Journal of Plant Biology, April 2006, 49(2) : 186-192 **SHORT COMMUNICATION**

Response to Red and Blue Lights by Electrical Currents on the Surface of Intact Leaves

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Exposure to red and blue lights caused an increase in electrical currents (0.14 μA cm^{-2} for red and 0.05 μA cm^{-2} for blue, respectively) flowing on the lower surface of leaves from *Commelina communis*. However, no changes were measured in currents from isolated epidermal cells. To determine the influence of the mesophyll on such electrical changes, those cells were infiltrated with photosynthesis inhibitors. Both DCCD treated and control leaf discs showed the same level of response to red light. Epidermal strips were also removed to measure the currents above partially exposed mesophyll cells in order to elucidate the relationship between intact leaves and those mesophyll cells. Changes in current were smaller in the latter type. The partially exposed mesophyll cells of a leaf also showed electrical current changes, but smaller than those of the intact leaf. In DCMU-infiltrated leaf discs, the electrical currents of intact leaves were increased to 0.05 μA cm^{-2} in response to red light. For sodium azide-infiltrated leaf discs, however, intact leaves showed no response. Likewise, a measure of photosynthetic efficiency, the Fv/Fm ratio, was reduced to that measured in the control, thereby indicating that photosynthetic activity significantly altered the electrical current for intact leaves. Therefore, these results demonstrate that the current observed from the lower side of intact leaves is related to photosynthetic activity in the mesophyll cells.

Keywords: Commelina communis, electrical currents, guard cells, mesophyll cells

Driven by the turgor changes in guard cells, stomata open in the light and close in the dark. Increases in turgor pressure results from the accumulation of ions when a primary electrochemical potential gradient is generated across the plasmalemma. This gradient presumably originates through the electrogenic activity of a proton efflux pump driven by ATP, which then creates both a pH and potential-difference gradients across the membrane (Spanswick, 1981). The stomatal guard cells have independent sensory transduction pathways for environmental factors including the influence of light and CO_2 concentrations (Assmann, 1999; Hetherington and Woodward, 2003; Frechilla et al., 2004). However, it is not yet fully understood how these signals are sensed and transduced into driving the ion fluxes that control stomatal movements.

The carotenoid pigment zeaxantin has been suggested as the signal transduction chain for a blue light photoreceptor in guard cells (Zeiger et al., 2002; Talbott et al., 2003). Phototropin has also been postulated as well (Kinoshita et al., 2001). However, neither the location nor the nature of the red-light responses in the stomatal guard cells is very clear. Chloroplasts which are present in the guard cells of most species show photosynthetic electron transport (Willmer and Fricker, 1996; Tsionsky et al., 1997). These guard-cell chloroplasts are generally smaller and less numerous, and have fewer grana than mesophyll chloroplasts (Sack, 1987; Willmer and Fricke, 1996). However, photophosphorylation, on a chlorophyll basis, can be up to 80% higher than in the mesophyll cells (Shimazaki and Zeiger, 1985). Although guard cells have much lower chlorophyll contents than do mesophyll cells (25- to 100-fold less), they are also considerably (approximately 10-fold) smaller (Willmer and Fricker, 1996). Zeiger (1990) has demonstrated that the red light receptors are contained in the guard cell, where chloroplasts are able to do photosynthesis. Furthermore, photosynthetic carbon fixation in the guard cell chloroplasts has been implicated as sensing site (Zeiger et al., 2002; Frechilla et al., 2004).

Although the role of guard cell chloroplasts in CO_2 fixation is still controversial (Lee and Bowling, 1995; Willmer and Fricker, 1996; Lu et al., 1997; Asai et al., 2000; Lawson et al., 2002, 2003; von Caemmerer et al., 2004), a correlation has been reported between photosynthesis and stomatal conductance (Outlaw, 1989, 1996; Lee and Bowling, 1995; Shirke and Pathre, 2004). Outlaw (1989) has reviewed the evidence for such CO_2 fixation by guard cells but has dis-

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missed most of it on the basis of flawed techniques.

