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Photosynthetic HCO_3^- Utilization and OH^- Excretion in Aquatic Angiosperms

LIGHT-INDUCED pH CHANGES AT THE LEAF SURFACE[1]

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ABSTRACT

The utilization of HCO_3^- as carbon source for photosynthesis by aquatic angiosperms results in the production of 1 mole OH^- for each mole CO_2 assimilated. The OH^- ions are subsequently released to the medium. In several *Potamogeton* and *Elodea* species, the site of the HCO_3^- influx and OH^- efflux are spatially separated. Described here are light- and dark-induced pH changes at the lower and upper epidermis of the leaves of *Potamogeton lucens*, *Elodea densa*, and *Elodea canadensis*.

In the light, two phases could be discerned. During the first phase, the pH increased at both sides of the leaves. This pH increase apparently resulted from CO_2 fixation. During the second, so-called polar phase, the pH at the upper side increased further, but the pH at the lower side dropped below the pH of the ambient solution. The pH drop at the lower epidermis indicates that the K^+ influx exceeds the net CO_2 (HCO_3^- + CO_2) influx slightly. This may result either from a proton pump driving an extra K^+ influx or from CO_2 diffusion from the cells into the outer medium previously taken up as HCO_3^-. In the dark, a CO_2 gush was observed at both sides. During the polar phase, the upper side becomes electrically negative with respect to the lower side. Subsequent depolarization in the dark revealed that this potential difference consisted of a fast and a slow component.

A number of aquatic plants can use HCO_3^- as well as CO_2[2] as C source for photosynthetic C assimilation. Photosynthetic reduction of HCO_3^- by these aquatics produces one OH^- for each CO_2 molecule assimilated. The OH^- ions are subsequently released by the cells into the medium. This process plus the normal CO_2 fixation may raise the pH of the surrounding solution considerably, especially near the leaf surface. Several species of *Potamogeton* and *Elodea* have a spatial separation between the site of HCO_3^- influx and OH^- efflux. These so-called polar plants take up HCO_3^- at the morphologically lower side of the leaf and release OH^- at the upper side (1, 2, 8, 27). A similar phenomenon has been observed in Characeae in which the two ion fluxes are spatially separated as small bands along the cell (11-18, 26, 28).

Polar HCO_3^- uptake and subsequent OH^- release in *Potamogeton* and *Elodea* are accompanied by cation transport from the lower to the upper side of the leaf and by the formation of an electrical PD across the leaf, making the upper side negative with respect to the lower side (5, 6, 7). Comparable PD were also observed in *Chara corallina* cells. In these latter cells, electric currents are generated between the alkaline and acid regions and may result in electrical PD of 7 mv between the centers of the bands (4, 18, 28). The highest value we observed so far with a *Potamogeton lucens* leaf was 40 mv, upper side negative.

We analyzed the time course of the light induced pH changes at both sides and the trans-leaf PD of leaves of *Potamogeton* and *Elodea* with miniature pH and reference electrodes positioned against the leaf surface.

For comparison, we studied the pH changes at the leaf surface of *Vallisneria spiralis*, a HCO_3^--fixing aquatic species with no separation of the HCO_3^- and OH^- transport sites, and of *Ludwigia natans*, a waterplant that used CO_2 only as a C source for photosynthesis.

MATERIALS AND METHODS

P. lucens and *Elodea canadensis* were grown in concrete tanks outside the building, whereas *Elodea densa*, *V. spiralis* and *L. natans* were grown inside under artificial light as described earlier (21). All plants were cultivated in H_2O at pH 7.8 to 9.5.

The pH near the surface on both sides of the leaves was measured simultaneously with two miniature pH electrodes (Microelectrodes M1 440) with the sensing bulb touching gently on the leaf surface. Two reference electrodes (Microelectrodes M1 401) filled with 3 M KCl were also positioned with their tips very near the leaf surface on both sides. These reference electrodes were also used to measure the electrical PD across the leaf (Fig. 1). The pH-sensitive part of the glass electrode is a hemisphere of radius 0.75 mm and the wall is not of uniform thickness. The end which touches the leaf surface is much thinner and, thus, is the most sensitive part. To enlarge the contact between the sensing bulb and the leaf surface, we folded the leaf slightly around the bulbs (Fig. 1B) in a number of experiments. This did not significantly change the pH values measured at both sides. The experimental solution was in open connection with both sides of the leaf. There was no physical separation between upper and lower side. Thus diffusion from one side to the other was possible. However, the diffusion pathway is very long compared with the distance between the electrodes and the leaf surface. Using a different set-up, comparable to that used by Helder in earlier experiments (5-8), in which the solutions in contact with both sides were separated, we measured identical pH changes at the leaf surface as with the open system described here.

