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〈Mini-review〉

The Possible Participation of the Mesophyll on Stomatal Opening

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Abstract - Many researchers have been studied with guard cell protoplasts and detached epidermis as they think that properly stabilized protoplasts and detached epidermis retain many of the properties of intact guard cells. However, some studies have shown that stomata in detached epidermis behave differently, both quantitatively and qualitatively, from those in the intact leaf. Stomata in the intact leaf are very sensitive to environmental factors such as light, CO_2 and osmotic stress, but stomata in detached epidermis are less sensitive to these factors than those in the intact leaf. The clearest evidence to suggest the different response between detached epidermis and intact leaf obtained from the experiments with heavy metal, cadmium. 3-weeks old *Commelina. communis* was transferred to and grown in Hoagland solution in the presence or absence of 5 mM Cd^{2+} for 4 days. The application of Cd^{2+} showed about 70% inhibition of stomatal conductance when measured at various light intensity (100~1,000 μmole $m^{-2}s^{-1}$). However, stomata in detached epidermis floated on an incubation medium containing 100 μM Cd^{2+} opened to a degree of about 8.38 μm, but the stomata treated with no cadmium opened to 3.74 μm. These results were unexpected as the intact leaf grown in a Hoagland solution containing cadmium showed very negative physiological responses. These results showed that stomata in detached epidermis and in the intact leaf could respond reversely. Therefore, it is possible that we now misunderstand how stomata open in real natural condition.

Key words : Detached epidermis, Isolated epidermis, Stomata

Introduction

Environmental factors such as light and low CO_2 concentrations trigger events which may result in stomatal opening. How these signals are sensed and how they are transduced into driving the ion fluxes which control stomatal movements in the intact leaf is not fully understood.

The hypothesis which is now widely accepted to explain stomatal activity involves fluxes of inorganic cations and anions across the plasmalemma and tonoplast of guard cells associated with the synthesis and degradation of organic anions. When stomata open, protons are first pumped out from the guard cell, resulting in hyperpolarization of the plasmalemma potential difference. K^+ may then passively enter the guard cell to higher osmotic potential. The primary osmotic species involved in stomatal activity is now accepted as being the potassium ion. Some Cl^- also enters the guard cells, but complete charge balance of the excess K^+ is accomplished by synthesis of malate (MacRobbie 1987).

This theory supports a direct response of stomata to light initially demonstrated by the work of Heath and Russell (1954) and more recently studied by Zeiger *et al.* (1987), Zeiger (1990) and Zeiger and Zhu (1998). They suggested that stomatal responses to white light are the combined expression of two distinct photoreceptor systems: the guard cell chloroplasts and a blue light-dependent photosystem. Zeiger and Zhu (1998) reported

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that xanthophyll, zeaxanthin has recently been identified as a blue light photoreceptor in guard cells. Assman and Zeiger (1987) reported that guard cell chloroplasts themselves could supply all the requirements of ATP to fuel the plasma membrane proton pump coupled to potassium influx thereby leading to stomatal opening.

If we accept the theory of a direct response of stomata to light, it means that guard cells itself have the total potential of stomatal control without any relation or influence from the mesophyll cells. However, there are accumulating reports indicating the possibilities of mesophyll participation on stomatal control in the intact leaf. Heath and Russell (1954) suggested that there was an indirect effect transmitted either from the epidermal cells or through them from the mesophyll cells by some agent of a chemical or electrical nature. Wong *et al.* (1979) found that the diffusive conductance of leaf epidermis to CO_2 transfer changed proportionately with the rate of assimilation. They considered that this suggested that the stomata responded to a metabolite of photosynthesis in the mesophyll. Lee and Bowling (1992, 1993a, b, 1995) proposed continually that stomatal opening in the intact leaf could be dependent on an electrical signal or a chemical propagated from the mesophyll, however, these factors have still to be identified.