Nevertheless, although the sensory transduction chain for red light is not yet clear, it is apparent that red-light photoreceptors are situated in the guard cell itself or in the mesophyll cells. The basic role of the stomata is to regulate transpiration and photosynthesis (Lee and Bowling, 1995). The latter process is central to plant physiology, such that an understanding of its response to light is critical to any discussion of how plants sense and react to that stimulus. However, research on stomatal physiology has been relatively negligible, and more studies are necessary to evaluate the role of mesophyll cells both in fixing carbon dioxide via photosynthesis and in contributing that carbon to epidermal cells over a long period.

It is possible that, if the mesophyll cells are necessary for stomatal opening, then changes in the electrical properties of guard cells may depend on the mesophyll cells through electrical energy or signal metabolites. Fairy rapid hyperpolarization (at a potential difference by 10-15 mV) has been observed in three types of cells (guard, subsidiary and epidermal) when intact leaves of *Tradescantia albiflora* are illuminated (Gunar et al., 1975). Such research has suggested that, because the epidermal and subsidiary cells do not contain chlorophyll, those light-induced changes in potential differences could be associated with the electrical excitation propagated from the mesophyll cells.

A vibrating probe was used to measure electrical current over the surface of detached leaves and isolated portions of the leaf epidermis from *Commelina*. Bowling et al. (1986) have found a linear relationship between electrical current and stomatal apertures.

The main aim of this study was to investigate the role of the mesophyll cells in responding to red and blue lights. Changes in electrical current were measured on the lower sides of intact leaves as well from the isolated epidermis. In addition, mesophyll cells were infiltrated with chemical inhibitors that reduce photosynthetic activity. Afterward, alterations in electrical current were assessed on the lower side of the intact leaf and over the partially exposed mesophyll cells.

MATERIALS AND METHODS

Plant Materials and Growing Conditions

Commelina communis L. was reared from seeds in a growth chamber (minimum day/night temperatures of 20 and 15°C) under a 16-h photoperiod (photon flux density was 300 μmol m^{-2} s^{-1} from mercury lamps). At all stages of development, moisture stress was averted by periodic watering, and 1 g L^{-1} Phostrogen fertilizer was applied twice a week. Three-week-old healthy plants were used as experimental materials.

Measurements of PSII Photochemical Efficiency

Chlorophyll fluorescence was measured with a PAM-2000 fluorometer (Walz, Germany) after dark-adaptation for 1 h. Minimal fluorescence (Fo), with all open reaction centers, was obtained by determining the amount of modulated light sufficiently low enough (<0.1 μmol m^{-2} s^{-1}) not to induce any significantly variable fluorescence. Chlorophyll fluorescence (Fm), with all closed PSII reaction centers, was determined by applying 0.8-s saturation pulse at 1000 μmol m^{-2} s^{-1} to dark-adapted leaves. Variable fluorescence (Fv) was equal to Fm minus Fo, hence, the photochemical efficiency of PSII was defined as Fv/Fm (Kitajima and Butler, 1975).

Infiltration of the Mesophyll Cells with Photosynthesis Inhibitors

The leaves of *C. communis* were infiltrated for 48 h with various chemical inhibitors (Sigma, UK) of photosynthesis (DCMU, DCCD, NaN_3) for 48 h. Mature (second bifoliate leaf) leaves were excised and their petioles immediately dipped in a solution of 0.5 mM $CaCl_2$ and 0.25 mM $MgSO_4$ buffered at pH 5.9 with 5 mM sodium citrate/10 mM sodium, which also contained either the inhibitors or a standard medium. DCMU and DCCD were dissolved in 80% ethanol. The leaves were kept in a growth chamber as described. During the infiltration period, efficiency was monitored according to uptake rate from 0.9 mL of medium over 24 h. Afterward, the effects of red light were examined by measuring the electrical current flowing on the lower sides of intact leaves. Epidermal strips were then removed to measure the currents above the partially exposed mesophyll cells in an effort to elucidate the relationship of electrical flow between intact leaves and mesophyll cells.