The pH near the leaf surface depends on the balance between

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[2] Abbreviations: CO_2, CO_2 in its molecular form, including both CO_2 and the hydrated form H_2CO_3; ΣCO_2, sum of CO_2, H_2CO_3, HCO_3^-, and CO_3^{2-}; PD, potential difference.

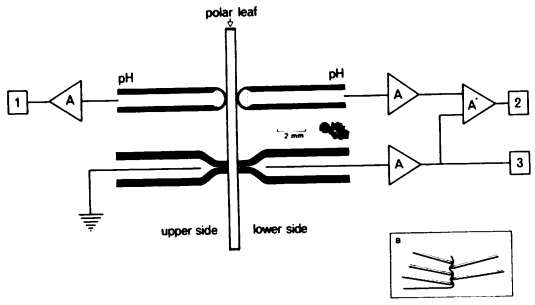

FIG. 1. Experimental set up. A: high impedance amplifier; A' operational amplifier. 1, 2, and 3: pH at the upper leaf surface, pH at the lower surface, and electrical PD across the leaf, respectively; these are all connected to a multichannel recorder. The electrodes are drawn to scale. B: in a number of experiments, the leaf was slightly folded to increase the surface area in contact with the sensitive area of the pH electrodes.

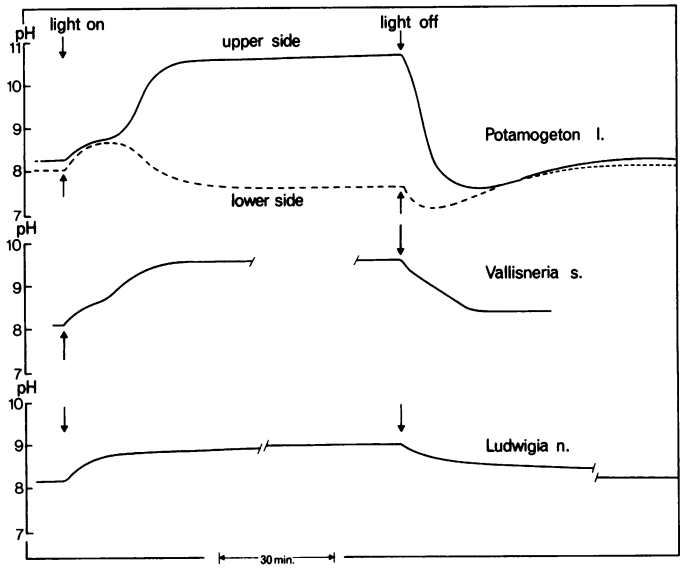

FIG. 2. Light- and dark-induced pH changes near the surface of *P. lucens, V. spiralis,* and *L. natans* leaves. pH changes observed with *Vallisneria* and *Ludwigia* leaves were the same at both sides.

the diffusion of the substances involved and the rate of the pH affecting process in the leaf. During the experiments the pH of the bulk solution did not change. Therefore, a constant ΔpH between the leaf surface and the solution indicates a constant rate of the latter process. In experiments, whole leaves of *Elodea* and leaf strips about 4 mm wide of the other species were used. In most of the experiments, a 1 mM $KHCO_3$ solution was used. Normally this solution was in contact with the surrounding air. Gaseous CO_2 diffusion, however, between the air and experimental solution must have been insignificant. The CO_2 pressure of a 1 mM $KHCO_3$ solution (pH 8.3) equals that of normal air. The experiments with different ambient pH values were done in a closed vessel. Control experiments at pH 8.3 in closed and open vessels showed identical pH changes. All experiments were done at ambient room temperature (20–23 C).

RESULTS AND DISCUSSION

Effects of Light and Darkness. Figure 2 shows the effect of light on the surface pH of a CO_2-absorbing leaf of *L. natans* [1 mM $KHCO_3$ experimental solution (pH 8.3)]. The pH near the leaf surface increased in the light to a steady value of 9 due to CO_2 uptake. At this pH, the CO_2 concentration can be calculated to be 2.2 μM, assuming that the K^+ concentration remains 1 mM near the leaf surface. This corresponds to 66 μl/l CO_2 in a gas phase, which is in equilibrium with this solution, and is very near the CO_2 compensation point as observed with C_3 plants (30). The pH gradually decreased to 8.1 after turning off the light. Time courses in light and darkness were similar. Presumably, the slightly lower pH in the dark was caused by respiratory CO_2.

