Planta (1999) 208: 188–195

Planta

The effect of light on membrane potential, apoplastic pH and cell expansion in leaves of *Pisum sativum* L. var. Argenteum.

Role of the plasma-membrane H^+-ATPase and photosynthesis

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Received: 3 September 1998 / Accepted: 15 October 1998

Abstract. The connection between three light responses of green leaf cells-membrane potential (V_m), H^+ net efflux and growth, was analyzed. Illumination of mesophyll cells in leaves from Argenteum peas caused two rapid responses: (i) a de- and repolarization of V_m and (ii) an alkalinization of the apoplast. The rapid responses were completely eliminated by the photosynthetic inhibitor 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU) but not affected by *ortho*-vanadate, an inhibitor of the plasma membrane (PM) H^+-ATPase. The rapid changes were followed by a set of delayed responses: (i) a slow, gradual hyperpolarization of V_m, (ii) a gradual acidification of the mesophyll apoplast and (iii) an increased rate of elongation. These three light responses persisted under DCMU but were completely eliminated by vanadate. The data show that the delayed (in contrast to the rapid) responses were due to a stimulation of PM H^+ pumps which occurred independently of non-cyclic photosynthetic electron transport and the "dark" processes depending on it. When the rapid responses were blocked by DCMU, light-induced acidification, hyperpolarization of the membrane potential and growth proceeded simultaneously. A shared (4-min) lag phase indicated slower signal processing in mesophyll than in epidermal cells where light stimulation of PM H^+ pumps was rapid.

Key words: Cell expansion – Electric photoresponse – Mesophyll – Photosynthesis – *Pisum* (Argenteum mutant) – Plasma-membrane H^+-ATPase

Introduction

One of the fundamental photomorphogenic adaptations of developing plants is the stimulation of leaf growth (Mohr and Shropshire 1983). The photobiology of this stimulation shows large differences between etiolated and de-etiolated plants. Whereas the growth of etiolated leaves responds to low-fluence irradiation and exhibits a red/far red reversibility (Went 1941; Downs 1955), light-stimulated growth of de-etiolated leaves has the typical characteristics of a High Intensity Response (HIR; Mancinelli 1980). Maximal expansion of green pea and bean leaves requires at least 100 μmol photons cm^{-2} s^{-1} white light and continuous illumination (Sale and Vince 1963; Van Volkenburgh et al. 1990), and is for the most part due to cell expansion (VanVolkenburgh and Cleland 1980). The presence of both photomorphogenic and photosynthetic processes in de-etiolated plants obstructs a clear classification of the light-stimulated growth of dicot leaves as a photomorphogenic process until the responsible signal transduction path has been characterized. The signal transduction chain can be unraveled from both ends: (i) by the identification of the responsible photoreceptors (e.g. Van Volkenburgh et al. 1990; Blum et al. 1992; Neff and Van Volkenburgh 1994) and (ii) by deciphering the direct mechanism of light-induced cell expansion in leaves (Van Volkenburgh and Cleland 1980; 1990).

The mechanism of cell expansion in leaves (as in other plant organs) requires apoplastic acidification (Keller and Cosgrove 1996). It has been shown that light-induced growth stimulation of bean leaves depends on an increased H^+ net efflux into the apoplast (Van Volkenburgh and Cleland 1980; Brock and Cleland 1989). It is not known, however, how these findings relate to the observed changes in the photoelectric response of the membrane potential (V_m) of leaf cells (reviewed by Luttge and Higinbotham 1979). In order to make the connection to H^+ net efflux and growth this study determined the role and place of the light-stimulated H^+ pump in the photoelectric V_m of leaf cells. By suppressing photosynthetically mediated light

Abbreviations and symbols: DCMU = 3-(3′, 4′-dichlorophenyl)-1, 1-dimethylurea; PM = plasma membrane; V_m = membrane potential

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responses with 3-(3′,4′-dichlorophenyl)-1,1-dimethylurea (DCMU), we could show that light-induced acidification starts simultaneously with a vanadate-sensitive hyperpolarization of the V_m response after about 5 min of illumination.

Several studies have indicated that the H^+ pump may also have a role in the first phase of the photoelectric response, the rapid depolarization of green plant cells. It has previously been suggested that the rapid light-induced depolarization of the plasma membrane (PM) reflects a transient inhibition of the PM H^+ pump caused (i) by a rapid and transient reduction of cytoplasmic ATP levels by the raised demand of illuminated chloroplasts (Mimura and Tazawa 1986; Bulychev and Vredenberg 1994), and/or (ii) by a transient alkalinization of the cytoplasm causing a reduced substrate (i.e. H^+) availability for the PM H^+ pump (e.g. Hansen et al. 1993). We tested whether the PM H^+ pump was involved in the light-induced depolarization. Since the occurrence and size of the light-induced depolarization in pea mesophyll cells with a vanadate-inhibited PM H^+ pump was unchanged, our results do not support this notion.

