and malate) that in turn drives water fluxes. However, how light is sensed and how it is transduced into driving the ion fluxes which control stomatal movement is still not fully understood.

There have been several reports to support a mesophyll contribution to stomatal control (Willmer and Mansfield, 1969; Fischer, 1970; Nelson and Mayo, 1975; Zeiger and Hepler, 1977; Grantz and Schwartz, 1988; Atkinson *et al.*, 1989; Fricker *et al.*, 1991; Lee and Bowling, 1992, 1993). Those reports mainly suggest that stomata in detached epidermis behave both quantitatively and qualitatively differently from those in the intact leaf. Some of the reports also demonstrated that the responses of guard cells in detached epidermis to environmental stimuli were less sensitive than those in the intact leaf. It is possible that if mesophyll cells are necessary for stomatal opening, the change of electrical properties in guard cells could be dependent on the mesophyll cells by way of electrical energy or signal metabolites. Gunar *et al.* (1975) showed that a fairly rapid hyperpolarization of the three types of cells (guard, subsidiary and epidermal) PD by 10~15 mV was observed upon the switching-on of the light in intact leaves of *Tradescantia albiflora*. They suggested that since epidermal and subsidiary cells do not have chlorophyll, changes in their PD induced by light could be associated with electrical excitation propagated from the mesophyll cells. Cheesman *et al.* (1982) also found that membrane potentials in isolated strips were considerably lower than those in intact leaves and were insensitive to light. Furthermore, there are a number of reports suggesting that photoinduced ion fluxes in green plant tissue are probably associated with pumps regulated by photosynthesis (Jeschke, 1970; Higinbotham, 1973), photo-synthetic electron transport (Hartmann, 1975) and cyclic-photophosphorylation (Spanswick, 1973). The above reports evoke the possibility that membrane hyperpolarization of guard cells in response to light could be related to the mesophyll cells.

However, no attempts to investigate how the mesopbyll can contribute to guard cell membrane hyperpolarization has been made. Therefore, this study was carried out to investigate the influence of the mesophyll on the guard cell membrane hyperpolarization in response to light and CO_2.

MATERIALS AND METHODS

Plant material. *Commelina communis* and *Tradescantia virginiana* were potted in John Innes No. 2 compost supplemented with Phostrogen and watered every morning. They were grown in a glass house (minimum temperature of 20℃ during the day and 15℃ at night) under a light regime of 18 hours day and 6 hours night (natural daylight supplemented by high pressure sodium lighting (150 μmol $m^{-2}s^{-1}$).

Vacuolar PD measurements. The measurements were conducted on the abaxial surface of the leaves of *Tradescantia* and *Commelina*. Four or five week-old fully expanded leaves were excised early in the morning and floated on distilled water in the dark for 1 h to ensure that the stomata were tightly closed. After 1 h, the leaf was put, abaxial side uppermost, on a slide which was placed in a chamber with two holes provided for the air stream. The slide was inclined at 30° to facilitate micro-electrode implantation. The cut end of the leaf was dipped in KCl (10 mM). The chamber was installed on the stage of a stereomicroscope and the leaf surface was viewed at a magnification of ×100 with the microscope lamp giving 100 μmol $m^{-2}s^{-1}$. Light intensity used in the measurements was 550 μmol $m^{-2}s^{-1}$, provided by a Lux 150 4-Port Projector. In light quality experiments, the micro-electrode was inserted under dim green light to avoid any side effects of other light qualities. Red light (100 μmol $m^{-2}s^{-1}$) was obtained by passing light from a Lux 150 4-Port Projector through a plastic filter (transmitting above 625 nm); blue light and green light were obtained using a plastic filter (blue: transmitting at 512~412 nm; green: transmitting at 575~475 nm). When a steady reading for the PD had been obtained, CO_2 was given to the leaf. A stream of CO_2, obtained from a cylinder (pure CO_2) passed into the box chamber via air flow meter.