Measurements of Electrical Current on the Surfaces of Intact Leaves and from the Isolated Epidermis

Electrical current at the leaf surface was measured with a vibrating probe (Model NJ 806; The Vibrating

Probe, USA), which was mounted on an XYZ micromanipulator (Line Tool, USA) on a vibration-free bench. The probe was calibrated using a constant current source of 15 μA cm^{-2} in 10 mM MES-KOH (pH 6.15) and 100 mM KCl, at a resistivity of 540 cm^{-2} and with a vibration amplitude of 30 μm apart. Thus:

$$\text{Current density} = \frac{2.83}{3 \times 10^{-3} \times 540} \text{ A cm}^{-2}$$

Leaves from greenhouse-grown *C. communis* were first held for 1 h in darkness. Afterward, both the isolated epidermis and whole leaves were rolled and then fixed to the base of a plastic Petri dish, filled with 10 mM MES-KOH buffer (pH 6.15) and 100 mM KCl. The dish was then mounted under a stereomicroscope for viewing at 25X magnification. The vibrating probe was lowered from 30 to 40 μm above the sample surface, and currents were listed on a chart recorder. When a steady reading for the currents had been obtained, the leaf surface was illuminated (100 μmol m^{-2} s^{-1}) by a Lux 150 4-port projector (assembled in the author's laboratory). Red light was obtained with a glass filter (transmitting above 625 nm, maximum peak: 650 nm) while blue light was produced through a plastic filter (transmitting 512-412 nm, maximum peak: 450 nm).

Measurements of Stomatal Apertures

Fully expanded leaves were detached and laid, abaxial surface uppermost, in plastic Petri dishes lined with wet filter paper. The dishes were first placed in the dark for 1 h so that the stomata would close. Leaves were then segmented and again laid abaxial side up in plastic Petri dishes lined with filter paper moistened with distilled water. At various intervals, the intact segments were transferred to liquid paraffin and their epidermal strips were peeled. To measure the stomatal apertures, an Olympus microscope (Japan) with a camera (200X magnification) was connected to a monitor video and a printer calibrated by an ocular micrometer disc. After incubation, the isolated epidermis was also mounted under the microscope. Stomatal apertures screened in the video could be photographed directly within one minute, after which they were accurately measured with a scaler.

RESULTS

Measurements of Electrical Currents

A vibrating probe is a very sensitive instrument that can detect electrical changes of less than 1 μA. As the first to utilize this probe for stomatal studies, Bowling et al. (1986) found a linear relationship between currents and stomatal apertures. Here, the vibrating probe was used to detect red and blue light-induced changes in electrical currents for both intact leaves and isolated epidermis (Fig. 1). Under darkness, readings for excised intact leaves were ranged from 0.3 to 0.6 μA cm^{-2}. These currents were relatively stable for the intact leaf surface in the dark, but slowly increased in response to red light. This initial rise was maintained for about 2 min, and then slowly declined to nearly the start point while the red light was on. In that tissue type, red and blue lights increased surface current by about 0.15 and 0.05 μA cm^{-2}, respectively. In contrast, no changes in currents were recorded for the isolated epidermal tissue.

Measurements of PSII Photochemical Efficiency

To understand the pathway and directional movement of electrical components, it is important to trace changes in currents on the surfaces of excised intact leaves. Therefore, mesophyll cells were infiltrated with inhibitors to elucidate the correlation between current and photosynthetic activity (Fig. 2). DCMU is known to block electron flow from PSII to PSI, thereby preventing the reduction of NADP (Taiz and Zeiger, 2002). DCCD is an energy-transfer inhibitor that stops H^+ extrusion in *Streptococcus faecalis* (Harold and Papineau, 1972). It appears to bind covalently to the membrane-bound ATPase system from *Streptococcus* (Harold et al., 1969) and to inhibit plasma-membrane-bound ATPase from oat roots (Leonard and Hodges, 1973). Finally, although its site of action is unknown, NaN_3 reportedly inhibits the Hill reaction