This study also tests the role of photosynthetic light perception in the signal transduction chain that stimulates the PM H^+ pump. The effect of light on the PM H^+ pump in green leaf cells could be an exclusively photosynthetic response. In the aquatic plant *Elodea densa*, light-induced activation of the H^+ pump is completely suppressed by the photosynthetic inhibitor DCMU as well as by the removal of CO_2 from the gas phase (Marre et al. 1989). Similarly, the rapid, light-induced, and pump-dependent hyperpolarization in charophytic cells was shown to depend on the presence of chloroplasts (Tazawa and Shimmen 1980). In leaf cells of several higher plants, blocking of linear electron flow in the photosynthetic apparatus with DCMU (Vasil'ev and Venedictov 1993) leads to a reduced PM H^+ pump activity and indicates a partial dependency on photosynthetic processes or products (e.g. Kelly 1983; Petzold and Dahse 1988; Blum et al. 1992). It was, however, also concluded that light can stimulate growth without photosynthesis (Van Volkenburgh and Cleland 1990). The controversy over photosynthesis as a primary process (chlorophyll acting as the responsible photoreceptor) or as a mere support process (providing some of the necessary ATP) for the stimulation of the PM H^+ pump is essentially a controversy over whether it is primarily a photosynthetic or a photomorphogenic process. We address this question in short-term studies of the light effect on leaf cells with or without functional photosynthesis.

Materials and methods

All experiments were carried out with young, partially unfolded leaflets of the Argenteum mutant of *Pisum sativum* L. Handling and preincubation proceeded under dim green light with a photon fluence rate of less than 1 μmol m^{-2} s^{-1}, to which we refer as darkness. Pea plants were grown for 3 weeks in a growth chamber (E 15; Environmental Growth Chambers, Chagrin Falls, Ohio, USA) at 21 °C with a 12-h photoperiod provided by mixed fluorescent and incandescent lamps yielding about 100 μmol m^{-2} s^{-1} photons of white light.

Measurements of the extracellular pH of mesophyll cells. Leaflets were excised and for 2–4 h floated in darkness on incubation solution containing 10 mM KCl, 1 mM $CaCl_2$, and 0.1 mM Mes/1,3 bis[tris(hydroxymethyl)methylamino]propane (BTP) at pH 6.0. With finely pointed forceps, the abaxial epidermis was peeled away from the central region of one half of the Argenteum leaflets exposing an area of about 40 mm^2 of undamaged mesophyll as well as epidermal cells (Hrazdina et al. 1982). The exposed mesophyll area was rapidly covered with 50 μl of incubation solution. The leaflet was placed adaxial side down upon 10 layers of water-soaked filter paper stacked in a small plastic petri dish (55 mm diameter). Through a small hole in the cover of the petri dish the tip of a small-volume (gel) combination pH electrode (Model MI-410; Microelectrodes Inc., Bedford, N.H., USA) was lowered onto the mesophyll where surface tension leads to a covering of the electrode tip with an amount of solution which remains constant over time. After the electrode read a constant pH for about 1 h, white light of 150 μmol m^{-2} s^{-1} was applied from a projector (Techni-Quip Corp., Hollywood, Calif., USA) equipped with a 150-W bulb (Quartzline lamp; General Electric Co., Cleveland, Ohio, USA) and a fiberglass light guide. Similarly, the peeled abaxial epidermis layer was placed cuticular side down in the petri dish and rapidly covered with incubation solution to which 1 mM sucrose had been added.

Measurements of membrane potential (V_m) of mesophyll cells. Individual leaf strips (20 mm × 4 mm) were cut parallel to the midvein of young leaflets and floated upon a solution containing 10 mM KCl, 1 mM $CaCl_2$, and 1 mM Mes/BTP (pH 6.0). After an incubation period of 3–10 h in the dark (in which the leaf cells recovered from excision and regained a sizable V_m) the strips were secured against a Plexiglas stage with strands of Terostat (Teroson Werke, Heidelberg, Germany) and mounted into a perfusion chamber on a microscope stage which contained the reference electrode. The mounted strip was continuously perfused with the incubation solution (see above) and a microelectrode was inserted under microscopic control using perpendicular green light of less than 1 μmol m^{-2} s^{-1}. The microelectrodes were pulled from borosilicate glass capillaries (Kwik-Fil; World Precision Instruments, Sarasota, Fla., USA), backfilled with 300 mM KCl and had tips with resistances ranging between 10 and 30 MΩ, and tip potentials of less than 10 mV. Continuous recordings of the membrane potential were made of the second or third cell encountered during the impalement, process and therefore assumed to be mesophyll cells (unlike leaves in most dicot species with a strict differentiation between palisade and spongy mesophyll, pea leaves have a more homogeneous chlorenchyma; see also Bolhar-Nordenkampf and Draxler 1993). After impalement, mesophyll cells were required to show a steady V_m value for at least 30 min before white light of 150 μmol m^{-2} s^{-1} was applied from a projector through a fiberglass light guide.

