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Rapid, Blue-Light-Induced Acidifications at the Surface of *Ectocarpus* and Other Marine Macroalgae[1]

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In most brown algae, photosynthesis saturated with red light can be stimulated by continuous blue light. Pulses of blue light lead to transient increases in photosynthetic rate. When a CO_2-sensitive electrode was used, occasionally blue light was observed to cause an apparent increase of CO_2 instead of the expected decrease. This was changed by buffering the seawater medium and, under these conditions, blue light caused stimulation of CO_2 consumption. These results led to investigations of blue-light-dependent pH changes at the outer surface of the plants. Shifts of the pH were recorded in the presence of the photosynthetic inhibitor 3-(3,4-dichlorophenyl)-1,1-dimethylurea. In all brown algae tested and in the green algae *Ulva* and *Enteromorpha*, blue-light pulses caused transient acidification of 0.03 to 0.18 pH units, depending on the species. The kinetics showed lag phases of a few seconds and the minimum was reached after 5 to 9 min. Fluence response relationships indicated that the sensitivity (threshold) to blue light was very similar in all species. The responses in *Ectocarpus* changed with time, and about 5 h after the beginning of red light or darkness, a second component became evident, which peaked 20 min after the blue-light pulse. The refractory period of the whole system was about 3 h in *Ectocarpus*. The blue-light-dependent pH changes show striking similarities to those of higher plant guard cells, and it is possible that similar responses may occur in other tissues of higher plants. In red algae, however, no blue-light-dependent acidifications could be detected. The possible role of the observed pH shifts in a mechanism of CO_2 acquisition is discussed.

Morphogenetic and physiological responses to blue light are widespread in plants and fungi (for reviews, see Senger and Schmidt, 1986; Senger, 1987). However, the identity of the photoreceptor and the details of the signal transduction mechanism for these processes have eluded investigators for many years.

It is generally accepted that responses that rapidly follow a light stimulus are closer to the primary event of photoreception than those exhibiting long lag phases, and investigations of fast responses may well provide clues about the mechanism of signal transduction and lead finally to an identification of the photoreceptor. There are only a limited number of blue-light responses available whose lag phases are in the range of a few seconds. One of the fastest responses to blue light reported so far is the recently discovered transient stimulation of red-light-saturated photosynthetic rates in brown algae (Dring, 1989; Schmid and Dring, 1992). The lag phases after a saturating pulse of blue light in *Ectocarpus* were about 15 s (Schmid and Dring, 1992). Blue-light stimulation appears to affect the availability of CO_2 for photosynthesis, because increasing the external CO_2 concentration of seawater by either adding bicarbonate or lowering the pH leads to increased photosynthetic rates and a corresponding reduction of the extent of blue-light stimulation (Forster and Dring, 1992). Although we could not find a corresponding behavior in *Ectocarpus* when using artificial seawater medium, recent results from experiments with enriched natural seawater show that, in this species, also, the blue-light response is suppressed as the overall photosynthetic rates increase.

There are a number of different ways by which aquatic plants can increase CO_2 availability (for a recent review, see Madsen and Sand-Jensen, 1991). One of the simplest possibilities, which is found in *Chara* (Lucas, 1983; Takeshige et al., 1992) and in higher aquatic plants (Elzenga and Prins, 1989), is to acidify the outer plant surface by proton extrusion and to compensate for this by a locally separated, simultaneous influx of protons (Fisahn and Lucas, 1990) or release of hydroxyl ions (Elzenga and Prins, 1989).

Initial experiments with the brown algal species *Sphacelaria* also indicated blue-light-dependent lowering of the pH outside the plant (see below). Therefore, we have investigated pH changes at the plant surface of various brown algae, with particular emphasis on *Ectocarpus*, and found transient decreases of the pH after blue-light stimuli in darkness or in red light in the presence of the photosynthetic inhibitor DCMU. The experiments described in the present report characterize these pH changes.