The effect of light on the leaf surface pH was more complicated with the polar leaves of *Potamogeton* and *Elodea.* The pH changes at both sides in the light showed two distinct phases (Fig. 2). The first phase lasted from 5 to 10 min, depending on the light intensity used. During this phase, the pH increased upon illumination to a semiconstant level of pH 8.7 and 8.9 for the lower and upper side, respectively, showing about the same rate for both sides. After this a-polar phase, the pH at the upper side increased further, whereas the pH at the lower side decreased to a value well below that of the experimental solution (pH 7.6) as in Figure 2.

Final pH values varied from leaf to leaf, depending on its physiological condition. At the upper side, the pH ranged between 10.0 and 10.8 and at the lower side, between 6.5 and 8 [bathing solution, 1 mM $KHCO_3$ (pH 8.3)]. The light-induced pH changes we observed in *Potamogeton* looked quite like the light-induced pH changes seen in the alkaline and acid regions along the cell wall of *C. corallina* cells (12–15).

The secondary rise of the pH at the upper side must result from OH^- release at this side following HCO_3^- assimilation at the lower side which is accompanied by a pH drop here. In most of the experiments, the second phase started simultaneously at both sides of the leaf. Occasionally, we observed that one of the phases started a few minutes later than the other (Fig. 3). This observation indicates that both processes are not directly and intimately coupled. During the second phase, the polarity of the leaves also becomes apparent in the electrical PD across the leaf which is generated during this phase (Fig. 4). Shown, too, is a small electrical PD across the leaf with reversed polarity during the first phase, rendering the upper side slightly positive with respect to the lower side. This small initial reversed PD was not always present but seems to be a normal pattern since it was quite often observed. In *C. corallina,* the utilization of HCO_3^- and the subsequent release of OH^- is accompanied by electric currents between the acid and alkaline bands (4, 18, 28). In *C. corallina,* too, the cell surface PD is generated during the second phase and is absent during the initial phase. From this cell surface PD, it has been concluded that OH^- transport in *C. corallina* is electrogenic (15). From cell membrane potential measurements in *E. densa* and simultaneous pH measurements, the same was concluded for this plant (22). Preliminary experiments with K^+ electrodes showed that K^+ transport through the leaf occurred only during the second phase.

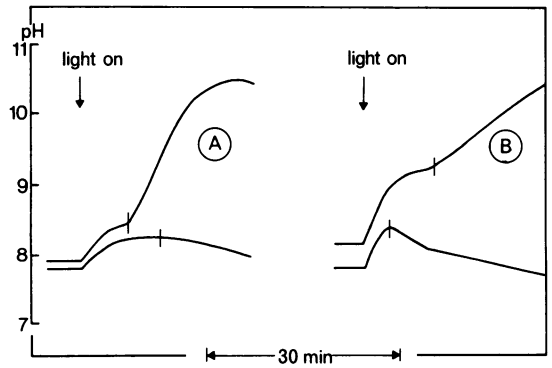

FIG. 3. Examples of experiments in which the pH drop at the lower side of a polar leaf preceded the secondary increase of pH at the upper side (A) and the reverse (B). A was a rare exception but B was quite often observed with a time lag up to 5 min in some experiments.

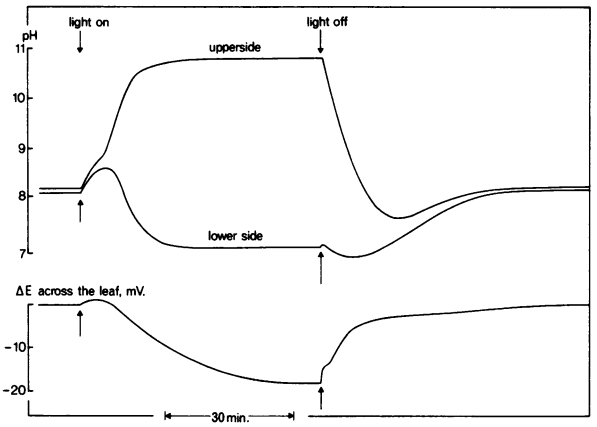

FIG. 4. Comparison of the light-induced pH changes which occurred at the upper and lower side of a *P. lucens* leaf with the light-induced electrical PD across it (upper side − lower side).