Growth measurements. Leaflets were excised and for 2–4 h floated in darkness on incubation solution containing 10 mM KCl, 1.0 mM $CaCl_2$, and 1.0 mM Mes/BTP (pH 6.0). Leaf strips (15 mm × 4 mm) were cut parallel to the midvein from partially folded, growing leaflets and mounted between a fixed and a mobile clamp while floating abaxial side down on the incubation medium. The mobile clamp was connected to the axis of a rotary position transducer (RD 30; Schaevitz, Pennsauken, Pa., USA) which recorded the expansion of the strip under a load of 2 g keeping the strip in a stretched position. Upon mounting between the clamps under dim green light (< 1 μmol m^{-2} s^{-1}) the leaf strips showed initially a rapid expansion (data not shown) which after 20–60 min converted into a steady-state growth rate. At least 60 min of a steady rate was observed before white light of 150 μmol m^{-2} s^{-1} was applied.

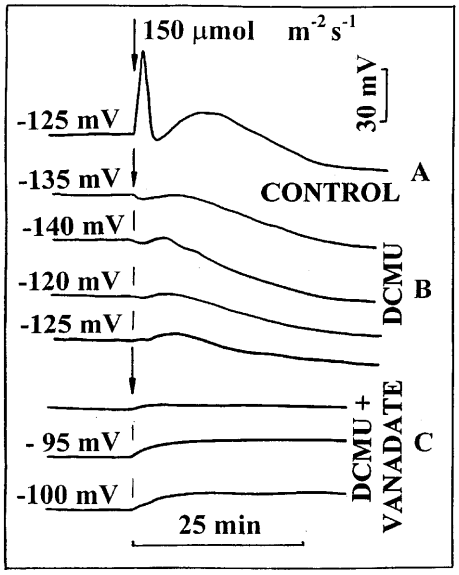

Fig. 1A–C. The effect of 5×10^{-5} M DCMU on the light-induced changes in the V_m of mesophyll cells from leaves of Argenteum peas. The leaf strips were floated for 6–12 h on incubation solution which did (**B**) or did not (**A**) contain DCMU + 1.0 mM sucrose. A third group of strips was incubated in a solution containing both DCMU + sucrose and 5×10^{-4} M sodium *ortho*-vanadate (**C**). Leaf strips were submersed and perfused in a flow-through chamber and a conventional microelectrode was inserted into the first or second layer of mesophyll. After reading a steady value in a mesophyll cell for more than 30 min in the dark, the leaf strip was illuminated with 150 μmol m^{-2} s^{-1} photons of white light (*arrows*)

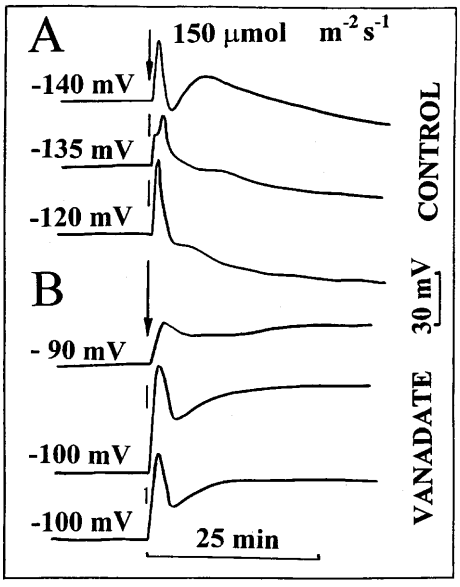

Fig. 2A,B. The action of light (**A**) upon the V_m of pea mesophyll cells and the effect of *ortho*-vanadate (**B**) upon the light-induced V_m changes in the same mesophyll cells 60–100 min after adding 5×10^{-5} M sodium *ortho*-vanadate to the perfusing solution. Note that although adding vanadate depolarized the steady-state level of V_m, it did not reduce the size of the light-induced rapid depolarization. Light-on is indicated by *arrows*