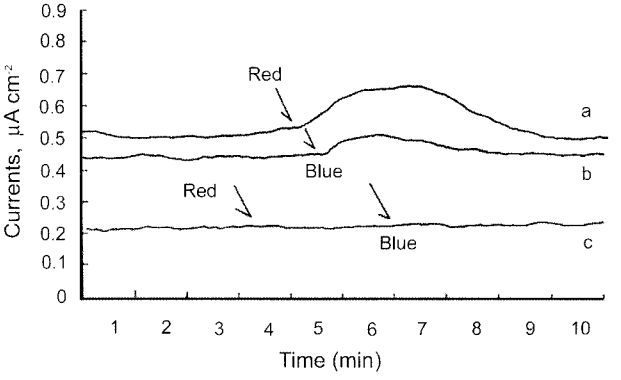

Figure 1. Effects of red and blue lights on changes in electrical currents on lower side of the intact leaf (a, b) and isolated epidermis (c) of *C. communis*.

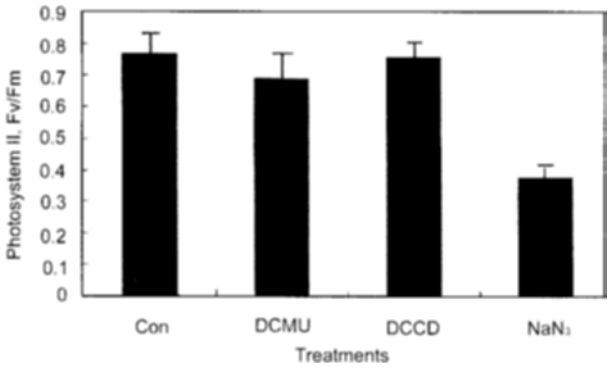

Figure 2. Leaves of *C. communis* were infiltrated for 48 h with 1 mM of photosynthesis inhibitors (DCMU, DCCD, or NaN_3). Afterward, their effects of DCMU, DCCD, and NaN_3 on quantum yield of Photosystem II, Fv/Fm, for the intact leaves were determined. Each value is mean (± SE) of three replicate experiments (3 plants each).

(Spanswick, 1981).

In the present study, infiltration with DCMU caused a 6% decrease in chlorophyll fluorescence (Fv/Fm) when mesophyll cells were exposed to red light. The ratio of Fv/Fm is proportional to the activity of the photosynthetic reaction centers and in particular, PSII (Demmig and Bjorkman, 1987). In other experiments, when mesophyll cells infiltrated with DCCD, red light did not significantly alter Fv/Fm, whereas, for NaN_3 infiltrated leaf discs, photosynthetic activity was reduced nearly by half, with the Fv/Fm ratio being only 55% compared with the control. This demonstrates, therefore, if the crucial response to red light by partially exposed mesophyll cells that are treated with NaN_3.

Electrical Current Measurements for Leaves Infiltrated with Photosynthetic Inhibitors

Mesophyll cells were infiltrated for 48 h before measurements were made. For the control, red light clearly affected electrical currents at the lower side of intact leaves (Fig. 3). There, in order to examine how mesophyll cells would influence those surface currents, epidermal strips were carefully removed. Surprisingly, the partially exposed mesophyll cells did exhibit some changes in current, although to a lesser extent than those recorded for intact leaves. This is a further evidence for mesophyll participation in altering current at the leaf surface.

Infiltration with DCMU caused an approximately 73% drop in electrical currents for leaf surfaces and