Micro-electrodes for PD measurements were pulled from glass capillaries (2.0 mm outside diameter, 1.6 mm inside diameter with internal filament, Clark Electromedical Instruments, Pangbourne, Berks, U.K.) filled with 3 M KCl and connected by way of a Ag/AgCl half cell to a voltage follower and chart recorder. The circuit was completed by a Ag/AgCl reference electrode dipping into the bathing solution. Microelectrodes were inserted into the cells using a micromanipulator (Research Instruments TVC 300). In some experiments, the membrane PD of guard cells of detached epidermis in response to light was measured. A small piece of strip, about 1.0 cm^2, was

held in 5.0 cm^3 of the medium (10 mM KCl) by a fixed plastic ring in a Petri-dish so that the strip was just submerged. The PD was measured under an Olympus CK2 inverted microscope (Olympus Optical Co. Ltd., Tokyo, Japan) at a magnification of ×200.

Infiltration of the mesophyll cells with photosynthetic inhibitors. The mature leaves (second bifoliate leaf) were excised, and their petioles were immediately dipped into a solution containing 50 μM $CaCl_2$, 250 μM $MgSO_4$, buffered at pH 5.9 with 5 mM sodium citrate/10 mM sodium phosphate (standard medium), with (treated samples) or without (control) 100 μM DCMU (3(3,4-dichlorophenyl)-1,1-dimethylurea), 1 μM CCCP (carbonylcyanide m-chlorophenylhydrazone), 50 μM DCCD (dicyclohexylcarbodiimide), or 1 μM NaN_3. The leaves were kept in a glass house (minimum temperature of 20℃ during the day and 15℃ at light) under a light regime of 18 hours day and 6 hours night (natural daylight supplemented by high pressure sodium lighting (150 μmol $m^{-2}s^{-1}$). This infiltration lasted 48 hours, and its efficiency was checked by measuring the rates of uptake of medium. The volume of uptake of medium was around 0.9 mL for 24 hours.

Micro-electrode determination of vacuolar pH. Direct measurement of vacuolar pH was carried out using pH sensitive micro-electrodes. pH sensitive micro-electrodes were made by the same method used by Bowling (1989). Microelectrode resistancewas typically 0.4~0.5 gigaohms. The calibration of the pH sensitive micro-electrode was carried out with standard pH buffer solutions (pH 4, 5, 6, 7 and 9.2). The slope of the calibration curve between pH 4.0 and pH 9.2 was always greater than 50 mV per pH unit. Reference electrodes were made by pulling micropipepttes as before and filling with 3 M KCl. The pH and reference micro-electrodes were connected via Ag/AgCl half-cells to a high impedance electrometer (437 Electrometer, Pitman Instruments, Weybridge, England).

The samples of intact leaf for pH measurements were prepared using the same method described for the PD experiments. The pH was measured by lowering both micro-electrodes on to the same cell as close together as possible without touching (5~10 μm apart) using Zeiss micromanipulators (C-J, Jena). When a steady reading for the pH had been obtained, a stream of CO_2 or light was given to the leaf disc. The source of light and CO_2 was the same as for the PD experiments. The micro-electrode tips could be clearly seen under the microscope, but when the micro-electrode was inserted into the cell in order to measure vacuolar pH, its tip could not be seen. However, its location in the cell could be inferred from the reading obtained. pH values between 5.0 and 6.0 indicated that the tip was in the vacuole (Bowling and Edwards, 1984). Micro-electrodes were calibrated before and after readings. The pH of the suspension medium buffer (10 mM MES-KOH, pH 6.15, 100 mM KCl) did not vary over the course of the measurement. In some experiments with detached epidermis this buffer was used with the same method as the PD measurements.

RESULTS

The effect of light and CO_2 on the membrane PD of guard cells in a closed stoma in the intact leaf is shown in Fig. 1. Fast hyperpolarization of guard cell membrane PD was recorded reaching up to 13 mV both for *Tradescantia* and *Commelina* in response to light. The initial responses were the fastest and the saturation point of hyperpolarization was reached within 30 sec. It then slowly depolarized to near the start point while the light was on. In darkness, a stable base reading of PD was recorded and sometimes, there was a very slow depolarization.

When a steady reading for the PD had been obtained, a stream of CO_2 was passed into a chamber which the leaf disc was put inside. At the onset of CO_2, the PD showed a dramatic hyperpolarization of between 10 and 15 mV in *T. virginiana* or 42 mV in *C. communis* (Fig. 1).