Results

Contribution of photosynthesis and PM H^+ pump to light-induced changes in the V_m of mesophyll cells. After the mesophyll cells in pea leaf strips had been given sufficient time to recover from excision and handling procedures and restore a sizable V_m, the first V_m response to light was a rapid depolarization (as seen in Figs. 1A, 2A) which was immediately followed by a repolarization process starting within 1–2 min of illumination and reaching its peak at 2–3 min. Transient depolarizations occurred in all our recorded photoelectric responses although they varied in individual amplitudes and hence in proportion (see examples in Fig. 2A). Similar responses are found in many other monocot and dicot leaf cells (reviewed by Luttge and Higinbotham 1979). Subsequent V_m responses were a slow depolarization of variable size (which peaked between 7 and 12 min) and a slow gradual hyperpolarization.

Rapid V_m transients have been shown to depend on photosynthesis (reviewed by Luttge and Higinbotham 1979; Spalding et al. 1992). In pea mesophyll cells, DCMU completely removed the rapid transient depolarization as the first step of the photoelectric response (Fig. 1B). After this elimination the first light response was a small transient depolarization (peaking after 4–7 min exposure). The major V_m change, however, was then a slow, gradual and sustained hyperpolarization of 10–40 mV which started after 4–6 min of illumination (Fig. 1B, Table 1). The ionic basis of this hyperpolarization seems to be the light-activated PM H^+ pump. When the pump inhibitor ortho-vanadate was applied in addition to DCMU, the hyperpolarization disappeared (Fig. 1C). Even after applying both inhibitors there remained a V_m response to light: a small and sustained depolarization (Fig. 1C).

Vanadate depolarized the steady-state V_m to values between −85 and −100 mV (Fig. 2). In spite of the depolarized state of V_m, illumination caused a depolarization unabridged in size (compare each of the examples in Fig. 2A with those in Fig. 2B, which show the photoresponses of the same cells before and after vanadate application). This result contradicts the notion that the PM H^+ pump participates in the light-induced depolarization (see Introduction). It indicates that the rapid light-induced depolarization appears to be independent of V_m. Vanadate pretreatment reduced the amplitude of the light-induced repolarization and completely abolished the slow hyperpolarization. Therefore the steady-state V_m of vanadate-treated cells is more depolarized (about 30 mV) in the light than in the dark.

The contribution of photosynthesis and PM H^+ pump to light-induced extracellular pH changes. Unlike normal pea leaflets, the Argenteum mutant can be stripped of the epidermis without apparent wounding, yielding both viable epidermis and mesophyll layers (Hrazdina et al. 1982; Staal et al. 1994). This fact is conducive to the measurement and comparison of light-induced pH changes in the apoplast of epidermal and mesophyll layers. While mesophyll cells showed a rapid

Table 1. The effect of DCMU on the time of appearance and the amplitude of light effects on growth (increase in growth rate above dark level), apoplastic acidification (rate of steady pH decrease) and V_m hyperpolarization (above steady-state dark level) in mesophyll cells from young pea leaves. Except for growth, lag phases become meaningful estimates only after DCMU eliminated simultaneous, antagonistic processes driven by photosynthesis. The table provides arithmetic means and standard errors of no less than eight separate experiments

	Δ growth rate (mm h^{-1})	Lag time (min)	Acidification rate (pH units h^{-1})	Lag time (min)	V_m hyper-polarization (mV)	Lag time (min)
CONTROL	0.25 ± 0.02	3.9 ± 0.58	0.80 ± 0.06	15.5 ± 1.2	24 ± 2.5	10 ± 0.25
+ DCMU	0.06 ± 0.01	4.0 ± 0.76	0.35 ± 0.02	4.5 ± 0.2	16 ± 2.5	4.7 ± 0.3