MATERIALS AND METHODS

The same isolate of *Ectocarpus siliculosus* (Dillwyn) Lyngb. was used as in our previous investigations (Schmid and Dring, 1992; Schmid et al., 1992). However, it is now cultured in Provasoli's enriched seawater medium (Provasoli, 1968) in a 12-/12-h light/dark regimen (daylight fluorescent tubes, 70 μmol m^{-2} s^{-1}) at 18°C. *Sphacelaria* sp. was a 2-year-old isolate from plants collected in Strangford Lough (Northern Ireland, near Portaferry) cultured under the same conditions. All other species (*Pilayella littoralis* [L.] Kjellman, *Leathesia difformis* [L.] Aresch., *Dictyota dichotoma* [Hudson] Lamouroux, *Laminaria saccharina* [L.] Lamouroux, *Laminaria digitata* [Hudson] Lamouroux, *Fucus vesiculosus* L., *Ceramium rubrum* [Huds.] Agardh, *Porphyra umbilicalis* [L.] J. Agardh, *Bonnemaisonia hamifera* Hariot, *Cladophora* sp., *Ulva* sp., and *Enteromorpha* sp.) were collected in the field and maintained in filtered

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seawater under the above culture conditions for a maximum of 7 d.

Rates of photosynthetic O_2 evolution and CO_2 consumption were recorded in an open-flow system with the plants mounted directly on the electrodes (Schmid and Dring, 1992). For measuring changes of pH, samples were also attached directly to a glass combination pH electrode by a dialysis membrane that was fixed by a rubber ring. Although care was taken to cover the electrode tip as evenly as possible, the extent of the pH shifts varied considerably, particularly in the filamentous species, possibly because of variations in the area of contact between the glass membrane and the sample surface. The pH electrode was inserted into a glass dish with a total volume of 120 mL of Provasoli's enriched seawater medium containing 10^{-5} M DCMU and slightly buffered by 1 mM Tris/HCl (pH 8). The medium was stirred to ensure a continuous fast flow of medium past the electrode tip. The whole setup was kept in a water bath at 18°C in a dark box.

Because of limitations of the electrical components of the measuring system, the pH could not be recorded on an absolute scale. However, the pH at the beginning of the recordings was always between 7.8 and 8.2.

Samples were irradiated from below using two projectors and a cold-light mirror (Balzers, Vaduz, Liechtenstein). Red light was obtained by insertion of a red plexiglass filter (No. 501; Röhm and Haas, Darmstadt, Germany). For the blue light, a broad band blue interference filter (maximum emission at 402 nm, half-bandwidth 120 nm; Schott, Mainz, Germany) was used, and the light was attenuated by neutral density filters (Schott). Irradiances were measured using a Li-Cor quantum meter.

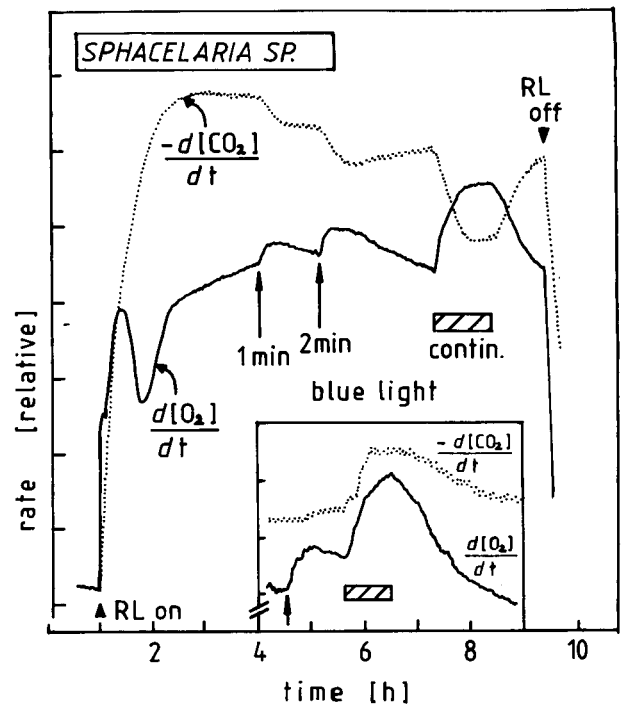

Figure 1. *Sphacelaria* sp.: Photosynthetic rates in red light and after induction by blue light, measured with the O_2 electrode (solid lines) and a CO_2-sensitive electrode system (dotted lines). Rates of gas exchange were measured with the plants being directly attached to the electrodes and held in place with a dialysis membrane while the seawater medium flowed across the electrode. Photosynthesis was recorded under continuous red light (RL) that saturated photosynthesis (45 W m^{-2}). At the times indicated by the arrows and by the hatched bar, blue light (2.7 W m^{-2}) was given. The inset shows a corresponding trace in the presence of 0.1 M Tris, pH 7.8. The arrow indicates an irradiation with blue light of 2 min duration.

