Calcium-calmodulin signalling is involved in light-induced acidification by epidermal leaf cells of pea, *Pisum sativum* L.

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Received 20 January 1997; Accepted 26 August 1997

Abstract

Pathways of signal transduction of red and blue light-dependent acidification by leaf epidermal cells were studied using epidermal strips of the Argenteum mutant of *Pisum sativum*. In these preparations the contribution of guard cells to the acidification is minimal. The hydroxypyridine nifedipine, a Ca\(^{2+}\)-channel blocker, partly inhibited the response to both blue and red light, while the phenylalkylamine, verapamil, a Ca\(^{2+}\)-channel blocker that has been shown in plant cells also to block K\(^{+}\)-channels, caused nearly complete inhibition. The Ca\(^{2+}\)-channel activator S(−)Bay K 8644 induced acidification when added in the dark and diminished the light-induced lowering of the extracellular pH. The Ca\(^{2+}\)-ionophores, ionomycin and A23187, also reduced the light response. Furthermore, the light-induced acidification was inhibited by the calmodulin antagonists W-7 and trifluoperazine, but not by W-5. These calmodulin inhibitors completely inhibited the red light-induced acidification, but inhibited the response to blue light by only 60–70%. In general, inhibition by compounds affecting Ca-calmodulin signalling was always stronger on the red light response than that on the blue light response (with the exception of verapamil that blocked both the red and blue light responses equally well). This differential effect on red and blue light-induced responses indicates a role for Ca\(^{2+}\)-CalM signalling in both the red and blue light responses, while a second process, independent of Ca\(^{2+}\) is activated by blue light.

Key words: Signal transduction, light-induced acidification, epidermal cells, pea.

Introduction

Light controls expansion growth of leaves in an intricate way, involving several light receptor systems and affecting multiple physiological processes. In *Phaseolus vulgaris* both phytochrome and a blue light receptor are involved (Van Volkenburgh *et al.*, 1990) and activation of these systems can stimulate growth independently of photosynthesis. In isolated epidermal strips of *Pisum sativum* red and blue light are effective in stimulating proton pumping across the membrane (Staal *et al.*, 1994). Extension growth is dependent on loosening of the cell wall and maintenance of turgor pressure. Activation of the plasma membrane proton pumping ATPase will have a stimulatory effect on both these processes. Acidification of the extracellular space will increase the extensibility of the cell wall (Cosgrove, 1996) and by increasing the proton motive force across the membrane facilitate uptake of solutes adding to the osmotic potential of the cytoplasm. Light-stimulated extracellular acidification is fluence rate-dependent, exhibits additive effects of red and blue light, is dependent on millimolar concentrations of K\(^{+}\) in the medium, is blocked by Ba\(^{2+}\), is inhibited by DCCD, and is insensitive to DCMU (Staal *et al.*, 1994).

Both blue and red light seem to act as signals for activation of the proton pump, not as a direct energy source (Van Volkenburgh *et al.*, 1990). Both phytochrome and the blue light-specific signalling systems are ubiquit-
ous in plant cells. Involvement of both the sensor pigments for blue and red/far red light is found in photomorphogenic processes as diverse as hypocotyl growth, anthocyanin synthesis, glutamate synthase accumulation (Mohr, 1994) and inhibition of antheridiogenesis (Gomphirc, 1988). Blue light-specific responses have been studied extensively in guard cells (Assmann et al., 1985; Shimazaki et al., 1986; Gautier et al., 1992), in extensor/flexor cells in pulvini of Samanea (Satter et al., 1981) and Phaseolus (Okazaki et al., 1995) and in hypocotyl cells (Cosgrove, 1988). In Arabidopsis a blue light photoreceptor mutant is known and the gene for that photoreceptor (Cry1) has now been characterized (Ahmad and Cashmore, 1993; Lin et al., 1995), but our understanding of the signalling processes of blue light responses is still very limited.