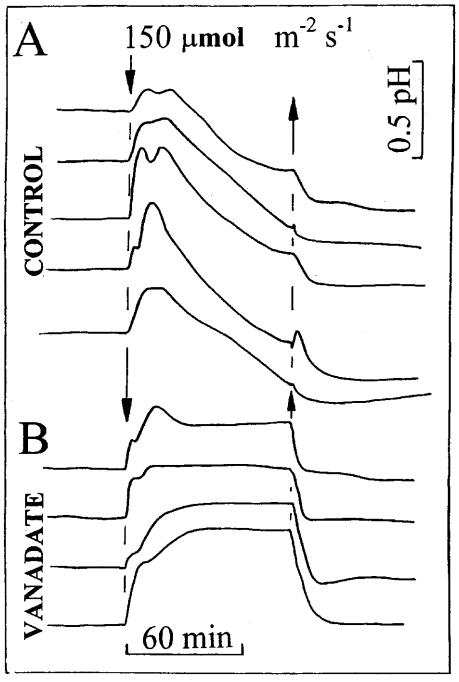

Fig. 3A,B. The effect of illumination with white light on the extracellular pH of mesophyll cells from growing Argenteum leaves (**A**) and its modification after pretreatment with *ortho*-vanadate (**B**). After peeling off the abaxial epidermis, 30–40 mm^2 of the exposed mesophyll layer was covered with 50 µl incubation solution where the apoplastic pH was continuously recorded with a gel-type pH mini-electrode. After reading a steady-value for more than 30 min in the dark, the exposed mesophyll layer was illuminated with 150 µmol m^{-2} s^{-1} photons of white light (as indicated by the *first arrow;* the moment when illumination ceased is indicated by the *second arrow*). The incubation solution for **B** contained an addition of 5×10^{-4} M sodium *ortho*-vanadate

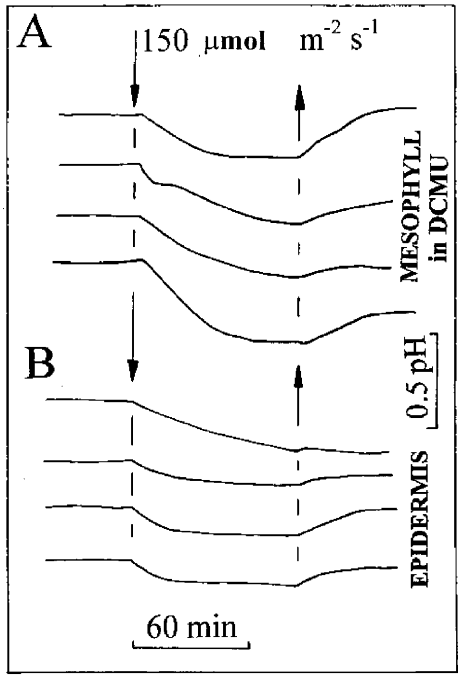

Fig. 4A,B. The effect of DCMU in the incubation solution on the light-induced pH changes in the apoplast of growing pea mesophyll cells (**A**) and the effect of illumination with white light on the extracellular pH of epidermal cells from growing Argenteum leaves (**B**). Experimental conditions for mesophyll/epidermal cells were as described in Fig. 3 for mesophyll layer. The incubation solution for the mesophyll layer contained an addition of 5×10^{-5} M DCMU + 1 mM sucrose, and for the epidermal cell layer an addition of 1 mM sucrose

alkalinization and subsequently a gradual acidification starting after about 20 min (Fig. 3A, Table 1), epidermal cells started to acidify their apoplast within 10 s (Fig. 4B). Addition of DCMU totally eliminated the rapid alkalinization response (Fig. 4A) which therefore should depend on photosynthetically mediated electron and intracellular H^+ transport. Accordingly, DCMU-treated mesophyll cells responded to light with an acidification, just as epidermal cells did (Fig. 4). However, mesophyll cells did not extrude protons as rapidly as epidermal cells. The DCMU-treated mesophyll showed that acidification starts after 3–6 min (Fig. 4A). This is much earlier though than is apparent from untreated tissue (Fig. 3A) where early proton extrusion is hidden by the rapid and strong alkalinization. This may explain the biphasic shape of many alkalinization processes and also why "attempts to identify an early light-induced increase in H^+ extrusion in isolated leaves from *Commelina* and *Pisum* gave negative or inconclusive results" (Marre and Rasi-Caldogno 1989).

Although DCMU completely eliminated the alkalinization, it did not suppress but only weakened the subsequent acidification (similar results were obtained by Petzold and Dahse 1988, and by Van Volkenburgh and Cleland 1980) relative to the untreated control (Table 1; Fig. 4A). This result suggests that photosynthesis modulates the intensity and duration of the H^+ net efflux.

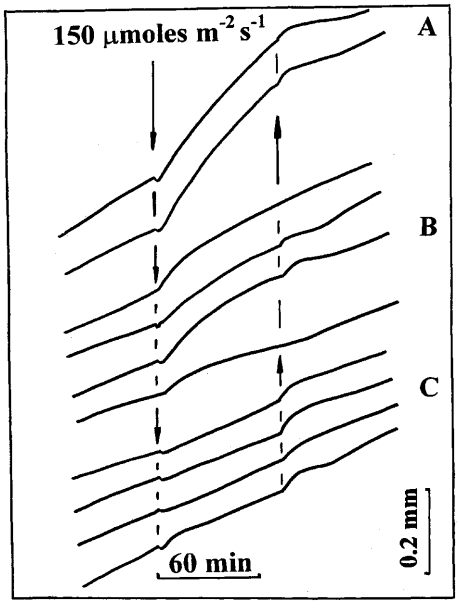

Fig. 5A–C. The effect of illumination with 150 μmol m^{-2} s^{-1} photons of white light (as indicated by the *first arrow*; the moment when illumination ceased is indicated by the *second arrow*) upon the growth of Argenteum leaf strips which were floated for 12 h on incubation solution (**A**), incubation solution plus 5×10^{-5} M DCMU and 1.0 mM sucrose (**B**), and incubation solution plus 5×10^{-4} M sodium *ortho*-vanadate (**C**)