The saturation point of hyperpolarization was reached with a lag time between 1 and 6 sec. The effect of CO_2 on the PD was greater and faster than that of light. In

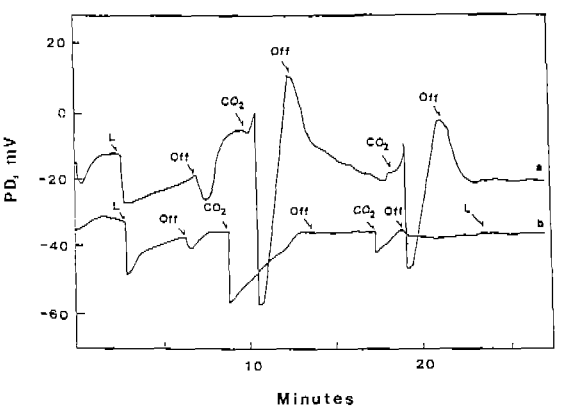

Fig. 1. The effect of white light (L) and CO_2 on the change of membrane PD of the guard cell in a closed stoma in the intact leaf of *C. commelina* (a) and *T. Virginiana* (b).

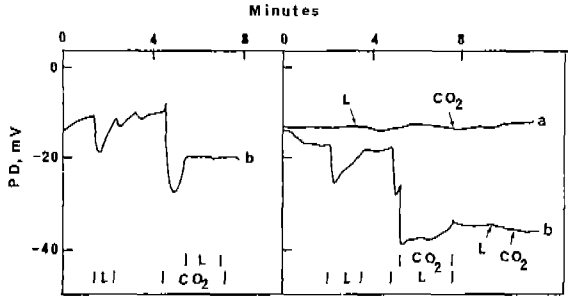

Fig. 2. The relationship between white light and CO_2 on the change of membrane PD of the guard cell of a closed stoma in the detached epidermis (a) and the intact leaf (b) of *T. virginiana*.

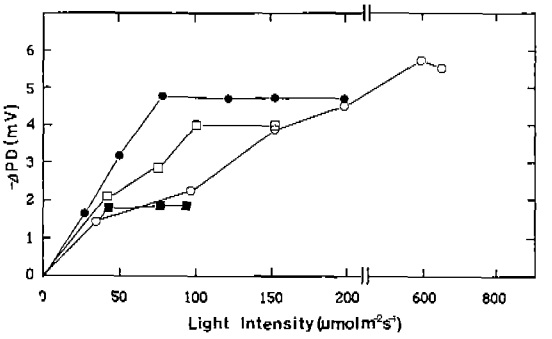

Fig. 3. The effect of the light intensity according to light quality on the change of membrane PD of the guard cell in a closed stoma in the intact leaf of *T. Virginiana*. Each point is the mean of 3 or 4 measurements. Open circles, white light; closed circles, red light; open rectangles, green light; closed rectangles, blue light.

these experiments, if the guard cells were given two separate CO_2 pulses, the second response was smaller than the first and if light was given after CO_2 treatment, there was no response (Figs. 1b and 2). When light and CO_2 were given together, the light effect was masked by the CO_2 effect (Fig. 2).

The effect of CO_2 was so dramatic that the possibility of it being an artifact was investigated. The effect of normal air and CO_2 free air on the change of guard cell membrane PD was investigated. When they passed over the leaf, these air currents caused no more than small fluctuations in the PD (data not shown).

Whenever this experiment was attempted with detached epidermis, there was no response to light or CO_2 (Fig. 2a). This could be due to the different conditions for the measurements in the intact leaf and in the detached epidermis and difficulties in penetrating detached epidermal cells. Even though agar gel was used to support the epidermis, enabling easy penetration of the electrode, there was still no response. Considering that peeled epidermis can be injured upon electrode penetration the vibrating probe which can measure small currents without penetrating the epidermis has been applied (data not shown). However, there were no currents changes by light in detached epidermis.

Fig. 3 shows the effect of light intensity according to lightquality on the change of membrane PD of the guard cell. The saturation point of the membrane PD was around 500 $\mu mol\ m^{-2}s^{-1}$ in response to white light, but in response to red and blue light, it was 80 and 40 $\mu mol\ m^{-2}s^{-1}$. The mean PD change caused by white, red, green and blue light at the saturation point of light intensity was respectively 5.3, 5.2, 4.0 and 3.2 mV. Likewise, the change of membrane PD of the guard cell in response to blue light was relatively small and saturated at the low light intensity. However, even though the saturation points between white and red light were different, the magnitude of white and red induced hyperpolarization was almost same, indicating that white light response mainly came from red light effect. This suggested that photosynthesis in the mesophyll could be related to the guard cell membrane hyperpolarization. Therefore, the effects of photosynthetic inhibitors have been used to study the relationship between the guard cell membrane hyperpolarization and photosynthesis. Infiltration of the mesophyll cells with DCMU, CCCP, DCCD and NaN_3 for 48 hours was performed. The effect of blue light on the change of membrane PD was too small to allow comparisons between inhibitor treatments, therefore red-light and CO_2 only were used in these experiments (Fig. 4).