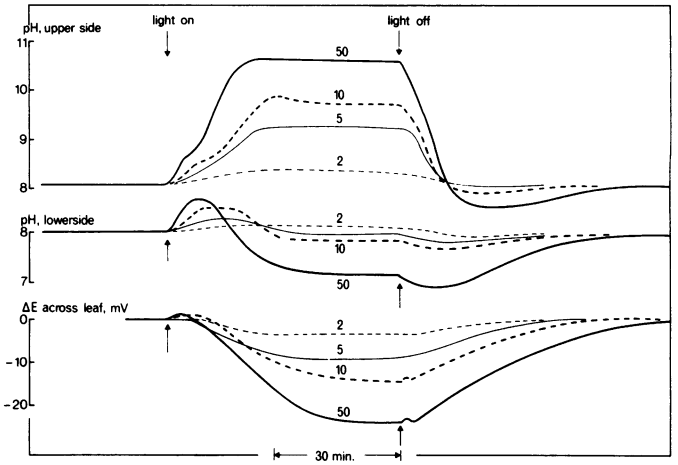

FIG. 5. Effect of light intensity on the light-induced pH changes and electrical PD across a leaf of *P. lucens*. Incident light intensities are given as w m^{-2}. True light intensities at the leaf were less as the leaf was mounted more or less parallel to the direction of the light beam.

When the light was turned off the pH at both sides decreased. At the lower side, the pH reached a minimum after 5 to 10 min, then it returned gradually to a value slightly below that of the surrounding solution. At the upper side, the pH dropped at a rather constant speed for a few minutes, often followed by a slight acceleration before a slowing down to a minimum. (This acceleration is just visible in Figure 2 *P. lucens,* but not in Fig. 4.) A comparable bending was observed in the OH^- transport deactivation curve in *C. corallina* (15). The minimum was reached after about 15 min. Thereafter, the pH increased slowly and finally stabilized after 50 min to a value somewhat higher than that at the lower side. Eventually, the pH at both sides reached the same value. The final pH value in the dark was somewhat below the value of the bathing solution, apparently due to respiration.

The electrical trans-leaf PD showed a rapid depolarization of a few mv in the dark, followed by a shoulder or a small transient repolarization (Fig. 4) and then a further depolarization at about the same proportion as the decline of the pH gradients at the upper and lower side. Identical pH induction curves and trans-leaf PDs as in *P. lucens* were observed also in *E. densa* and *E. canadensis.*

Effect of Light Intensity. The typical shoulder or transient repolarization of the trans-leaf PD was not always observed in the dark. Notably, it was absent after a low light intensity. Also, the pH induction curves depended very much on the light intensity used (Fig. 5). At the lowest light intensity (2 w m^{-2}), only a slight increase of the pH at both sides was observed; the secondary decrease of the pH at the lower side and the continued increase at the upper side were absent. The existence of a small electrical PD across the leaf indicated that the leaf was still polar under these conditions. At the higher light intensities, the characteristic pH induction pattern was recognizable again. The lag period before the onset of the second phase decreased with increasing light intensity in such a way that, at the highest light intensity (50 w m^{-2}), the shoulder of the first phase, normally observed at the upper side, was often hardly visible. This occurred especially with *E. canadensis* leaves. In *C. corallina,* too, the lag period before the onset of the major pH rise depends on the light intensity. In this alga, there is a strong correlation between the HCO_3^- and OH^- fluxes and membrane potential hyperpolarization. Both transport systems may be voltage dependent. The role of the membrane potential may be especially important for the activation of OH^- transport (16, 18). We also consider this for *Elodea* and *Potamogeton.* The light-induced membrane hyperpolarization in these plants is nearly completed before the onset of the major pH increase (22). The time course of this hyperpolarization depends on the light intensity (23). Membrane potentials in *Elodea* and *Potamogeton* were −200 to −250 mv in the light. Assuming a cytoplasmic pH of 7 and 10.5 at the upper leaf surface, the Nernst equation predicts an equilibrium potential for OH^- of about −200 mv. Thus, OH^- excretion may be a downhill process. On the other hand, the HCO_3^- influx obviously requires energy under most circumstances, considering the highly negative membrane potential. A minimum estimate of the CO_2 pressure inside a photosynthesizing cell is the CO_2 compensation point, which is about 60 μl/l or 2 μM. Again, assuming the cytoplasmic pH to be 7, we can calculate the minimum HCO_3^- concentration to be 8.3 μM, but actually the concentration is much higher as the cells perform net photosynthesis. Given a membrane potential of −200 mv, the Nernst equation predicts a $[HCO_3^-]$ in the medium of 23 mM for thermodynamic equilibrium. Actual HCO_3^- concentrations of the experimental solutions were much lower and still induced rapid photosynthesis. The same conclusion was reached for *Chara* (29).