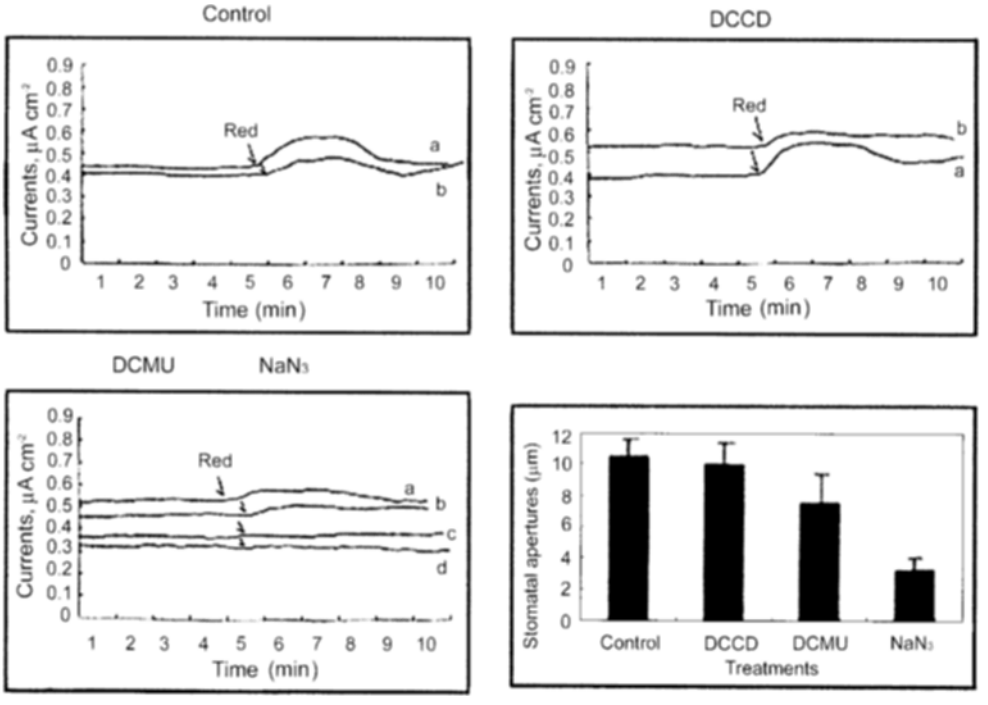

Figure 3. Leaves of *C. communis* were infiltrated for 48 h with photosynthesis inhibitors (DCMU, DCCD, NaN_3). Afterward, effects of red light on flow of electrical current and stomatal opening were determined for intact leaves (a). Epidermal strips were then removed to measure electrical currents above partially exposed mesophyll cells (b). NaN_3 was sampled for intact leaf (c) and mesophyll cells (d). For stomatal measurements, each point is mean (± SE) of three replicate experiments (60 stomatal apertures).

partially exposed mesophyll cells in response to red light. DCCD had no effect on current at the leaf surface. Likewise, when infiltrated with NaN_3, neither intact leaves nor mesophyll cells showed any change in current after red light exposure. Nevertheless, for those NaN_3-infiltrated leaf discs, the more easily determined Fv/Fm ratio was reduced to 55% of the control even though altered currents were undetectable at the intact surfaces of excised leaves.

Measurements of Stomatal Apertures

All tested mesophyll cells were held under darkness for 1 h. When transferred to the light, stomata from the control tissues opened to a maximum aperture of about 10.5 μm after 1.5 h (Fig. 3). However, pre-infiltration of the mesophyll cells with DCMU caused a 29% decrease in aperture size for intact leaves. In NaN_3-infiltrated leaf discs, that reduction was 70%. This further demonstrates that NaN_3 was a very crucial chemical in terms of the inhibition of all physiological responses in this study.

DISCUSSION

Weyers and Meidner (1990) have suggested the use of isolated epidermal cells as a valuable technique tool for advancing our understanding of the stomatal mechanism. However, attempts to study stomatal behaviour have had varing success, casting doubts on the worthless of such the techniques. For instance, microelectrodes are used to measure cellular pH and membrane potentials, but their penetration into the thick guard cell walls can damage those cells, as well as result in broken electrode tips and improper insertion inside the guard cell space. Therefore, this present study utilized a vibrating probe to avoid such risks. Red light was three times more effective than blue in changing electrical current at the surfaces of excised intact leaves, but neither influenced electrical flow for isolated epidermal cells. Two Blue light photoreceptors, carotenoid pigment zeaxantin or phototropin, have been suggested for stomatal responses (Kinoshita et al., 2001; Zeiger et al., 2002; Talbott et al., 2003). However, in this study, the red light-induced changes in electrical current observed at the surface of the excised intact leaf were related to mesophyll photosynthetic activity.