100 μM DCMU and 50 μM DCCD had no effect on guard cell membrane hyperpolarization in response to light and CO_2. In 1 μM CCCP infiltrated leaf discs the guard cell membrane was depolarized slightly in response to light, but CO_2 caused abrupt hyperpolarization, similar to the effect on control leaves. In 1 μM azide infiltrated leaf discs the guard cell membrane showed no response to light and there was a much reduced membrane hyperpolarization by CO_2 compared to other infiltrated treatments, indicating that the activity of whole plant metabolism was reduced.

To investigate if membrane hyperpolarization is the result of the proton efflux, the effect of CO_2 on the pH of the vacuolar sap of guard cells in intact leaves was

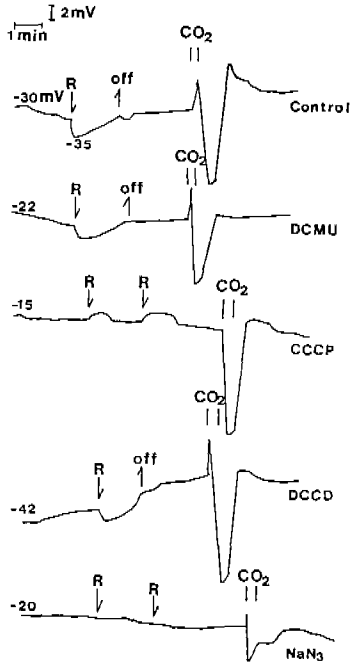

Fig. 4. The effect of mesophyll cell infiltration by photosynthetic inhibitors (DCMU, CCCP, DCCD and NaN_3) in the change of membrane PD of the guard cell of a closed stoma in the intact leaf of *C. communis* when the leaf was exposed to red-light and CO_2.

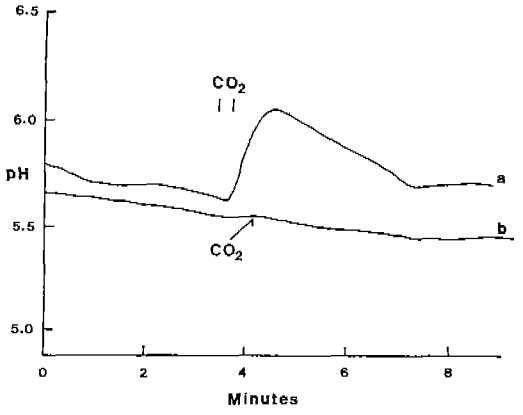

Fig. 5. The effect of CO_2 on the change of vacuolar pH of a closed guard cell in the intact leaf (a) and the detached epidermis (b) of *C. communis*.

investigated (Fig. 5). CO_2 caused the alkalization of guard cell up to about 0.3 pH unit in the intact leaf. However, this change was absent in detached epidermis.

DISCUSSION

This study suggests that guard cell membrane hyperpolarization by light could be associated with the mesophyll cells. It was found that the membrane hyperpolarization by white light had been mostly obtained by the contribution of photosynthetic red-light, suggesting that guard cell membrane hyperpolarization was related to mesophyll photosynthesis. This was clarified by the results of the photosynthetic inhibitor infiltration experiments.

In CCCP infiltrated leaf, the guard cell membrane was not hyperpolarized in response to red-light, but CO_2 caused abrupt hyperpolarization, similar to the effect on control leaves. CCCP is generally recognized as an effective inhibitor of cyclic-photophosphorylation (Klob *et al.*, 1973). This suggested that the guard cell membrane hyperpolarization could be related to ATP produced by cyclic-photophosphorylation in the mesophyll. Spanswick (1973, 1981) also reported that photoinduced ion fluxes in green plant tissue are probably associated with cyclic-photophosphorylation.