RESULTS

Evidence for Blue-Light-Dependent pH Changes Using a CO_2-Sensitive Electrode

When photosynthetic rates were recorded in a flow system using an O_2 electrode and a CO_2-sensitive electrode in an unbuffered seawater medium, both O_2 evolution and CO_2 consumption increased as soon as red light was switched on (Fig. 1). The kinetics of the increase in CO_2 consumption were slightly slower because of the slowness of the CO_2 electrode, which is based on a glass pH electrode. However, when blue light was given either as a pulse or as a continuous irradiation, O_2 evolution was stimulated (as is typical for brown algae; see Dring, 1989; Forster et al., 1990; Schmid and Dring, 1992; Forster, 1992) but CO_2 consumption seemed to be inhibited. The downturn of the traces from the CO_2 electrode could also mean, however, that blue light caused an increase of the CO_2 concentration at the plant surface (experimental setup permits only relative rates to be recorded, and absolute changes of CO_2 concentrations cannot be measured).

Similar experiments with *Ectocarpus*, on the other hand, had shown that O_2 evolution and CO_2 consumption were both stimulated by blue light. The discrepancy between these results was resolved when the experiment with *Sphacelaria* was repeated in buffered seawater medium (0.1 M Tris/HCl, pH 7.8). Under these conditions, blue light caused a stimulation of CO_2 consumption (Fig. 1, inset). Therefore, the increase of the ambient CO_2 concentration must have been due to a blue-light-induced acidification at the plant surface that released CO_2 from bicarbonate (approximately 98% of the inorganic carbon species in seawater at pH 7.8). This phenomenon was investigated further in the following experiments.

Shifts of the pH in Various Species Caused by Blue Light

The photosynthesis of aquatic plants in a closed cell is accompanied by an increase of pH because of the withdrawal of CO_2 from the CO_2/bicarbonate/carbonate equilibrium in the medium. This was also observed in our open system when the plants were directly mounted onto the glass membrane of the pH electrode (not shown). At least in *Ectocarpus* these pH changes due to the uptake of CO_2 were up to 2 orders of magnitude greater than the pH shifts described below, and the latter were completely hidden unless photosynthesis was blocked. Therefore, DCMU was added to the enriched seawater medium for all of the measurements described below. The concentration used (10^{-5} M) inhibited

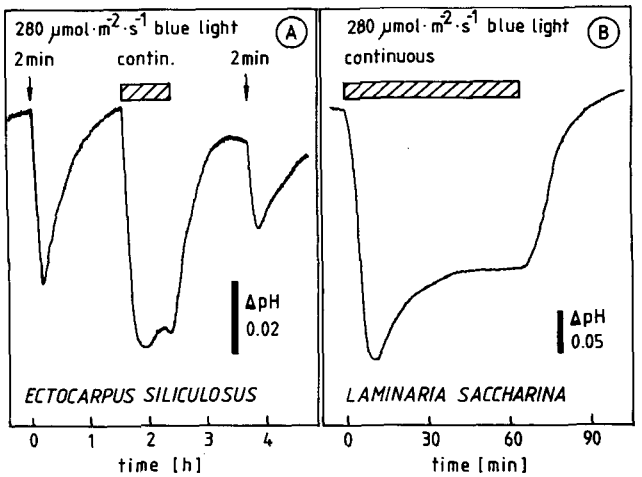

Figure 2. Effects of continuous blue irradiation (280 μmol m^{-2} s^{-1}) on the pH at the plant surface in the presence of 10^{-5} M DCMU. Red light (400 μmol m^{-2} s^{-1}) was given as a continuous background illumination. A, *Ectocarpus siliculosus*; B, *L. saccharina*. In A, the responses to pulse irradiations are also shown.

photosynthesis by >99.5%, and switching the standard red light on or off caused pH shifts of ≤0.005 units.

It should also be noted that, during the long-term measurements used in the open system, the pH in darkness or continuous red light always exhibited irregular slow shifts. Therefore, the baseline in the figures is rarely parallel to the time axis (see Fig. 2 for an example).