More is known about the transduction chain for phytochrome (Vierstra, 1993), but the responses have been described as varied and complex and may include protein kinases, G-proteins, lipid-derived signals (e.g. IP3) and Ca2+-signalling (Roux, 1994).

The Argenteum mutant of pea is deficient in a component of the cell wall middle lamella and as a result has a very loosely attached leaf epidermal cell layer. Peeling this epidermal layer minimally affects the viability of the epidermal cells (Hoch et al., 1980), enabling the study of the light response of this cell type without interference of the mesophyll. In the present study, red and blue light-dependent acidification was investigated using compounds interfering with Ca2+-signalling. The Ca2+-ionophores, Ca2+-channel blockers, Ca2+-channel activators, and CaM antagonists all inhibited the light-dependent acidification, but never blocked the blue light response completely, in contrast to the red light-induced acidification.

Materials and methods

Plant material

Seeds of the Argenteum mutant of Pisum sativum were obtained from the Plant Genetic Resources Unit, USDA/ARS, NYS Agricultural Experimental Station, Geneva, NY, USA. Plants were grown as described by Elzenga et al. (1991). Young unfolding leaves, about 40% of full-size, were used.

Extracellular acidification by epidermal strips

Epidermal strips were peeled from the lower leaf surface and mounted between two Plexiglas plates (5 x 7 cm) with access to the mesophyll side of the epidermal strip provided by a hole (7 mm diameter) drilled in one of the plates. Either the experimental solution was pumped along the surface of the epidermal strip at a rate of 0.6 ml min−1 with a peristaltic pump (2232 microperpex S, LKB, Upsalla, Sweden) or the hole was filled with 100 µl (in single wavelength experiments) or 400 µl (in double wavelength experiments, with high fluence rate red background light) of the experimental solution. Unless otherwise stated the experimental solution was 1 mol m−3 KCl. A flat-tipped combination pH-reference electrode (Ingold, Mettler-Toledo, Tiel, The Netherlands) was placed gently in contact with the tissue, which was then incubated in darkness for at least 45 min. The pH was recorded continuously on a chart recorder. The incubation solution was essentially unbuffered, resulting in small differences in initial pH. During incubation in darkness, a steady but very slow acidification was often observed. The acidification rates reported for light-treated tissue are the difference between the initial rate observed after exposure to light (averaged over the first 5 min) and the rate in the dark (averaged over the 10 min before light-on). The light from a projector lamp was filtered for blue light with a DT Blue filter (central wavelength 420 nm) and for red light with a K-65 filter (central wavelength 650 nm) (both filters from Balzers, Maarssen, The Netherlands). The fluence rate, 100 µmol m−2 s−1 unless otherwise stated, was measured with a quantum meter (SKP 200, Skye Instruments, Powys, UK) at the level of the epidermal strip.

Dose-response curves were fitted to the data using the fitting function of the Prism graphics software version 2.01 (Graphpad, San Diego, USA). Fluence rate response curves were fitted using an equation describing saturation (equation 1).

$$A = A_{\text{max}} \left(1 - e^{-\frac{L}{E}}\right)$$  

where $A$ is the observed rate of acidification, $A_{\text{max}}$ is the maximum rate of acidification, $I$ is the light intensity and $E$ is the light efficiency. To test significance of the effect of increasing concentrations of the compounds tested the Tukey–Kramer multiple comparisons test was used. To determine the $K_d$ of the compounds tested, equation 2 was fitted to the dose–response data points, using the fitting function of the Prism software package.

$$R = R_{\text{max}} + (R_{\text{max}} - R_{\text{min}})/(1 + 10^{(a - c/f)})$$  

Where $R$ is the response at concentration $c$ of the compound tested, $f$ is a slope factor and $R_{\text{min}}$ and $R_{\text{max}}$ are the responses at the highest concentration tested and the control, respectively. To test the significance of the difference between curves the method described by Ratkowsky (1983) was used.