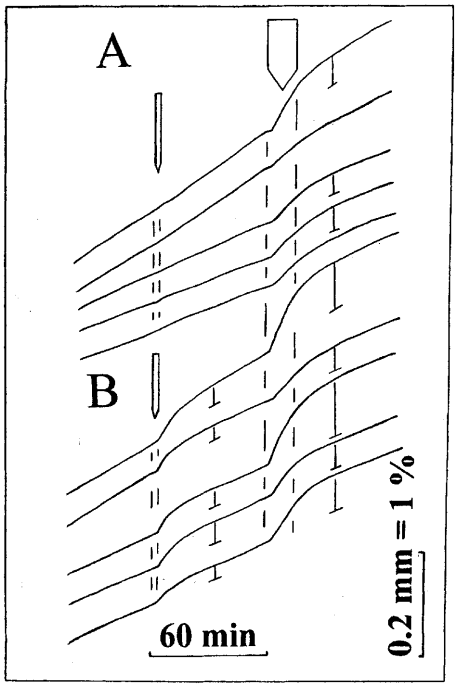

Fig. 6A,B. The growth effect of a 3-min (*first arrow*) versus a 15-min (*second arrow*) period of illumination with 30 (**A**) and 150 (**B**) μmol m^{-2} s^{-1} photons of white light. Data are shown for five leaf strips. The sequence of traces is the same in **A** and **B**, such that each trace in **B** represents a continuation of the trace from **A**

The contribution of photosynthesis and the PM H^+ pump to light-induced growth stimulation. Leaf strips (18 mm long) grew in the dark with stable expansion rates averaging 0.06 mm h^{-1} or about 0.3% h^{-1} (Fig. 5A). Upon illumination with white light, growth was inhibited (initially, even shrinking sometimes occurred) for 2–6 min before it accelerated to a new growth rate which was about 3–4 times higher than the dark level (Fig. 5A, Table 1). Ceasing illumination led transiently to a growth acceleration (lasting 4–15 mins) and a return to the dark level.

Leaf strips were preincubated in 5×10^{-5} M DCMU for 3–8 h to insure the complete suppression of photosynthesis (such strips no longer showed any light-induced oxygen release; data not shown). In the dark, the growth rate of these strips was like that of the untreated controls (Fig. 5A,B). Upon illumination both treated and untreated strips showed an increase in growth rate. This increase was smaller and transient in DCMU-treated strips (Table 1) in spite of supplemental sucrose and suggests that photosynthesis supports growth by other factors than just energy supply.

Light induced a rapid alkalinization in the mesophyll apoplast. If this process were causing the observed lag phase in the stimulation of leaf growth, the lags should disappear in DCMU-treated leaf strips. As this was is not so we conclude that the lags are neither caused nor affected by the alkalinization.

When preincubated for 3–8 h in vanadate, leaf strips still grew in the dark (Fig. 5C). The light-stimulated growth stimulation, however, completely disappeared. Still visible was light-induced shrinking (and dark-induced swelling) of the strips found only in young and not in older pea leaf strips (data not shown). These reversible changes are believed to reflect light-stimulated evaporation from the upper surface.

Light stimulation of leaf growth is a time-consuming process. The appearance of similar lag phases in the light stimulation of V_m hyperpolarization, H^+ net efflux and growth suggests that a minimal period of light exposure might be necessary to stimulate the PM H^+ pump. To test this notion we illuminated leaf strips for periods of either 3 min or 15 min with either 30 μmol m^{-2} s^{-1} (Fig. 6A) or 150 μmol m^{-2} s^{-1} photons of white light (Fig. 6B). At the lower fluence rate of 30 μmol m^{-2} s^{-1} a 3 min-period of illumination had no definitive effect on the growth of leaf strips while 15 min clearly elicited a growth stimulation after 4–5 min (Fig. 6A). With the fluence rate increased to 150 μmol m^{-2} s^{-1}, however, as little as 3 min of illumination clearly stimulated leaf growth (Fig. 6B). Growth did not accelerate during the 3-min illumination period. Instead it occurred after the leaf strips were returned to the dark. Higher fluence rates led to rapid, transient shrinking and after light-off we often observed the reverse phenomenon: an apparent growth stimulation (see Fig. 5). However, the growth stimulation at light-off in Fig. 6B is clearly more than compensating the light-on effect and must be interpreted as a delayed light stimulation. These results show that the observed lag phases reflect a slow signal transduction rather than the necessity for an extended exposure of a photoreceptor system.