pH Changes at *Vallisneria* Leaves. With leaf strips of *V. spiralis,* in which the sites of HCO_3^- influx and OH^- efflux are not spatially separated, essentially the same two-phasic pH change was observed at both sides of the leaf as with the upperside of a *Potamogeton* and *Elodea* leaf, except that the final pH reached in the light with *Vallisneria* was somewhat lower, *viz.* 9.4 (Fig. 2). In recent experiments, somewhat higher values were obtained. Similar pH changes were observed with other nonpolar leaves as *Ceratophyllum sp.* (results not shown here). These results show that the typical two-phasic pH induction curve is not a singularity of polar leaves but is somehow linked to the assimilation of HCO_3^- and release of OH^-.

Role of CO_2 and HCO_3^-. Comparison of the light-induced pH changes near the leaf surface of *L. natans* with those at the upper and lower sides of *Potamogeton* and *Elodea* leaves suggests that the initial pH rise observed with the latter two species also results from CO_2 fixation. In accordance with this view, the highest initial pH rise we observed with *Potamogeton* and *Elodea* leaves was up to 9 in a solution containing 1 mM $KHCO_3$. The CO_2 pressure at this pH is in equilibrium with 66 μl/l CO_2 in air. C_3 plants having CO_2 compensation points of this magnitude will never bring the pH of the surrounding solution much higher than 9 by mere CO_2 fixation without concomitant HCO_3^- uptake and accompanying OH^- release. The following experiment confirmed this conclusion. Using a bathing solution of pH 9.8, ΣCO_2 = 1 mM, the initial pH rise was absent (Fig. 6). The CO_2 concentration was 0.3 μM, which corresponds to 9 μl/l in a gas phase and is well below the CO_2 compensation point of C_3 plants. On the other hand, at low pH (6.3), the reversed situation occurred: the initial pH rise was very

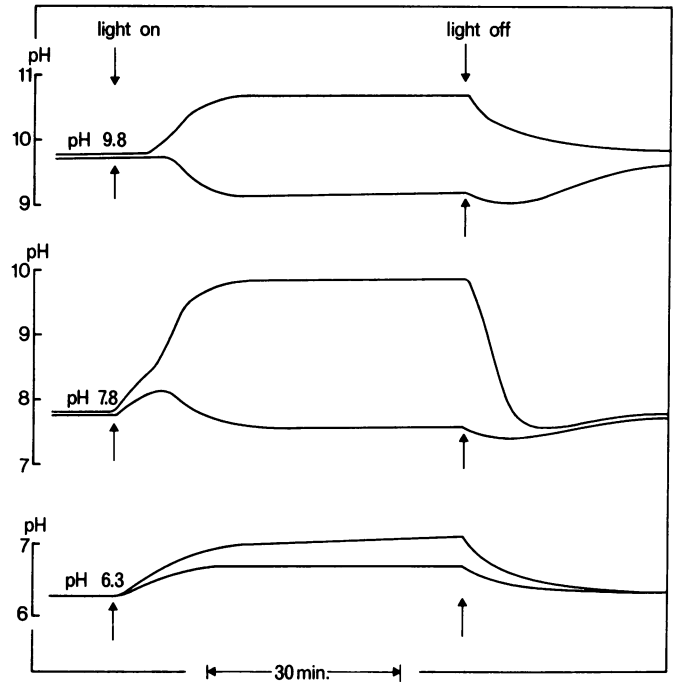

FIG. 6. Effect of ambient pH on the light-induced pH changes at the leaf surfaces of *P. lucens*. Above pH 8.3, the experimental solution contained 1 mM $KHCO_3$ + 1 mM (KCl + KOH); the pH was set by the ratio KCl/KOH. Below pH 8.3, the solution contained 1 mM $KHCO_3$ + 1 mM (KCl + HCl); the pH was adjusted by changing the ratio KCl/HCl.