Chemicals

Verapamil, nifedipine, TFP, W-7, and W-5 were obtained from Sigma (Zwijndrecht, The Netherlands), A23187 and ionomycin from Fluka (Bornem, Belgium) and S-(−)-Bay K 8644 from RBI (SanverTech, Breda, The Netherlands). For the stock solutions the chemicals were dissolved in DMSO (nifedipine and ionomycin), in ethanol (A23187 and S-(−)-Bay K 8644), or in 1 mol m−3 KCl (verapamil, W-7, W-5). The final concentrations of DMSO and ethanol never exceeded 0.2% and 2% (v/v), respectively. In control experiments these concentrations did not affect the response significantly, neither was the viability, as judged by the viability staining, reduced after 2 h in these solutions.

Viability staining

Viability of epidermal cells and guard cells in epidermal strips was judged by the FDA/PI double staining method (Oparka and Reid, 1994). Fluorescence of FDA and PI was observed using a Nikon Diaphot inverted microscope (Nikon Inc., Tokyo, Japan) equipped with an epifluorescence optical unit (filter combination Nikon B-2A: 510 nm dichroic mirror, 450–490 nm excitation filter, 520 barrier filter).

The ultrasonic disrupter used at 50 W was a Sonifier B12 (Branson Sonic Power Connecticut, USA) with a microtip.
Results

In Fig. 1A the effect of both blue and red light on the surface pH of an epidermal strip is shown. Blue light induces an almost immediate acidification (lag-time 0.8 ± 0.2 min, n = 4) that is essentially completed within 15 min and is on average 1.35 pH units h⁻¹ (with a SD of 0.32, largely reflecting seasonal changes). Red light also acidifies the surface layer of the epidermis, but has a longer lag-time (3.3 ± 0.4 min, n = 4), is less strong (0.51 ± 0.15 pH units h⁻¹) and does not saturate within 45 min.

Effect of compounds interfering with the cytoplasmic [Ca²⁺]

Both from membrane potential measurements (Elzenga et al., 1995) and from earlier studies on acidification (Staal et al., 1994) it is know that extracellular Ca²⁺ is involved in the light response of epidermal cells. Therefore, the effect of the hydroxypyridine nifedipin, a Ca²⁺-channel blocker, on the acidification was tested. Nifedipin blocks 70% of the red light-induced acidification and about 40% of the blue light-induced acidification (Table 1). Verapamil, a phenylalkylamine class Ca²⁺-channel blocker, also inhibited the acidification. Both the red and the blue light response were almost completely blocked (Table 1).

The Ca²⁺-channel activator S(-)Bay K 8644 also inhibits the light-induced acidification in a concentration-dependent way (Table 1). As the light-induced acidification is calculated from the difference between the rate of change of pH before and after the onset of illumination, a strong acidification in the dark will result in a reduction of the light-stimulation, as illumination can only increase acidification up to a maximal rate. The inhibition of the light-induced acidification could be the result of a stimulation of the acidification by S(-)Bay K 8644 in the dark. With a flow-through system the effect of S(-)Bay K 8644 in the dark could be measured. When S(-)Bay K 8644 was added to a solution flowing along the epidermis this resulted in an acidification of the medium (Fig. 2).

Identical inhibition of the light-induced acidification was obtained with the Ca²⁺-ionophores A23187 and ionomycin that increase the permeability of the membrane for Ca²⁺ (Table 1).

Effect of calmodulin inhibitors

The light-induced acidification was also inhibited by the CaM-antagonists trifluoperazin (TFP) (Fig. 1B) and W-7. An analogue of W-7 with a lower affinity for CaM, W-5, had no effect on the acidification when tested at a concentration at which W-7 blocked the response maximally (Table 1). The difference in inhibition by TFP of the red light and the blue light responses (95% versus 67%, respectively (Table 1)) is illustrated by Fig. 1B.