The comparison of Figs. 5 and 6 shows that continuous illumination is a necessary requirement for the continuing growth stimulation in pea leaves. Growth stimulation declines to the initial level 5–15 min after illumination ceased. To activate growth, pea leaves need an exposure to more than 5.4 mmol m^{-2} photons of white light. To cause growth acceleration, 270 mmol m^{-2} photons suffice whether they are derived from a 3-min exposure to 150 μmol m^{-2} s^{-1} photons or from a 15-min exposure to 30 μmol m^{-2} s^{-1} photons.

Discussion

Role of the PM H^+ pump in photoelectric and growth responses. In a pioneering study, Pallaghy and Luttge (1970) drew attention to a possible relatedness of light-induced H^+ fluxes and V_m changes in mesophyll cells of *Atriplex spongiosa* leaves. A light-induced and DCMU-insensitive hyperpolarization of V_m was first associated with a stimulated PM H^+ pump in *Nitella* (Spanswick 1974). In leaf cells of higher plants, however, the role and place of the PM H^+ pump in the photoelectric response had never been clearly identified (reviewed by Luttge and Higinbotham 1979).

With photosynthesis inhibited, pea mesophyll cells show the parallel occurrence of (1) an extracellular acidification, (2) a gradual hyperpolarization of V_m and (3) growth stimulation. These three light-induced processes share (i) vanadate-sensitivity, (ii) a slow, gradual increase and (iii) a lag period of comparable size. We conclude that they reflect a non-photosynthetic stimulation of the PM H^+ pump after 5 min of illumination. This result resolves the long-standing question of the role and place of the light-stimulated PM H^+ pump in the photoelectric response and helps us to understand how photoelectric changes relate to the light-induced responses in extracellular pH and growth.

What causes the lag phase in the activation of growth and the mesophyll PM H^+ pump? Light induces a rapid alkalinization in the mesophyll apoplast. Using DCMU we experimentally addressed the question of whether the observed lag phases in the light-induced growth stimulation are a consequence of the apoplastic alkalinization or a kinetic characteristic of the signal transduction process. As lag phases did not differ between DCMU-treated and untreated strips we conclude that they are not affected by the alkalinization.

Inhibition of photosynthesis uncovered comparable lag phases in all three delayed processes. On average it takes 4–5 min of light exposure to start apoplastic acidification, hyperpolarization and growth in DCMU-treated mesophyll cells. When illumination was stopped after 3 min, leaf strips completed the expected growth stimulation in the dark. This result indicates that 4 minutes are needed to complete a remarkably slow signal transduction from the non-photosynthetic light receptor to the stimulation of the H^+ pump. At present we do not comprehend why this signal process takes several minutes in mesophyll cells and how it differs from the rapid (< 1 min) light-stimulation of PM H^+ pumps in epidermal (see also Staal et al. 1994; Elzenga et al. 1997) and guard cells (Assmann et al. 1985).

The PM H^+ pump has no part in the light-induced membrane depolarization and alkalinization. Two rapid, light-induced processes were clearly mediated by photosynthesis: a transient depolarization of mesophyll cells and their extracellular alkalinization. Such alkalinizations have been reported before in lower and higher plants (e.g. Petzold and Dahse 1988; Muhling et al. 1995) as well as in green algae. The light-induced alkalinization in algae is due to the uptake of HCO_3^- ions (Merrett et al. 1996), in higher plants to the direct uptake of CO_2 (e.g. Espie and Colman 1982). The disappearance of HCO_3^- ions and CO_2 cause a similar shift to the left in the following equilibrium: $CO_2 + H_2O \Leftrightarrow H_2CO_3 \Leftrightarrow H^+ + HCO_3^- \Leftrightarrow CO_3^- + 2H^+$, which results in a drop in free H^+ ions. Accordingly, the light-induced alkalinization in suspensions of oat protoplasts showed parallel kinetics with O_2 evolution and decreased CO_2 concentration (Kelly 1983).