It is generally believed that the rapid electrical changes could be related to mechanism of stomatal opening and closing in several ways (Hsiao, 1976; Moody and Zeiger, 1978). Hence, it is possible that stomatal opening in the intact leaf to a greater or lesser extent is dependent on an electrical signal or an energy propagated from the mesophyll. In favour of this hypothesis Wong *et al.* (1979) suggested that the stomata responded to another metabolite of photosynthesis in the leaf of mesophyll tissue. They considered that ATP or NADPH could be the substances involved.

Outlaw (1989) in his review showed that most reports indicate that the photosynthetic carbon-reduction pathway is absent in guard cells or, at most, does not exceed about 5% of that in the mesophyll cells on a chlorophll basis. If the guard cell itself could not supply all the requirements of energy and osmotically active sugars for stomatal opening, then import from the mesophyll must occur. A number of biochemical data has demonstrated that there is fairly rapid exchange of metabolites and others, such as hormones, ATP and NADPH) between the mesophyll and epidermis (Thorpe and Milthorpe, 1984; Morison, 1987; Willmer *et al.*, 1978). Though the role of the movement of such metabolites and their rates and amounts *in vivo* has yet to be clarified, it can be suggestted that this movement affects the long-term, perhaps daily, stomatal response.

Raschke and Hedrich (1989) reported that blue light

activated pumps in the plasmalemma but red light had no effect on the change of PD of guard cell protoplasts. Zeiger and Hepler (1977) reported that stomata in isolated epidermis of onion were only sensitive to blue light. However, they found both red and blue light responses in paradermal slices which included several layers of mesophyll cells. Futhermore, guard cell protoplasts swelled when illuminated with blue light, but red light had no effect (Zeiger and Hepler, 1977; Lee and Bowling, 1993). This in turn favours the hypothesis that blue light is perceived by stomata at the guard cell itself whereas red light is sensed by the mesophyll. This hypothesis is broadly in line with Fischer (1970), Gunar *et al.* (1975) and Wong *et al.* (1979). In the leaves infiltrated with photosynthetic inhibitors the inhibition of guard cell photosynthesis could be also imagined, but its effect was negligible as stomata indetached epidermis did not show the membrane hyperpolarization by light and CO_2.

CO_2 induction of membrane hyperpolarization was unexpected because CO_2 is generally known as an inhibitor of stomatal opening. Spanswick (1973) reported that CO_2 inhibited membrane hyperpolarization. However, in that case hyperpolarization was stimulated when CO_2 was eliminated, while light was still present. This result could be interpreted as the light response being inhibited by CO_2. So then, how can the CO_2-induced membrane hyperpolarization be explained in connection with stomatal closing? The results in Figs. 1 and 2 can be explained in two points. Firstly, CO_2 inhibits the light response. Secondly, CO_2 may change membrane permeability and recovery of membrane structure may take some time. Likewise, CO_2 could inhibit stomatal opening by affecting light response and altering the membrane permeability. This view was supported by Edwards and Bowling (1985). Moody and Zeiger (1978) also suggested that the activity of a proton pump could cause changes in membrane permeability. This study also suggests that CO_2-induced membrane hyperpolarization is mainly the result of the proton pump. Therefore, it can be suggested that CO_2 affects membrane permeability after the passive proton efflux. Other rapid effects of CO_2 in stomatal movements could be mediated by an amplified acidification of guard cell cytoplasm, and a concomitant decrease in the pmf (proton motive force), or the use of HCO_3^- as a counter ion for K^+ (Edwards and Bowling, 1985; Gepstein *et al.*, 1982; Hsiao, 1976; Raschke, 1979; Zeiger *et al.*, 1978).