Contribution of guard cells to the light-induced acidification

As a blue light pulse also stimulates the plasma membrane proton pumping ATPase in guard cells (Assmann et al., 1985; Shimazaki et al., 1986), the possibility that the guard cells significantly contribute to the light-induced acidification by epidermal strips was examined. The phenotypic instability of the Argenteum mutation (Marx, 1982) offers a unique possibility to estimate the relative contribution of guard cells to the light-response. On Argenteum plants leaves with the wild type phenotype appear occasionally. When these leaves with wild-type phenotype are used, instead of leaves with the Argenteum phenotype, the epidermal peels contain no viable epidermal cells, whereas guard cells are >90% viable as judged by FDA/PI fluorescence. In these peels both the blue and the red light-induced acidification is strongly diminished compared to the peels obtained from leaves with the Argenteum phenotype (Fig. 3). Likewise, treatment with an ultrasonic disrupter for 4 x 5 s of an epidermal peel from Argenteum leaves leads to disruption of 72% of the epidermal cells and to a loss of viability of only 3% of the guard cells. This treatment leads to an almost complete loss of the acidification by red light (0.01 pH units h⁻¹) in disrupted strips compared to 0.51 pH units h⁻¹ in control strips.
Table 1. The effect of compounds interfering with Ca\(^{2+}\)-signalling on the rate of light-induced extracellular acidification by epidermal strips.

In the experiments with verapamil, nifedipine, TFP, W-7, and W-5 the control solution was 1 mol m\(^{-3}\) KCl and with A23187, ionomycin and S(-)Bay K 8644 the control solution was 1 mol m\(^{-3}\) KCl plus 1 mol m\(^{-3}\) CaCl\(_2\). For each treatment the average and standard deviation was calculated from at least three independent experiments. An asterisk indicates that the acidification rate in the presence of the indicated concentration of the effector is significantly different from the control value at the \(P<0.05\) level. In the first column \(k_d\) and \(k_R\) are the apparent binding constants (in mmol m\(^{-3}\)) in blue and red light, respectively, for the compound tested as determined by the fitting equation 2 (Materials and methods) to the data. (For verapamil a range is indicated as no concentrations were tested between those giving no inhibition and full inhibition.) The \(k_d\) were determined by fitting equation 2 (see Materials and methods) to the data.