Table I. *Characteristics of the blue-light-induced pH shift in various macroalgal species*

	Maximal Shift	Duration of Lag Phase[a]	Time to Reach Maximum
	ΔpH	*s*	*min*
Phaeophyta			
E. siliculosus	−0.04	7	5–6
P. littoralis	−0.03	4–6	5
Sphacelaria sp.	−0.07	~60	9
L. difformis	−0.04	15	5–6
D. dichotoma	−0.18	30–50	6
L. saccharina	−0.13	20[b]	6–7
L. digitata	−0.13	30	7
F. vesiculosus	−0.025	~30	8
Rhodophyta			
C. rubrum	0.00		
P. umbilicalis	0.00		
Palmaria palmata	0.00[c]		
B. hamifera (*Trailiella* phase)	0.00		
Chlorophyta			
Cladophora sp.	< −0.005		
Ulva sp.	−0.08	90[c]	8
Enteromorpha sp.	−0.035	60–90[c]	7

[a] Determined by first visible deviation from baseline at the standard blue-light irradiance (280 μmol m^{-2} s^{-1}). [b] After extrapolation (cf. Fig. 4B). [c] Trace starts with a deflection toward higher pH values during the blue-light pulse.

When added to red background light (at an irradiance that saturated photosynthesis in the absence of DCMU), continuous blue light caused a rapid reduction of the pH on the outer plant surface of *Ectocarpus* (Fig. 2A) and *L. saccharina* (Fig. 2B). The pH reached a minimum about 15 to 20 min after the onset of blue light and stabilized later at a slightly higher level. The initial maximum pH change caused by blue light was about 0.07 for *Ectocarpus* and 0.3 for *L. saccharina*. These values were considerably higher than those observed after a pulse of blue light (see below, cf. Table I). After the blue light was switched off, there was a short lag of about 2 min before the pH shifted back to higher values with first-order kinetics. Similar shifts occurred when blue light was given to plants in darkness (i.e. with no red background illumination). However, there was an initial increase in pH (probably due to residual photosynthetic activity, because it was also observed with red light; see Fig. 7B), that interfered with the acidification response to blue light.

When exposed to a single 2-min pulse of blue light, all species of brown algae so far investigated showed similar transient decreases of pH (shown for a number of species in Fig. 3 and Table I). The pH changes were acidifications of between 0.01 and 0.18 pH units, which started 4 to 40 s after the beginning of the blue-light pulse, depending on the species (shown on an expanded time scale for *Ectocarpus* and *L. saccharina* in Fig. 4 and Table I). The pH minimum was reached after about 5 to 8 min, followed by a decay with

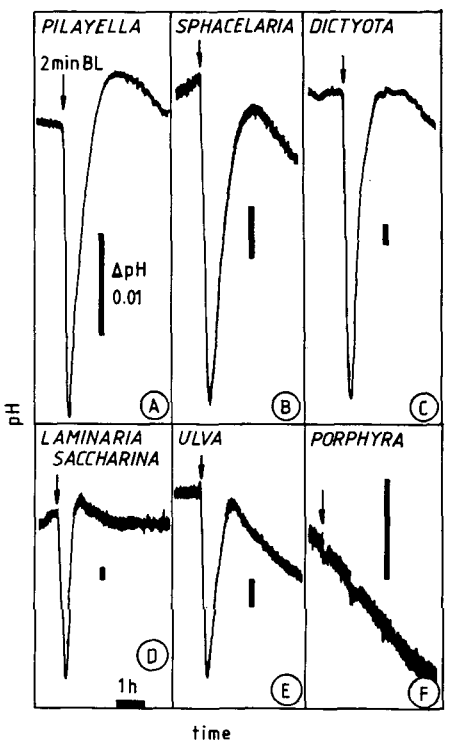

Figure 3. Effects of pulses of blue light (BL; arrows: 280 μmol m^{-2} s^{-1} for 2 min) on the pH at the surface of various species of brown algae, *Ulva* (Chlorophyta), and *Porphyra* (Rhodophyta), respectively. Other conditions are as described in Figure 2. Vertical bars indicate a change in pH of 0.01.