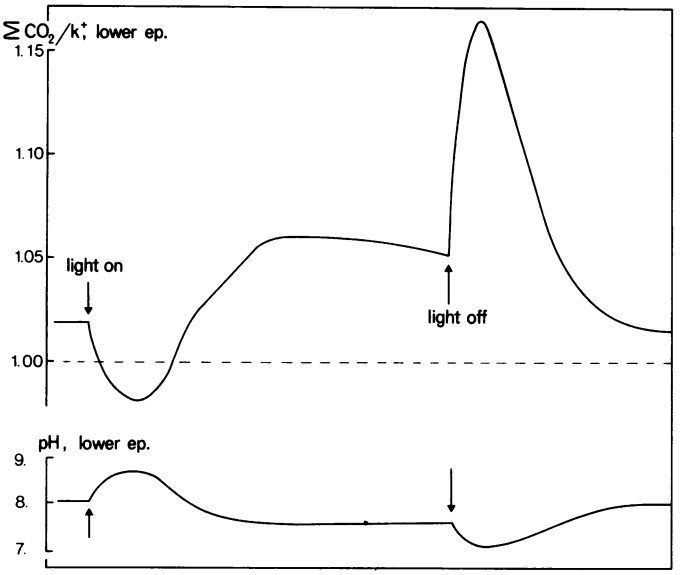

FIG. 7. The ratio $\Sigma CO_2/K^+$ was calculated from the pH for the lower epidermis of a *Potamogeton* leaf using the experimental data which is also given in Fig. 2.

clear, but the secondary further increase at the upper side was much reduced and the acidification at the lower side was absent. At a still lower pH (pH 5, not shown here), the increase at the upper side was reduced further and became practically equal to that at the lower side.

Also, in *C. corallina*, the main pH rise in the alkaline bands resulting from OH^- release is preceded by a small initial pH increase which may be caused by activation of an active OH^- transport system (15). However, this seems unlikely because of our results with different ambient pH values and because the initial pH rise occurred also at the lower side. An initial pH increase in the light has also been observed in the acid regions along the cell wall of *C. corallina* cells (14).

The further pH rise at the upper side during the second phase obviously results from OH^- excretion. The secondary pH decrease at the lower side is more difficult to interpret. A comparable acidification has been observed in the acid regions of *C. corallina* cells and near the ramps of the OH^- efflux site where it has been called the lateral OH^- sink (14, 15). The pH of a K^+- and HCO_3^--containing solution depends on the ratio $\Sigma CO_2/K^+$; an increase of this ratio is accompanied by a decrease of the pH. When pH values are not too high (pH < 9) and the K^+ concentration is not too low, the ratio becomes insensitive to small changes of the K^+ concentration and can be calculated directly from the pH [3] (8). At pH 8.35, the ratio is 1. In Figure 7, this ratio is shown for the lower side of a *Potamogeton* leaf calculated from the data in Figure 2. $\Sigma CO_2/K^+$ at the lower epidermis decreased from 1.02 in the dark to 0.98 in the light during the initial phase due to CO_2 uptake, whereas the pH increased to 8.7. In the second phase, $\Sigma CO_2/K^+$ increased to 1.06, whereas the pH dropped to 7.6. This increase in the ratio $\Sigma CO_2/K^+$ obviously resulted from a K^+ influx accompanying HCO_3^- uptake during this phase at the lower epidermis. As the ratio became greater than 1, the K^+ influx apparently exceeded the net CO_2 (*i.e.* HCO_3^- + CO_2) influx. To maintain electrical neutrality of the transport system, we expected the HCO_3^- influx to be balanced by an equal K^+ influx. Lucas *et al.* (17) showed that the HCO_3^- and cation influx, in their case Na^+, across the lower leaf surface of a *P. lucens* leaf were in close agreement. Spear *et al.* (26) assumed that the acidification observed in the acid regions of *Nitella* cells resulted from proton extrusion. Lucas and co-workers (14, 15) also considered the possibility of a proton extrusion pump operating next to the OH^- and HCO_3^- transport mechanism in *C. corallina* cells. A proton pump driving an extra K^+ influx operating simultaneously with the K^+ - HCO_3^- transport mechanism could be present in *Potamogeton* and *Elodea*, too. The light-induced membrane potential hyperpolarization observed in these and other photosynthesizing cells is generally thought to be generated by a light-dependent proton extrusion pump (9, 10, 20). Protonation of HCO_3^-, to H_2CO_3 and subsequently to CO_2 in the cell wall by the activity of proton pumps, has been considered as a means to transport bicarbonate electroneutrally across the plasmalemma in *Potamogeton* and *Chara* (24, 25, 29).

In the presence of inorganic C, the acidification was present at high pH but suppressed at low pH (Fig. 6) where HCO_3^- utilization is reduced; this points to an alternative explanation, namely, that of back diffusion of CO_2. An efficient photosynthesis at high pH implies that HCO_3^- uptake raises the cytoplasmic CO_2 concentration well above its compensation point, whereas the ambient CO_2 concentration is well below. The back diffusion of CO_2 from the cytoplasm into the surrounding medium results in a net uptake of OH^-, plus accompanying cation, causing acidification at the lower side. This mechanism may also play a role in *C. corallina* cells (15). At low pH, the reversed situation occurs. Here the cytoplasmic CO_2 concentration is lower than that of the medium, resulting in a net diffusion of CO_2 into the cells, rendering the solution near the leaf surface more alkaline.