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration (mmol m(^{-3}))</th>
<th>Blue Avg ± S.D. (n)</th>
<th>%</th>
<th>Red Avg ± S.D. (n)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Verapamil</td>
<td>Control</td>
<td>1.38 ± 0.15 (6)</td>
<td>103</td>
<td>0.54 ± 0.17 (5)</td>
<td>101</td>
</tr>
<tr>
<td></td>
<td>(k_d): B: 18</td>
<td>10</td>
<td>1.44 ± 0.61 (4)</td>
<td>26</td>
<td>0.55 ± 0.40 (4)</td>
</tr>
<tr>
<td></td>
<td>(k_d): R: 10–25</td>
<td>25</td>
<td>0.37* ± 0.05 (3)</td>
<td>6</td>
<td>0.08* ± 0.03 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
<td>0.45* ± 0.09 (3)</td>
<td>33</td>
<td>0.05* ± 0.04 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>100</td>
<td>0.36* ± 0.05 (4)</td>
<td>26</td>
<td>0.06* ± 0.05 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1000</td>
<td>0.09* ± 0.04 (4)</td>
<td>6</td>
<td>0.03* ± 0.02 (4)</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>Control</td>
<td>1.38 ± 0.15 (6)</td>
<td>74</td>
<td>0.54 ± 0.17 (5)</td>
<td>81</td>
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<tr>
<td></td>
<td>(k_d): B: 22</td>
<td>25</td>
<td>1.03* ± 0.20 (4)</td>
<td>66</td>
<td>0.26 ± 0.12 (3)</td>
</tr>
<tr>
<td></td>
<td>(k_d): R: 44</td>
<td>50</td>
<td>0.91* ± 0.16 (3)</td>
<td>59</td>
<td>0.12* ± 0.02 (3)</td>
</tr>
<tr>
<td>S(-)Bay K 8644</td>
<td>Control</td>
<td>1.78 ± 0.26 (4)</td>
<td>102</td>
<td>0.69 ± 0.25 (4)</td>
<td>52</td>
</tr>
<tr>
<td></td>
<td>(k_d): B: 2</td>
<td>2.5</td>
<td>1.04* ± 0.19 (3)</td>
<td>58</td>
<td>0.36* ± 0.06 (3)</td>
</tr>
<tr>
<td></td>
<td>(k_d): R: 1</td>
<td>10</td>
<td>0.90* ± 0.30 (3)</td>
<td>50</td>
<td>0.34* ± 0.20 (3)</td>
</tr>
<tr>
<td>A23187</td>
<td>Control</td>
<td>0.97 ± 0.06 (3)</td>
<td>84</td>
<td>0.25 ± 0.12 (3)</td>
<td>77</td>
</tr>
<tr>
<td></td>
<td>(k_d): B: 19</td>
<td>5</td>
<td>0.82* ± 0.06 (3)</td>
<td>59</td>
<td>0.06* ± 0.04 (4)</td>
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<tr>
<td></td>
<td>(k_d): R: 12</td>
<td>20</td>
<td>0.55* ± 0.10 (4)</td>
<td>32</td>
<td>-0.05* ± 0.06 (3)</td>
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<tr>
<td></td>
<td></td>
<td>50</td>
<td>0.31* ± 0.12 (3)</td>
<td>28</td>
<td>-0.01* ± 0.02 (3)</td>
</tr>
<tr>
<td>Ionomycin</td>
<td>Control</td>
<td>1.78 ± 0.26 (4)</td>
<td>36</td>
<td>0.69 ± 0.25 (4)</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>(k_d): B: 1</td>
<td>2.5</td>
<td>0.65* ± 0.24 (4)</td>
<td>81</td>
<td>0.37 ± 0.16 (9)</td>
</tr>
<tr>
<td></td>
<td>(k_d): R: 4</td>
<td>5</td>
<td>0.61* ± 0.08 (3)</td>
<td>57</td>
<td>0.16* ± 0.05 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>0.51* ± 0.01 (3)</td>
<td>48</td>
<td>0.06* ± 0.01 (3)</td>
</tr>
<tr>
<td>W-7</td>
<td>Control</td>
<td>1.77 ± 0.24 (5)</td>
<td>43</td>
<td>0.02* ± 0.03 (3)</td>
<td>5</td>
</tr>
<tr>
<td></td>
<td>(k_d): B: 7</td>
<td>5</td>
<td>1.50 ± 0.51 (4)</td>
<td>85</td>
<td>0.72 ± 0.23 (5)</td>
</tr>
<tr>
<td></td>
<td>(k_d): R: 10</td>
<td>10</td>
<td>0.84* ± 0.28 (3)</td>
<td>48</td>
<td>0.66 ± 0.30 (4)</td>
</tr>
<tr>
<td>W-5</td>
<td>Control</td>
<td>1.19 ± 0.14 (4)</td>
<td>33</td>
<td>0.11* ± 0.12 (4)</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td>(k_d): B: 7</td>
<td>5</td>
<td>1.25 ± 0.27 (4)</td>
<td>105</td>
<td>0.37 ± 0.07 (3)</td>
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<tr>
<td></td>
<td>(k_d): R: 10</td>
<td>25</td>
<td>0.58* ± 0.19 (3)</td>
<td>33</td>
<td>0.02* ± 0.02 (3)</td>
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</tbody>
</table>

units h\(^{-1}\) in intact strips) and to a reduction of the acidification rate induced by a blue light pulse to 0.13 pH units h\(^{-1}\), compared to 0.60 pH units h\(^{-1}\) in intact strips.

A second possibility to distinguish between the response of guard cells and epidermal cells was offered by the property of the blue light-dependent acidification in guard cells, that it is strongly enhanced by a red background illumination (Gautier et al., 1992). The effect of red background light (600 \(\mu\)mol m\(^{-2}\) s\(^{-1}\)) on the acidification induced by a 30 s blue light pulse of different fluence rates was determined. As with guard cells a 30 s pulse of blue light induces a temporary stimulation of the acidification by the epidermal strips. However, the dose-response curve of the blue light pulse with red light background did not differ significantly \((P=0.65)\) from the curve without red background illumination (Fig. 4).