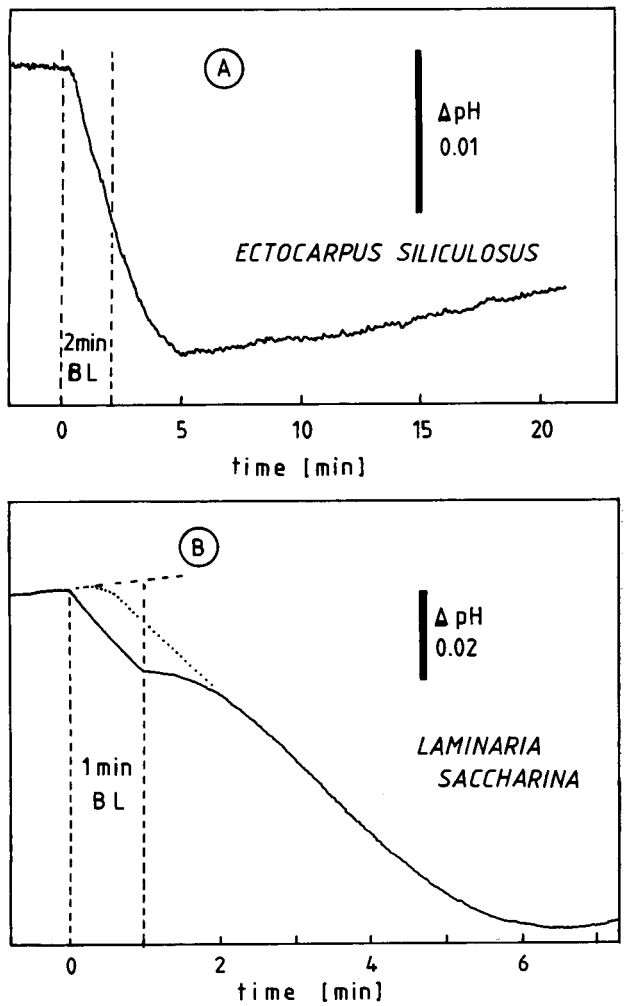

Figure 4. Shifts of the pH in *E. siliculosus* (A) and *L. saccharina* (B) following a pulse of blue light (BL) at higher time resolution. For experimental conditions see Figure 2.

half-lives that also seemed to be species dependent (not investigated in detail). Often an overshoot toward higher pH values was observed (Fig. 3, A and D).

In *L. saccharina* only, we occasionally observed an immediate acidification response during the blue-light pulse (Fig. 4B). At lower irradiances of blue light, this immediate response could be clearly distinguished from the later response that was common to all brown algae. This immediate decrease in pH stopped as soon as the blue light was switched off.

A few species from other macroalgal phyla were also analyzed (Table I and Fig. 3, E and F). There was no similar pH response to blue light in any red alga investigated so far. On the other hand, two of the three marine species of the Chlorophyta (*Ulva* [Fig. 3E and Table I] and *Enteromorpha* [Table I]) showed clear acidifications in response to blue-light pulses that matched the characteristics described for the brown algae. The only difference seemed to be an initial increase in pH during the blue-light irradiation. Therefore, the lengths of the lag phases in these species were determined by extrapolation. They appear to be longer than those of the Phaeophyta (Table I).

Dependence on the Blue-Light Fluence

Fluence-response relationships were measured for four members of the Phaeophyta: the filamentous species *Ectocarpus, Pilayella,* and *Sphacelaria* and the thalloid species *L. saccharina* (Fig. 5). The maximal pH shift was proportional to the logarithm of the blue-light fluence. The threshold sensitivities for blue light of the species ranged from 0.34 mmol m^{-2} for *L. saccharina* to 0.65 mmol m^{-2} for *Ectocarpus* and were remarkably close for all of the filamentous species. The threshold for the blue-light response of the green alga *Ulva* was also found to be in the same fluence range.

Refractory Period and Long-Term Changes

Further properties of the pH shift responses were studied in more detail with the *Ectocarpus* cultures.

It is evident from Figure 2 that a pulse of blue light given before a continuous irradiation caused a higher response than an identical second pulse given 90 min after the continuous irradiation, although, after that period, the pH had returned to its previous value. This indicated that a recovery of the complete system took longer than the return of the pH. The duration of the refractory period was tested by varying the interval duration between two pulses of blue light of equal fluences. With increasing interval length, the height of the responses increased (Fig. 6). After intervals >3 h the response was fully expressed, showing that after a pulse irradiation the system recovered within about 3 h. No attempts have been made so far to determine the refractory period after continuous blue irradiation.