Interest has been renewed in the study of sugar-related metabolism in guard cells (Lu et al., 1997). Gotow et al. (1988) have reported that sugar phosphates are formed by photosynthesis in the guard cells of broad bean (*Vicia faba* L.), while, in the same plant system, red light causes an increase in stomatal aperture size on epidermal peels as well as a decrease in guard cell Ψ_s without either a rise in guard cell K^+ concentration or a decline in guard cell starch content (Tallmann and Zeiger, 1988; Poffenroth et al., 1992; Talbott and Zeiger, 1993). Under other experimental conditions, the stomata also open without an increase in K^+ content but with a loss of starch. Those reports indicate that the data are not consistent with the theory that K^+ is the universal guard cell osmoticum, and include the suggestion that internal sugars arise as additional osmotica from the photosynthetic carbon reduction pathway or via starch breakdown.

Lu et al. (1997) have proposed a hypothesis in which sucrose in the guard cell walls is the physiological signal that integrates transpiration, photosynthesis, and translocation. It is generally believed that most evaporation of the transpiration stream occurs in or near the cell wall (Tyree and Yianoulis, 1980; Pickard, 1982; Maier-Maercker, 1983; Yianoulis and Tyree, 1984). Nevertheless, sucrose accumulates distally in the pathway, i.e., at the guard cell wall, because of the chromatography effect (Tyree and Yianoulis, 1980). This starch accumulation then increases due to two factors. First, the sucrose concentration in the apoplast is the net result of sucrose release from the mesophyll plus the efficiency of transport from the leaf (Ntsika and Delrot, 1986; van Bel, 1993; Lohaus et al., 1995).

The second factor is the rate of transpiration. From their research on *Pisum sativum,* Reckman et al. (1990) have reported that the chlorophyll content in guard cells is about 1/80 of that in the mesophyll cells, whereas their Rubisco activity (0.12 pmol $cell^{-1}$ h^{-1}) is just about 1/300 of that ordinary mesophyll cells. Therefore, the rate of hexose production through the photosynthetic pentose-phosphate cycle of the guard cells is not sufficient to deliver more than 2% of the flow of osmotic material required for stomatal opening. Pulse labeling, which follows the movement of solutes from labeled mesophyll into the epidermis as well as the much greater rate of accumulation of $^{14}CO_2$-fixation products in attached cells versus the isolated epidermis, all indicate a fairly rapid exchange of soluble metabolites between the mesophyll and the epidermis (Outlaw and Fisher, 1975; Outlaw et al., 1975; Dittrich and Rachke, 1977; Willmer et al., 1978; Thorpe and Milthorpe, 1984). These metabolites include glucose, sucrose, sugar phosphate, malate, glycine, serine, and the eth-

anol-insoluble HCl-hydrolysable fraction.

Lu et al. (1997) have reported that the guard cells import mesophyll-derived sucrose from the apoplast. In their study, levels of ^{14}C were low in the guard cells during the first 20 min after labeling, a result that discounts the guard cell photosynthetic carbon reduction pathway as a substantial source for increased sucrose in the apoplast. At 40 min after labeling, sucrose-specific radioactivity in the apoplast was high, a finding that eliminates starch breakdown as an important source of the rise in sucrose levels in the guard cell apoplast. In separate research, Loreti et al. (2000) have demonstrated that sugar-sensing signal transduction modulates the action of gibberellic acid.

Trejo et al. (1993) have found that rapid metabolism of abscisic acid (ABA) in the mesophyll can control ABA levels both there and in the epidermis. This is a novel view of the mesophyll influencing hormonal concentrations in the guard cells via metabolism. Although the movement of such metabolites (glucose, sucrose, and sugar phosphate) and hormones from the mesophyll to the guard cell seems quite apparent, further investigation is warranted to determine how these compounds affect stomatal control. Nevertheless, the present study clearly presents a correlation between photosynthetic activity and flow of the electrical current on the surfaces of excised intact leaves.

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