**Discussion**

Light-dependent acidification of the extracellular space is one of the processes necessary for extension growth of leaves. The insensitivity to DCMU and the delay between the onset of illumination and the start of acidification makes a role of light as a source of photochemical energy for the acidification response unlikely. A role of blue and red light in regulation of the proton pump activity seems more probable. Transduction of the signal from excited pigment to physiological response may involve the activa-
Ca$^{2+}$-signalling in light-induced acidification

Fig. 2. The effect on the extracellular pH of blue light and adding S(-)Bay K 8644 to the solution flowing along an epidermal peel. Representative recordings of three independent experiments with qualitatively identical results. The experimental solution (1 mol m$^{-3}$ KCl) was pumped along the surface of the epidermal strip at a rate of 0.6 ml min$^{-1}$ and at the indicated time 50 mmol m$^{-3}$ S(-)Bay K 8644 was added to the experimental solution. For comparison the effect of a blue light (blue on-blue off) and a red light (red on-red off) period (both with a fluence rate of 100 $\mu$mol m$^{-2}$ s$^{-1}$) on acidification in a flow-through experiment is given. The initial pH is indicated.

Fig. 3. The effect of red (red on) and blue (blue on) light on the surface pH of epidermal peels from leaves with the Argenteum phenotype (upper trace) and from leaves with wild-type phenotype (lower trace). Strips of epidermis were peeled from the lower surface of growing Argenteum leaves and the internal surface of the strips was placed in contact with a combination pH electrode moistened with 100 $\mu$l of 1 mol m$^{-3}$ KCl. Fluence rates were 100 $\mu$mol m$^{-2}$ s$^{-1}$; initial pH values are indicated.

Fig. 4. Effect of red background illumination on blue light-induced acidification. The rate of acidification of the medium (1 mol m$^{-3}$ KCl) in response to 30 s blue light pulses of different fluence rates was determined with and without red background illumination (600 $\mu$mol m$^{-2}$ s$^{-1}$). Equation 1 (see Materials and methods) was fitted to the data.

The effect of Ca$^{2+}$-channel blockers, Ca$^{2+}$-channel agonists and Ca$^{2+}$-ionophores. Exposure of the epidermal cells to the effectors in all cases inhibited the light-induced acidification. The result that both channel blockers and Ca$^{2+}$-ionophores do affect the light-induced acidification can be understood by considering the result of the experiments with the channel agonist Bay K 8644. When added in the dark the stimulation of Ca$^{2+}$-channels increases the rate of acidification as seen from the pH trace in Fig. 2, indicating that the increase in cytoplasmic [Ca$^{2+}$] stimulates proton pumping. The inhibition of the light-induced acidification by Bay K 8644 is assumed to be due to the already enhanced proton pumping in the absence of light, limiting the additional effect that light has in the absence of Bay K 8644. The inhibition by Ca$^{2+}$-ionophores of the light-stimulation of proton pumping can be interpreted similarly.

With the exception of verapamil at a very high concentration, the maximal inhibition of the blue light-dependent acidification was around 70%, whereas the red light-dependent acidification was inhibited completely or almost completely by W-7, TFP and the ionophores. This is a further indication that the signal transduction pathways for red and blue light are partly separate and that blue light acts in part through a Ca$^{2+}$-independent pathway as previously concluded from differential effects of Ba$^{2+}$ on red and blue light-stimulated acidification (Staal et al., 1994).