CO_2 diffusion into the medium also explains the acidity gush observed when the light is turned off. CO_2 gushes in the dark, following a light period, are a well known phenomenon in C_3

[3] As an example, the value of the ratio $\Sigma CO_2/K^+ = R$ is given here for pH 8.7 and 7 (the highest and lowest pH value shown in Fig. 7) for 1, 0.4, and 0.1 mM K^+. Preliminary experiments with K^+ electrodes showed that, with 1 mM $KHCO_3$ ambient solution, the lowest K^+ concentration observed at the leaf surface in the light was 0.4 mM. At pH 8.7, R = 0.981 for 1 mM K^+, R = 0.976 for 0.4 mM, and R = 0.951 for 0.1 mM; at pH 7, R = 1.240 for 1 mM K^+, R = 1.240 for 0.4 mM K^+, and R = 1.241 for 0.1 mM K^+.

terrestrial plants, although they last only a few minutes there (3, 30). An increased CO_2 production during 40 min darkness following a light period was observed in *Vallisneria* leaves (21). $\Sigma CO_2/K^+$ near the lower surface of a *Potamogeton* leaf showed a very marked transient increase in the dark (Fig. 7). An increased K^+ influx under these conditions seems unlikely, therefore, indicating a CO_2 gush during the first 10 to 20 min in the dark.

CONCLUSIONS

In the leaves of *Potamogeton* and *Elodea* CO_2 and HCO_3^- can be used simultaneously for photosynthetic C assimilation. The ratio between the two processes depends on the pH near the lower side of the leaf. The pH at this side is not solely determined by that of the medium but depends on the relative rates of CO_2 and HCO_3^- influxes, CO_2 efflux and other ion fluxes, notably of the cations. There are two possible mechanisms to explain the observed acidification at the lower epidermis: a light-driven proton extrusion pump or back diffusion of CO_2 previously taken up as HCO_3^- by the cells. The present experiments do not allow a definite conclusion as yet.

Considering the very negative membrane potential in the light, OH^- transport at the upper epidermis may be a downhill process. On the other hand, HCO_3^- utilization obviously requires energy considering this same highly negative electrical potential. The observation that cells of the lower epidermis are transfer cells lends support to the view that the active step is HCO_3^- utilization rather than OH^- excretion (19). The role of K^+ or any other accompanying cation remains to be elucidated.

Experiments, where pH measurements are combined with K^+ determinations, are in progress in order to test this model further. These will allow us to make more precise calculations of the relative rates of the CO_2 and HCO_3^- fluxes. The light induced pH changes reported here for some higher aquatic species are very similar to those observed in *C. corallina*. Preliminary experiments with a number of other aquatic species showed that similar pH changes can be observed in most of them. Future models will have to take into account that the underlying mechanisms for the observed pH changes apparently are the same in algae and higher species, polar as well as nonpolar.