Talbot and Zeiger (1998) reported that both K^+ and sucrose are primary guard cell osmotica, and that the use of these two solutes is separated into two distinct phases in which one or the other constitutes the dominant osmoticum. In the intact leaf, opening at the beginning of a day cycle is supported by K^+. In the second half of the daily cycle, K^+ content in guard cells decreases dramatically and sucrose becomes the dominant solute. Gerhard *et al.* (1999) also reported that sucrose could replace potassium and malate as the osmoticum for the maintenance of stomatal opening. Their reports invoke two aspects. The first is that the impotance of sucrose as a osmotica of a day cycle on stomatal opening in the intact condition of the plant implies that we need to reexamine the starch-sugar hypothesis which has been replaced by the present paradigm of guard cell osmoregulation by K^+ and its counterions. The second aspect is that if sucrose is the important osmotica on stomatal opening in the intact leaf, where the sucrose come from. Does the sucrose come from the mesophyll cells which are the most active site of the photosynthesis or from guard cell photosynthesis. Outlaw (1989), in his review, disputes the notion of the operation of the Calvin cycle in guard cells. Some researchers reported that guard cells have low levels of Rubisco activity (Gotow *et al.* 1988; Reckmann *et al.* 1990). More interesting reports come from studies of lady's slipper orchid, *Paphiopedilum*. In the case of *Paphiopedilum* (Nelson and Mayo 1975; Rutter and Willmer 1979), the Calvin cycle is not present in guard cells since chloroplasts are absent although the stomata are functional. This species provides evidence that guard cell chloroplasts may not be necessary as a source of ATP and reducing power for ion transport and other processes essential to the functioning of stomata. Nelson and Mayo (1975) observed that the stomata of *Paphiopedilum* opened normally in light. This raises the possibility that red photo-receptor may be located in the chloroplasts of the mesophyll.

Here, it is suggested that guard cells respond differently from their conditions to environmental signals and the possibility for the participation of the mesopyll on stomatal opening in the intact leaf.

Stomata in detached epidermis behave differently from those in the intact leaf

The basic role of stomata is to regulate transpiration and photosynthesis. Photosynthesis plays a central role in the physiology of plants and an understanding of its response to light is, therefore, critical to any discussion of how plant sense and respond to light. It is likely that many responses exhibited by plants to light are in fact mediated by the response of photosynthesis to light. This suggests the idea that stomata should be controlled according to the demand of the mesophyll cells.

Analyses of stomatal responses to light led to the conclusion that, in many cases, stomata in detached epidermis behave differently from those in the intact leaf. Attention in stomatal mechanism has concentrated on the mechanisms of ion uptake in guard cells as flux measurements are only possible in isolated epidermal strips, detached epidermis or guard cell protoplasts. If guard cells in isolated epidermal strips, detached epidermis or guard cell protoplasts are stably alive, many researchers think that they show responses as a cell to

environmental or mechanical exposures. However, many reports suggest that stomata in detached epidermis behave both quantitatively and qualitatively differently from those in the intact leaf. Some of the reports demonstrated that the responses of guard cell in detached epidermis to environmental stimuli were less sensitive than those in the intact leaf (Willmer and Mansfield 1969; Travis and Mansfield 1979; Cheesman *et al.* 1982; Grantz and Schwartz 1988; Fricker *et al.* 1991; Lee and Bowling 1992, 1993a, b, 1995; Lee, 2000b).

Intact guard cells swell against the back pressure from subsidiary or epidermal cells and require enough turgor to overcome the resistance to expansion provided both by the guard cell wall and by the turgor of surrounding cells. Stomata in isolated epidermal strips tend not to close completely in the absence of back pressure, and openings of 4 to 6 μm, or more, are commonly found (MacRobbie 1987). The term 'isolated' refers to guard cells in epidermal strips in which only guard cells are alive, but in detached epidermis, guard cells, subsidiary and epidermal cells are all alive.

In *Vicia*, guard cell K^+ concentrations of 460 to 880 mmol l^{-1} are associated with opening against turgid epidermal cells, whereas opening is achieved in isolated guard cells with only 276 to 357 mmol l^{-1} K^+ (Humble and Raschke 1971; Allaway and Hsiao 1973; Outlaw and Lowly 1977). In *Commelina*, open intact guard cells contain 385 to 600, compared with only 157 to 174 mmol l^{-1} in open isolated guard cells (Penny and Bowling 1974; MacRobbie and Lettau 1980a, b). This difference may be come from the physical difference between intact and isolated guard cells. This difference also imply that they behave differently.