적 요

Intact leaf과 detached epidermis에 있는 공변세포의 전기 생리학적 특성에 대한 빛과 이산화탄소의 효과를 조사하였다. 빛을 intact leaf의 abaxial side에 처리하면 공변세포막이 과분극(hyperpolarization)되었다. 닭의장풀의 intact leaf에 있는 공변세포들은 빛에 의해 최대 13 mV 그리고 이산화탄소에 의해 42 mV까지 membrane potential difference(MPD)가 negative하게 변했다. 자주달개비에서도 비슷한 결과를 얻었다. 그러나, 빛과 이산화탄소를 detached epidermis에 있는 공변세포에 처리할 경우에는 공변세포의 MPD가 변하지 않았다. 위의 결과들로부터, 엽육세포가 공변세포의 MPD 변화에 영향을 주는 것으로 사료되어, 엽육세포들을 광합성 억제제들을 침윤시켜 엽육세포 광합성의 어느 기작이 공변세포 MPD 변화에 영향을 주는지 조사하였다. CCCP로 침윤한 잎의 공변세포막은 적색광에 의해 약간 탈분극(depolarization)되었고 이산화탄소에 의해 과분극되었다. 반면에, DCCD와 DCMU로 침윤한 경우에는 대조구 잎과 마찬가지로 적색광과 이산화탄소에 의해 과분극되었다. Azide로 침윤한 잎에 적색광을 처리하면 공변세포의 MPD는 변하지 않았고, 이산화탄소를 처리하면 다른 처리구들에 비해 훨씬 감소한 막의 과분극을 보였다. 이는 azide가 잎에 손상을 유도하며 세포내 대사활성을 감소시킨 결과 이산화탄소에 의한 MPD 변화가 작았고, 적색광은 아무 효과도 보이지 않은 것으로 사료된다. 따라서, 엽육세포가 적색광을 감지하며 빛에 의해 유도된 공변세포막 과분극은 순환적 광인산화 반응에 의해 생성된 에너지에 의존하나 이산화탄소에 의해 유도된 공변세포막 과분극은 광합성과 무관하다고 볼 수 있다. 또한 이산화탄소를 intact leaf에 처리하면 공변세포 액포가 알칼리화되는 것을 관찰하였는데, 이는 막의 과분극이 양성자 이온의 방출에 의해 일어난다는 것을 의미한다.

ACKNOWLEDGEMENT

Joonsang Lee was an recipient of grants from Korean Ministry of Education during 1989-1992.

REFERENCES

Assmann, S.M., and E. Zeiger. 1987. Guard cell bioenergetics. *In*, Stomatal Function, E. Zeiger, G.D. Farquhar and I.R. Cowan (eds.). Stanford University Press, Stanford. pp. 163-193.

Atkinson, C.J., T.A. Mansfield, A.M. Kean and W.J. Davies. 1989. Control of stomatal aperture by calcium in isolated epidermal tissue and whole leaves of *Commelina communis* L. *New Phytologist* **111**: 9-17.

Bowling, D.J.F. 1989. Intracellular and intercellular pH measurement with microelectrodes. *Methods In Enzymology* **174**: 331-338.

Bowling, D.J.F. and A. Edwards. 1984. pH gradients in stomatal complex of *Tradescantia Virginiana*. *J. Exp. Bot.* **35**: 1641-1645.

Cheesman, J. M., M. Edwards and H. Meidner. 1982. Cell potentials and turgor pressures in epidermal cells of *Tradescantia* and *Commelina*. *J. Exp. Bot.* **33**: 761-770.

Edwards, A. and D.J.F. Bowling. 1985. Evidence for a CO_2 inhibited proton extrusion pump in the stomatal cells of *Tradescantia virginiana*. *J. Exp. Bot.* **36**: 91-98.

Fischer, R.A. 1970. After-effects of water stress on stomatal opening potential. II. Possible causes. *J. Exp. Bot.* **21**: 386-404.

Fricker, M.D., D.A. Grantz and C.M. Willmer. 1991. Stomatal responses measured using a viscous Flow (Liquid) porometer. *J. Exp. Bot.* **42**: 747-735.

Gepstein, S., M. Jacobs and L. Taiz. 1982. Inhibition of stomatal opening in *Vicia faba* epidermal tissue by vanadate and abscisic acid. *Plant Science Letter* **28**: 63-72.

Grantz, D.A. and A. Schwartz. 1988. Guard cells of *Commelina communis* L. do not respond metabolically to osmotic stress in isolated epidermis: Implications for stomatal responses to drought and humidity. *Planta* **174**: 166-173.

Gunar, I.I., I.F. Zlotnikova and L.A. Panichkin. 1975. Electrophysiological investigation of cells of the stomata complex in Spiderwort. *Soviet Plant Physiol.* **22**: 704-707.

Hartmann, E. 1975. Influence of light on the bioelectric potential of the bean (*Phaseolus vulgaris*) hypocotyl hook. *Physiol. Plant.* **33**: 266-275.