The effect of Ca$^{2+}$-channel blockers cannot be interpreted unequivocally as the specificity of the block is not very high. The Ca$^{2+}$-channel blockers verapamil and nifedipine also block the outward rectifying K$^{+}$-channel in plant plasma membrane (Thomine et al., 1994). Treatments that grossly perturb the Ca$^{2+}$ homeostasis, such as the addition of Ca$^{2+}$-ionophores, can also inter-
fere with cellular responses that are not directly mediated by Ca\(^{2+}\) and, therefore, cannot be taken as conclusive evidence that Ca\(^{2+}\)-signalling is involved (Bush, 1995). Nevertheless, the inhibition found with A23187 and ionomycin is remarkable, as these compounds do not affect blue light-stimulated acidification in guard cells (Shimazaki, 1995). The specificity of TFP as a CaM antagonist in plants is questionable. Phenothiazines, such as TFP, can affect numerous processes at a concentration higher than, but sometimes similar to, that required to activate CaM (for a list of these side-effects see González-Darós et al., 1993). However, both TFP and W-7, belonging to different classes of CaM-antagonists, phenothiazine- and naphthalene-sulphonamide-derivatives, respectively, inhibit the light-induced acidification. Furthermore, the concentration at which the CaM antagonists, TFP and W-7, inhibit the acidification half maximal, is very close to the \(k_d\) of isolated brain CaM (4 compared to 1.5 mmol m\(^{-3}\) for TFP and 10 compared to 11 mmol m\(^{-3}\) for W-7; Tanaka et al., 1982). The specificity of the inhibition by W-7 is evident from the lack of effect of W-5 an analogue with a lower affinity for CaM. This implies that CaM is the specific target of action for these inhibitors in the light-dependent acidification. However, the possibility that the light signal transduction is mediated by CaM-like proteins, instead of CaM sensu stricto, cannot be excluded, since CaM-related proteins with strong similarity in amino acid sequence to the regulatory domain of CaM have been identified in plants (Braam and Davis, 1990).

Taken together with the circumstantial evidence provided by the results with channel blockers, channel agonists and Ca\(^{2+}\)-ionophores, the effect of W-7 and TFP gives a strong indication for a role of Ca\(^{2+}\) in light signalling.

As guard cells also survive mechanical peeling it was essential to establish how much these cells contribute to the light response. The finding that acidification is inhibited by the disruption of most of the epidermal cells excludes the fact that the normal response of intact epidermal strips is dominated by the guard cells. The fact that red background illumination does not have a stimulatory effect on acidification induced by a pulse of blue light further excludes a major role for guard cells. In guard cells blue light hardly has any effect in the absence of red light (Gautier et al., 1992). In a previous study, it was reported that the light response of epidermal strips is insensitive to the photosynthetic inhibitor DCMU (Staal et al., 1994) which is also different from the response of guard cells. Another indication that the light-induced acidification originates in the epidermal cells, is that the light response apparently depends on extracellular Ca\(^{2+}\). In contrast, in guard cells proton pumping is not inhibited by ionophores or Ca\(^{2+}\)-channel blockers, but depends on the calcium stores in the endoplasmic reticulum (Shimazaki, 1995).

It is concluded that the light response measured with epidermal strips originates mainly in the epidermal cells and that this response is mediated by both a blue light photoreceptor and phytochrome. The results also indicate that Ca\(^{2+}\)-signalling is involved in the light-induced acidification and that this is mediated through CaM (or CaM-like proteins, sensitive to W-7 and TFP). The response to red light probably fully depends on these Ca\(^{2+}\)-signalling processes and the blue light response is partly independent of Ca\(^{2+}\)-signalling. In guard cells blue light activates a plasma membrane proton pump and regulation of the pumping activity is mediated by cytoplasmic Ca\(^{2+}\) (Kinoshiba et al., 1995). The involvement of Ca\(^{2+}\)-CaM signalling and two separate photoreceptors is also found in pulvinate movement in Cassia fasciculata (Roblin et al., 1989). These similarities in light signalling in the different tissues that respond by osmotic adjustment and solute translocation to light, could indicate that this is a more general mechanism.

Acknowledgement

We thank Professor E Van Volkenburgh for inspiring discussions. This is Ecotrans publication number 60.

References