Lee and Bowling (1992) observed that stomata in detached epidermis showed no response to CO_2. On the other hand, stomata in the intact leaf of *C. communis* responded markedly to experimentally manipulated CO_2 concentrations. In addition, stomata in the intact leaf opened to a maximum aperture of about 16 μm after about 70 min. in the light and closed rapidly on transfer to the dark. In contrast, stomata in the detached epidermis floating on an incubation solution containing 100 mmol l^{-1} did not respond to light.

Willmer and Mansfield (1969) reported that in *Vicia faba* the light effect was very apparent on attached epidermis, but on detached epidermis the effect was largely obscured by stomatal opening that occurred in darkness. They also found that effects of CO_2 concentration were detectable on detached epidermis, particularly in darkness, but were of a smaller magnitude than those on attached epidermis.

Travis and Mansfield (1979) found that stomatal responses to light and CO_2 in detached epidermis from *Commelina* were dependent on the KCl concentration in the incubation medium. They could eliminate the light and CO_2 effects altogether by manipulation of the medium. Grantz and Schwartz (1988) demonstrated that detached epidermis may show rather different stomatal responses from those found in the intact leaf. They found that guard cells of *C. communis* did not respond metabolically to osmotic stress in detached epidermis. However, in intact discs stomata exhibited clear, hydroactive stomatal responses. Fricker *et al.* (1991) measured stomatal aperture in detached epidermis of *C. communis* using a liquid flow porometer and observed that there was no response by the stomata to light. They suggested that the lack of light-stimulated opening is not unique to *C. communis* or to their system as similar results were found with *Vicia faba*. Hedrich and Neher (1987) reported that blue light activated pumps in the plasmalemma but red light had no effect on the change of the potential difference in guard cell protoplasts. This indicates that red light does not stimulate proton efflux across the guard cell membrane.

Lee (1994) investigated that the effects of light quality on the electrical characteristics of guard cells in the intact leaf. Guard cell showed the membrane hyperpolarization in response to white, red, green, and blue. The change of membrane potential difference (PD) of the guard cell in response to blue light was relatively small and saturated at the low light intensity. Even though the saturation points between white and red light were different, the magnitude of white- and red-light induced hyperpolarization was almost same, indicating that white light response mainly came from red light effect. This favours the hypothesis that in the intact leaf, red light might be more important on stomatal opening than blue light. Lee and Bowling (1993) also found that the guard cell membrane was hyperpolarised

by light and CO_2 in the intact leaf, but not in detached epidermis. Lee (1994) also used a vibrating probe (Model NJ 806, The Vibrating Probe Co., New York, U.S.A) to detect and measure electrical currents at the surface of excised leaf and detached epidermis. When intact leaf was illuminated by white light (550 μmole $m^{-2}s^{-1}$), currents up to 3.5 μA cm^{-2} moving out from the surface were observed, however these currents were absent in detached epidermis. Sergy (1997) investigated bioelectric responses of plant leaves to rhythmical variation of irradiance using a extracellular electrophysiological technique. He observed that all of the species studied responded in a frequency-dependent manner to rhythmical light. He used a resonant analysis approach to understand the composite bioelectric response on the leaf and concluded that the mesophyll components is substantial at higher frequencies.

The clearest evidence to suggest the different response between detached epidermis and intact leaf came from the recent study from Lee (2000a, b). In his experiments, the effect of Cd^{2+} on stomatal conductance in *Commelina communis* was investigated. 3-weeks old *Commelina. communis* was transferred to and grown in Hoagland solution in the presence or absence of 5 mM Cd^{2+} for 4 days. Cd^{2+} was accumulated in all parts of the organs including leaves, epidermis, roots and stem. The proximity from the root and the age of leaf were significant factors responsible for the distribution of cadmium. Most of Cd^{2+} was accumulated in the first leaf which was the nearest from the root. Cd^{2+} accumulation in the leaves led to significant reductions in a series of physiological metabolism. The treatment of Cd^{2+} showed about 70% inhibition of stomatal conductance when measured at various light intensity (Fig. 1).