Repeated pulses given at intervals greater than the refractory period showed that the time required to return to the original pH values increased with time (usually seen as a broadening of the peaks, Fig. 7A). This indicated that the

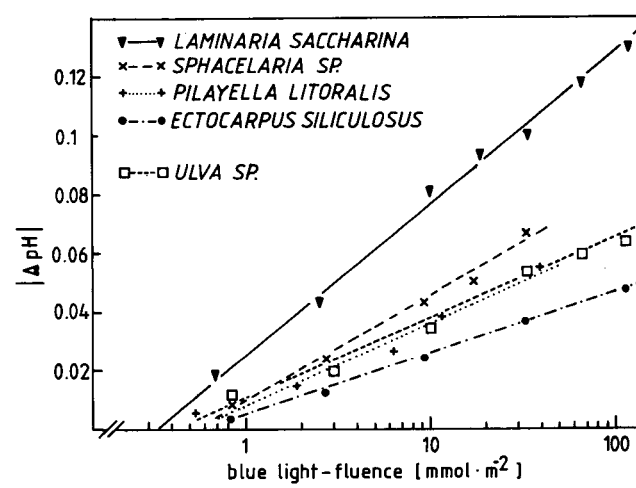

Figure 5. Fluence response curves for the pH shifts induced by 2-min pulses of blue light in various macroalgae. The pulses were applied as 2-min irradiations. The irradiance of blue light was varied using neutral density filters. For other details see Figure 2.

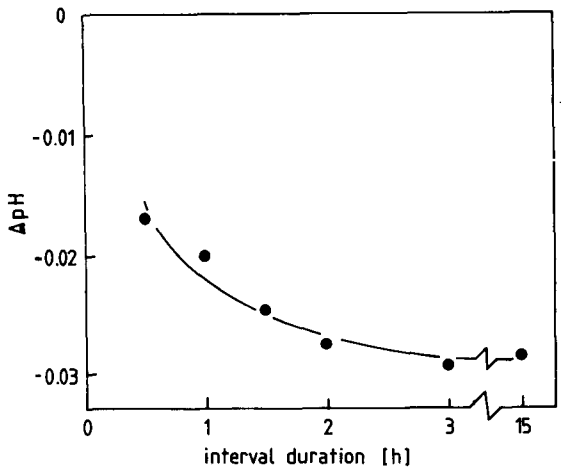

Figure 6. Recovery of the responsiveness to blue light of the pH-shifting system in *Ectocarpus*. The interval between two pulses of blue light (2 min at 280 μmol m^{-2} s^{-1}) was varied, and the extent of the pH shifts was determined. For other experimental conditions, see Figure 2.

response to blue light was subject to time-dependent changes. Time-dependent alterations were also observed when no red background illumination was used (Fig. 7B). Under these conditions, a second pH-shifting activity that peaked about 20 to 25 min after the blue-light pulse and had a longer half-life became apparent. Subtraction of an early peak from a later one (Fig. 7A, bottom) clearly shows that this second activity also develops in continuous red light. However, the first component appears to be enhanced in the presence of red light, and this hides the appearance of the second component.

DISCUSSION

At the normal pH of seawater, when <2% of the total inorganic carbon is available as free CO_2, photosynthesis of many aquatic macrophytes is CO_2 limited (for reviews, see Johnston, 1990; Madsen and Sand-Jensen, 1991). Uptake of bicarbonate has been detected in only about 50% of aquatic plants, and, in many of those species, the capacity for bicarbonate uptake is lower than that for CO_2 uptake (Madsen and Sand-Jensen, 1991). If CO_2 is taken up preferentially for photosynthesis in brown algae, acidification of the external cell space (cell walls/unstirred layer) to release CO_2 from bicarbonate would provide an excellent strategy for acquiring CO_2 from seawater. Indeed, the best explanation of the results obtained with the CO_2-sensitive electrode on *Sphacelaria* (Fig. 1) would be a blue-light-dependent change of the plants' external pH. This does not necessarily mean that the stimulation of light-saturated photosynthetic O_2 evolution in the brown algae is the consequence of such acidification, although we favor this as a working hypothesis at present.

At the normal pH of seawater (about 8.2), >98% of the inorganic carbon is present as bicarbonate or carbonate. A decrease of pH by only 0.25 units doubles the concentration of CO_2 available for photosynthesis at the plant surface (Skirrow, 1975). The magnitude of the pH shifts observed, at least in the thalloid species *Laminaria* and *Dictyota*, were almost of that order. However, although samples were mounted directly onto the electrode, cell walls and mucilage would still intervene between the electrode and the plasma membranes of the cells. We assume, therefore, that pH changes at the plasma membrane itself are even greater than those observed. Furthermore, we expect the pH gradient toward the plasmalemma to be very steep because of the buffering capacity of the CO_2/HCO_3^- system in seawater. The lower magnitude of the pH changes observed with the filamentous brown algae may have been due to the incomplete covering of the electrode surface.