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LITERATURE CITED

1. ARENS K 1933 Physiologisch Polarisierter Massenaustausch und Photosynthese bei submersen Wasserpflanzen. Planta 20: 621–658
2. ARENS K 1938 Manganablagerungen bei Wasserpflanzen als folge des physiologisch polarisierten Massenaustausch. Protoplasma 30: 104–129
3. DECKER JP 1955 A rapid, postillumination deceleration of respiration in green leaves. Plant Physiol 30: 82–84
4. FERRIER JM, WJ LUCAS 1979 Plasmalemma transport of OH^- in *Chara corallina*. Further analysis of the diffusion system associated with OH^- efflux. J Exp Bot 30: 705–718
5. HELDER RJ 1975 Polar potassium transport and electrical potential difference across the leaf of *Potamogeton lucens* L. Proc Kon Ned Akad Wet C78: 189–197
6. HELDER RJ 1975 Flux ratios and concentration ratios in relation to electrical potential differences and transport of rubidium ions across the leaf of *Potamogeton lucens* L. Proc Kon Ned Akad Wet C78: 376–388
7. HELDER RJ, J BOERMA 1973 Exchange and polar transport of rubidium ions across the leaves of *Potamogeton*. Acta Bot Néerl 22: 686–693
8. HELDER RJ, PE ZANSTRA 1977 Changes of the pH at the upper and lower surface of bicarbonate assimilating leaves of *Potamogeton lucens* L. Proc Kon Ned Akad Wet C80: 421–436
9. JESCHKE WD 1970 Lichtabhängige Veränderungen des Membranpotentials bei Blattzellen von *Elodea densa*. Z Pflanzenphysiol 62: 158–172
10. JESCHKE WD 1972 Ueber den lichtgeförderten Influx von Lonen in Blättern von *Elodea densa*. Vergleich des Influx von K^+ and Cl^- ionen. Planta 103: 164–180
11. LUCAS WJ 1975 Photosynthetic fixation of ^{14}Carbon by internodal cells of *Chara corallina*. J Exp Bot 26: 331–346
12. LUCAS WJ 1975 The influence of light intensity on the activation and operation of the hydroxyl efflux system of *Chara corallina*. J Exp Bot 26: 347–360
13. LUCAS WJ 1976 Plasmalemma transport of HCO_3^- and OH^- in *Chara corallina*: non-antiporter systems. J Exp Bot 27: 19–31
14. LUCAS WJ, FA SMITH 1973 The formation of alkaline and acid regions at the surface of *Chara corallina* cells. J Exp Bot 24: 1–14
15. LUCAS WJ, JM FERRIER, J DAINTY 1977 Plasmalemma transport of OH^- in *Chara corallina*. J Membr Biol 32: 49–73
16. LUCAS WJ, RM SPANSWICK, J DAINTY 1978 HCO_3^- influx across the plasmalemma of *Chara corallina*. Physiological and biophysical influence of 10 mM K^+. Plant Physiol 61: 487–493
17. LUCAS WJ, MT TYREE, A PETROV 1978 Characterization of photosynthetic ^{14}Carbon assimilation by *Potamogeton lucens* L. J Exp Bot 29: 1409–1421
18. LUCAS WJ 1980 Control and synchronization of HCO_3^- and OH^- transport during photosynthetic assimilation of exogenous HCO_3^-. In RM Spanswick, WJ Lucas, J Dainty, eds, Membrane Transport in Plants: Current Conceptual Issues. Elsevier/North Holland, Amsterdam, pp 317–327
19. PATE JS, BES GUNNING 1972 Transfer cells. Annu Rev Plant Physiol 23: 173–196
20. POOLE RJ 1978 Energy coupling for membrane transport. Annu Rev Plant Physiol 29: 437–461
21. PRINS HBA 1974 Photosynthesis and ion uptake in leaves of *Vallisneria spiralis* L. PhD thesis, University of Groningen, Haren, The Netherlands
22. PRINS HBA, RJ HELDER, PJC KUIPER 1978 Bicarbonate fixation and polar ion transport in leaves of *Potamogeton* and *Elodea*. Proc FESPP Meet, pp 20–21
23. PRINS HBA, RJ HARPER, N HIGINBOTHAM 1980 Membrane potentials of *Vallisneria* leaf cells and their relation to photosynthesis. Plant Physiol 65: 1–5
24. PRINS HBA, RJ HELDER 1980 Photosynthetic use of HCO_3^- by *Elodea* and *Potamogeton*, pH changes induced by HCO_3^-, CO_2, K^+, and H^+/OH^- transport. *In* RM Spanswick, WJ Lucas, J Dainty, eds, Membrane Transport in Plants; Current Conceptual Issues. Elsevier/North Holland, Amsterdam, pp 625–626
25. PRINS HBA, JFH SNEL, RJ HELDER, PE ZANSTRA 1979 Photosynthetic bicarbonate utilization in the aquatic angiosperms *Potamogeton* and *Elodea*. Hydrobiol Bull 13: 106–111
26. SPEAR DG, JK BARR, CE BARR 1969 Localization of hydrogen ion and chloride ion fluxes in *Nitella*. J Gen Physiol 54: 397–414
27. STEEMANN NIELSEN E 1960 Uptake of CO_2 by the plant. *In* Encyclopedia of Plant Physiology, Vol 5, Part 1. Springer, Berlin, pp 78–84
28. WALKER NA, FA SMITH 1977 Circulating electric currents between acid and alkaline zones associated with HCO_3^- assimilation in *Chara*. J Exp Bot 28: 1190–1206
29. WALKER NA 1980 The transport systems of charophyte and chlorophyte giant algae and their integration into modes of behavior. *In* RM Spanswick, WJ Lucas, J Dainty, eds, Membrane Transport in Plants: Current Conceptual Issues. Elsevier/North Holland, Amsterdam, pp 287–300
30. ZELITCH I 1971 Photosynthesis, Photorespiration and Plant Productivity. Academic Press, New York, pp 145–160