An alternative and additional cause of apoplastic alkalinization (and depolarization) is believed to exist in a transient inhibition of the PM H^+ pump. Bulychev and Vredenberg (1994) could amplify light-induced

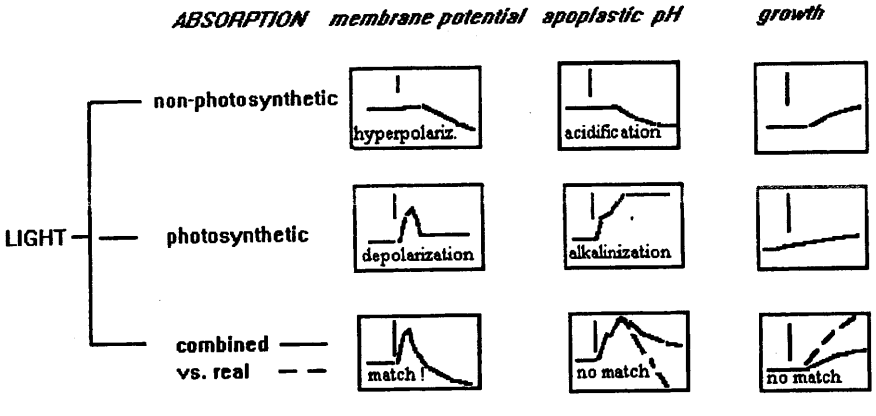

Fig. 7. Model of the contribution of photosynthetic (DCMU-inhibited) and non-photosynthetically modulated (vanadate-inhibited) effects to the natural light response of untreated pea mesophyll. The summation of photosynthetically and non-photosynthetically modulated events matches the real response in the case of the photoelectric V_m response of untreated mesophyll cells. The discrepancy between superimposed curves of the experimentally separated events with the real pH change (and real growth) of untreated mesophyll indicates an interdependent rather than independent action between the separated photosystems in normally functioning leaves

depolarization and alkalinization of moss cells by adding the ionophore nigericin and suggested that the increase must have been caused by a simultaneous, transient inactivation of the PM H^+ pump. When we blocked the PM H^+ pump in pea mesophyll cells by vanadate, however, neither light-induced depolarization nor extracellular alkalinization were affected. Therefore, rapid alkalinization and depolarization of V_m in leaf mesophyll were not at all affected by the activity of the PM H^+ pump. This result, however, does not exclude the possibility that both processes share another common cause.

Does photosynthesis stimulate the PM H^+ pump? Light induces a net efflux of H^+ in pea mesophyll cells, as in leaf cells of other species (Van Volkenburgh and Cleland 1980; Bown 1982; Kelly 1983; Petzold and Dahse 1988; Muhling et al. 1995). Inhibition of photosynthesis showed that light-induced depolarization and alkalinization are not required steps in the signaling process to H^+ net efflux, PM hyperpolarization and growth stimulation. This confirms earlier conclusions that DCMU has no direct effect on the PM H^+ pump (e.g. Bown 1982; Okazaki et al. 1994) and that photosynthesis is not essential for light-stimulated leaf growth (Van-Volkenburgh and Cleland 1990). On the other hand, our and other results (e.g. Kelly 1983; Blum et al. 1992) show clearly that photosynthetic processes promote and enhance H^+ extrusion and growth (Table 1).

Summarizing our results, Fig. 7 demonstrates that superposition of photosynthetically (vanadate-inhibited) and non-photosynthetically (DCMU-inhibited) mediated V_m responses suffices to explain the complete electric photoresponse in untreated mesophyll cells. The summation of the experimentally separated responses does not, however, match the normal light-stimulated H^+ extrusion and growth rate of untreated tissue. Such a mismatch is indicative of an interactive rather than an independent action of photosynthetic and morphogenetic light action under normal circumstances. It indicates that the influence of photosynthesis on leaf growth and H^+ extrusion in mesophyll cells deserves further research. Only a few out of the many possible effects of photosynthesis have been considered so far. The direct supply of sucrose is insufficient to explain the stimulatory effect of photosynthesis (see above). A redundant action of a chlorophyll-based photosystem on the PM H^+ pump (Marre et al. 1989; Marre and Rasi-Caldogno 1989) has not been experimentally excluded. Possible oxidizing effects of increasing oxygen release into cytoplasm and cell wall (Bown 1982; Takeshige et al. 1992) have not been explored. Effects of a photosynthetically increased apoplastic and cytoplasmic pH on the PM H^+ pump have rarely been tested (Felle and Bertl 1986). Using DCMU-inhibited pea leaf strips one can test which of these four factors will restore H^+ net efflux and growth to the level of the untreated controls.

The authors thank R.E. Cleland for his comments on the manuscript. This work was supported by a National Science Foundation grant (MCB-9220110) to E.V.

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