All of the brown algae examined so far have exhibited pH shifts that have similar kinetics and, among those species tested (Fig. 5), similar thresholds for their sensitivity to blue light. Two of the green algae tested also exhibited pH shifts with similar kinetics and thresholds to the majority of the brown algae, but no such responses could be detected in any species of red algae. The threshold measured for *L. saccharina* was about one-half of the values for the remaining species. We are not yet certain whether this difference is significant, but it could be related to the other exceptional feature of the

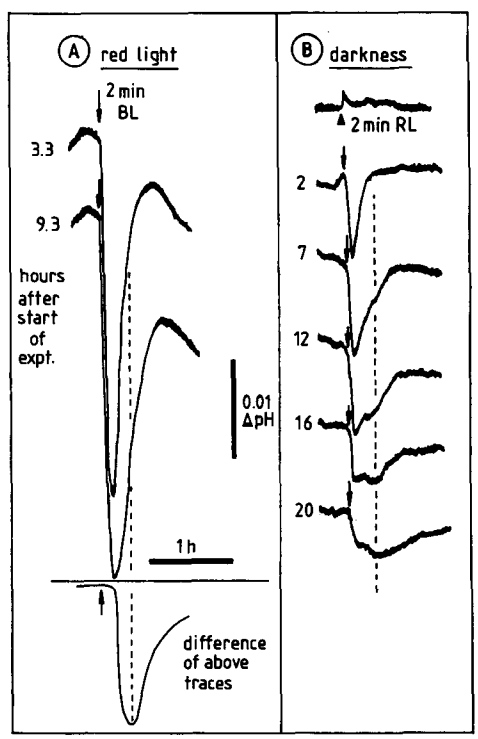

Figure 7. Time-dependent change of the kinetics of the blue-light (BL)-induced pH shifts in *Ectocarpus* in continuous red light (RL; A) or in darkness (B). Traces at various times after the beginning of the experiments are shown. Arrows indicate the times when 2 min of blue light (200 μmol m^{-2} s^{-1}) were given. The broken lines indicate the position of the second peak. A, The appearance of the second component is only visible as a broadening of the peak. Subtraction of the earlier from the later peak (bottom panel) reveals the second component clearly. The irradiance of red light was 240 μmol m^{-2} s^{-1}. B, Top panel shows the response to a red-light pulse of 2 min at 240 μmol m^{-2} s^{-1}.

response in *Laminaria*, which was the additional kinetic component, starting without a lag when the blue light was switched on and failing to persist beyond the end of the blue-light pulse.

The close similarity of the pH responses in brown and green macroalgae suggests that they are caused by a common basic mechanism. The wide distribution of the response among brown and green algae, coupled with its apparent absence from red algae, may also indicate that similar responses occur in higher plants, although they may be more difficult to detect. There is an obvious parallel with the acidification induced by blue light in stomatal guard cell protoplast suspensions (Zeiger, 1990). The threshold sensitivities of these responses (Shimazaki et al., 1986) are similar to those reported here, whereas the sensitivities of other responses of higher plants to blue light, such as first positive phototropism (e.g. pea epicotyls [Baskin, 1986]) and inhibition of stem elongation (e.g. pea epicotyls [Laskowski and Briggs, 1989]), are about 2 and 5 orders of magnitude lower. There could also be a connection between the acidifications in brown algae and the light-dependent extrusion of protons from the leaves of some flowering plants from freshwater habitats (*Elodea*, *Potamogeton*; Elzenga and Prins, 1989), although the wavelength dependence of the latter responses has not yet been investigated.

Guard cells represent one of the best-studied systems with respect to blue-light-driven apoplast acidifications. Their acidifications are caused by the activation of either a plasmalemma proton-ATPase (Assmann et al., 1985) or a redox chain in the plasmalemma (Raghavendra, 1990; Gautier et al., 1991). It is possible that the pH shifts in macroalgae are driven by similar mechanisms, although there is, as yet, no indication of the source of energy driving the responses. Because the lowered pH is maintained in continuous blue light (Fig. 2), there must be a steady input of energy under these conditions. It is unlikely that the energy comes from photosynthesis because our experiments were done in the presence of the photosynthetic inhibitor DCMU. This raises the possibility that the acidification mechanism has different characteristics in the presence of photosynthesis. At least the input of energy from photosynthesis could enhance the extent of the pH changes. Furthermore, blue light also stimulates photosynthetic capacity in brown algae, and, therefore, the presence of blue light could provide even more energy to drive the proton translocations than under continuous red light.