Higinbotham, N. 1973. Electropotentials of plant cells. *Ann. Rev. Plant Physiol.* **24**: 25-46.

Hsiao, T.C. 1976. Stomatal ion transport. *In*, Transport in plants, U. Luttge and M.G. Pitman (eds.). Encyclopedia Plant Physiology, Vol. 2B, Springer-Verlag, Berlin. pp. 195-221.

Jeschke, W.D. 1970. Lichtabhangige Veranderungen des Membranpotentiala bei Blattzellen von Elodea densa. *Zeitschrift fur Pflanzenphysiology* **62**: 158-172.

Klob, W., O. Kandler and W. Tanner. 1973. The role of cyclic photophosphorylation *in vivo*. *Plant Physiol.* **51**: 825-7.

Lee, J.S. and D.J.F. Bowling. 1992. Effect of the mesophyll on stomatal opening in *Commelina communis*. *J. Exp. Bot.* **43**: 951-957.

Lee, J.S. and D.J.F. Bowling. 1993. Effect of a mesophyll factor on the swelling of guard cell protoplasts of *Commelina communis* L. *J. Plant Physiol.* **142**: 203-207.

Nelson, S.D. and J.M. Mayo. 1975. The occurrence of functional non-chlorophyllous guard cells in *Paphiopedilum* spp. *Canadian J. Bot.* **53**: 1-7.

Morison, J.I.L. 1987. Intercellular CO_2 concentration and stomatal response to CO_2. *In*, Stomatal Function, E. Zeiger, G.D. Farquhar and I.R. Cowan (eds.). Stanford University Press, Stanford. pp. 229-251.

Moody, W. and E. Zeiger. 1978. Electrophysiological properties of onion guard cells. *Planta* **139**: 159-165.

Outlaw, W.H.Jr. 1989. Critical examination of the quantitative evidence for and against photosynthetic CO_2 fixation by guard cells. *Physiol. Plant.* 77: 275-281.

Raschke, K. 1979. Movements of stomata. *In*, Physiology of movements, W. Haupt and M.E. Feinleib (eds.). Encyclopedia of Plant Physiology, Vol. 7, springer-Verlag, Berlin. pp. 381-441.

Raschke, K. and R. Hedrich. 1989. Patch clamp measurements on isolated guard cell protoplasts and vacuoles. *Methods in Enzymology* **174**: 312-330.

Reckman, U., R. Scheibe and K. Raschke. 1990. Rubisco activity in guard cells compared with the solute requirement for stomatal opening. *Plant Physiol.* **92**: 246-253.

Spanswick, R.M. 1973. Evidence for an electrogenic ion pump in *Nitella translucence*. II. Control of the light-stimulated component of the membrane potential. *Biochim. Biophy. Acta* **289**: 387-398.

Spanswick, R.M. 1981. Electrogenic ion pumps. *Ann. Rev. Plant Physiol.* **67**: 1124-1132.

Thorpe, N. and F.L. Milthorpe. 1984. Transport of metabolites between the mesophyll and epidermis of *Commelina cyanea* R. *Br. Australian J. Plant Physiol.* **11**: 59-68.

Willmer, C.M. and T.A. Mansfield. 1969. A critical examination of the use of detached epidermis in studies of stomatal physiology. *New Phytologist* **68**: 363-375.

Willmer, C.M., N. Thorpe, J.C. Rutter and F.L. Milthorpe. 1978. Stomatal mechanism: carbon dioxide fixation in attached and detached epidermis of *Commelina*. *Australian J. Plant Physiol.* **5**: 767-778.

Wong, S.C., I.R. Cowan and G.D. Farquhar. 1979. Stomatal conductance correlates with photosynthetic capacity. *Nature* **282**: 424-426.

Zeiger, E. 1983. The biology of stomatal guard cells. *Ann. Rev. Plant Physiol.* **34**: 441-475.

Zeiger, E., A.J. Bloom and P.K. Hepler. 1978. Ion transport in stomatal guard cells: A chemiosmotic hypothesis. *What's New in Plant Physiology* **9**: 29-32.

Zeiger, E. and P.K. Hepler. 1977. Light and stomatal function: Blue light stimulates swelling of guard cell protoplasts. *Science* **196**: 887-889.

(Received October 25, 1993)