The effect of cadmium on stomatal apertures in detached epidermis was also investigated Fig. 2). Cadmium stimulated stomatal opening. The result was unexpected as the intact leaf grown in a Hoagland solution containing cadmium showed very negative physiological responses. The stomata, treated with 100 μM Cd^{2+} opened to a degree of about 8.38 μm, but the stomata treated with no cadmium opened to 3.74 μm. To understand how cadmium open stomata, the effect of cadmium on the K^+ influx into the epidermal strips was investigated (Lee 2000b). Cd^{2+}, SA, ABA inhibited 98%, 28%, 34% of K^+ uptake respectively. Lee (2000b) concluded that stomata in epidermal strips and intact leaves behave differently and cadmium-stimulated stomatal opening was due to the result of cadmium uptake into the epidermal strips instead of K^+. Heath and Russell (1954) also obtained some evidence for the possi-

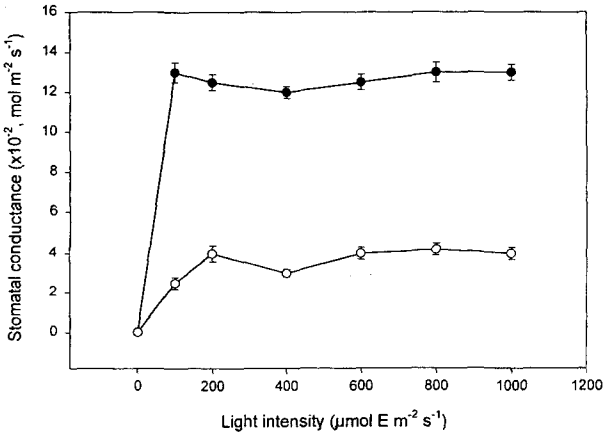

Fig. 1. Stomatal conductance as a function of light intensities in *Commelina communis* L. 3-weeks old *Commelina* was transferred to and grown in Hoagland solution (0 and 5 mM Cd^{2+}) for 4 days and measured stomatal conductance. Each point is the mean (±s.e.m.) of 2 measurements. Closed circles (control), open circles (5 mM Cd^{2+}) (Lee 2000a).

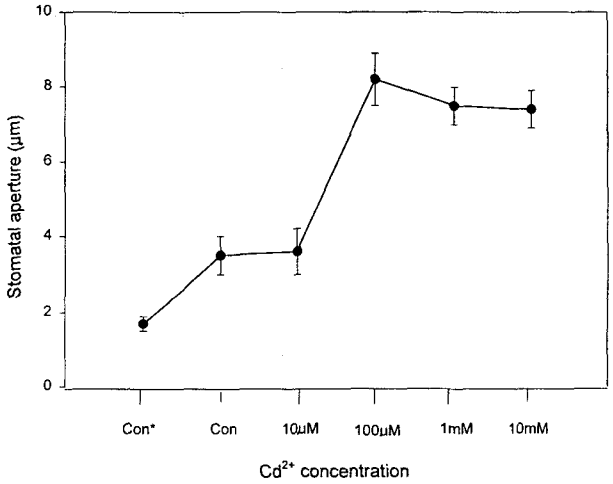

Fig. 2. The effect of Cd^{2+} on stomatal apertures of epidermal strips in *Commelina communis* L. Con* indicates the stomata aperture before the chemicals were treated and Con indicates the results after the chemicals were treated. Each point is the mean (±s.e.m.) of 2 measurements and 40 stomatal apertures were measured (Lee 2000b).

ble participation of the mesophyll cells on stomata. They suggested that there was an indirect effect transmitted either from the epidermal cells or through them from the mesophyll cells by some agent of a chemical or electrical nature. Since this possibility was put forward there have been hints from the results of other investigators of a link between the mesophyll and stomatal aperture. Wong *et al.* (1979) found that the diffusive conductance of leaf epidermis to CO_2 transfer changed proportionately with the rate of assimilation. They considered that this suggested the stomata responded to a metabolite of photosynthesis in the mesophyll. Lee and Bowling (1992, 1993a, b) reported that stomatal opening could be mediated by an electrical signal or a chemical propagated from the mesophyll. In conclusion, it is suggested that the accumulated results on stomatal responses obtained from guard cell protoplasts or epidermal strips may not be directly applied to the understanding of the stomatal responses in the intact leaf. All the above reports also suggest the possibility of mesophyll participation on stomatal control.

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