Our original idea was that blue-light-dependent acidifications of the apoplast would lead to higher CO_2 concentrations at the plant surface, which, in turn, would lead to the enhanced O_2 evolution found in various brown algae (Dring, 1989; Schmid and Dring, 1992; Forster, 1992). This hypothesis clearly requires that the two types of responses to blue light (i.e. pH shift and stimulation of O_2 evolution) have shared characteristics with respect to their blue-light dependencies and that they have kinetic properties that match.

An important requirement is that the pH response should be observed before the photosynthetic response. The situation is difficult to assess with most of the species of the brown algae investigated, because the lag phases for both responses are similar (e.g. 15 s for photosynthetic stimulation and 10 s for the pH shift). However, several arguments suggest that the lag phases for the pH response may be shorter. Because experiments were done while photosynthesis was inhibited, responses in the presence of photosynthesis might be faster. In addition, glass pH electrodes are usually slow when compared to other types of ion-sensitive electrodes, and this implies that response times may be delayed and that the pH shifts actually precede the stimulation of O_2 evolution. However, for the *Laminaria* sp. at least, lag phases for the pH response (20–30 s) were clearly shorter than those for stimulation of O_2 evolution, which starts only after about 2 min (R.M. Forster, M.J. Dring, and R. Schmid, unpublished data).

Complex kinetics have been described for the blue-light stimulation of photosynthesis in *Ectocarpus* (Schmid and Dring, 1992). Typically, there were two peaks with maxima at 5 and 20 min after the blue-light pulse, and the decay lasted for about 3 h. The decay of the pH shift, however, was complete within 30 to 40 min. On the other hand, measuring the refractory period for the pH response (Fig. 6) suggested that complete recovery was obtained only after about 3 h. This agrees with the observations on the stimulation of O_2 evolution. The traces of the pH measurements in darkness (Fig. 7B) showed a gradual appearance of two separate peaks, which also had maxima at about 5 and 20 min after the blue-light pulse. These two components may also be present when continuous red background irradiation is applied (Fig. 7A). Their timing suggests that they are related to the two peaks observed in the stimulation of O_2 evolution. However, the latter peaks could be observed very soon after the beginning of continuous red-light irradiation, whereas the second peak in the pH-shift experiments appeared only slowly. This difference may be due to different experimental conditions (e.g. running versus inhibited photosynthesis).

Some algae show large pH shifts but little stimulation of O_2 evolution. In *D. dichotoma*, for example, the maximum stimulation of photosynthesis observed so far was 25 to 30% above the levels in saturating red light (100%), and it was usually much lower (Forster et al., 1990; Forster, 1992). However, *Dictyota* produced the largest pH shift in response to a blue-light pulse of all algae tested (Table I). In *F. vesiculosus*, on the other hand, the pH shift was small (although it is a thalloid species with good contact between pH electrode and plant), and blue-light stimulation of photosynthesis was also small (about 5%). These two examples show that there is, at least, no direct correlation between these two types of response to blue light.

The threshold values for blue-light sensitivity of the pH shifts were generally higher than those of blue-light stimulation of photosynthesis. Threshold fluences for the acidification response were between 0.34 and 0.65 mmol m^{-2} (Fig. 5), but those for stimulation of red-light-saturated photosynthesis were 1 μmol m^{-2} in *Ectocarpus* (Schmid and Dring, 1992) and *Pilayella* (M.J. Dring and R. Schmid, unpublished data) and about 0.15 mmol m^{-2} in *L. saccharina* (M.J. Dring and R. Schmid, unpublished data; for *L. digitata*, see Dring, 1989).

At least for the two filamentous species, these differences must be regarded as a strong argument against a connection between the blue-light responses of acidification and of stimulation of photosynthetic capacity. Even the possibility of

reduced sensitivity of the electrode with the filamentous species (due to poor contact between electrode and sample) could not explain these >100-fold differences in threshold fluence for the two types of response because, for the thalloid species *L. saccharina*, the threshold sensitivity of the pH shift was only about twice that of the filamentous algae. In *Laminaria*, on the other hand, the possibility remains that there is a causal connection between the two responses.

Another result that argues against our working hypothesis is the taxonomic distribution of the two blue-light responses. As yet, no green alga has shown stimulation of light-saturated photosynthesis, but there is a very clear blue-light-induced pH shift in *Ulva* and *Enteromorpha*.

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