Plant Physiol. (1989) 91, 62–67
0032-0889/89/91/0062/06/$01.00/0

Received for publication August 3, 1988
and in revised form April 3, 1989

Light-Induced Polar pH Changes in Leaves of *Elodea canadensis*[1]

I. Effects of Carbon Concentration and Light Intensity

J. Theo M. Elzenga* and Hidde B. A. Prins

University of Groningen, Department of Plant Physiology, P. O. Box 14, 9750 AA Haren, The Netherlands

ABSTRACT

Leaves of the submerged aquatic *Elodea canadensis* Michx. exhibit a light induced polar pH reaction. In this study, the effects of light intensity and dissolved inorganic carbon concentration on this polar reaction were examined. At a light intensity of 100 watts per square meter the leaf showed a polar pH response when the dissolved inorganic carbon concentration was less than about 1 millimolar. The polar reaction was suppressed at a higher dissolved inorganic carbon concentration. This suppression was not due to the buffering capacity of bicarbonate. Because another weak acid, acetate, did not inhibit the polarity, but even had a small stimulatory effect, the effect of bicarbonate is also not due to acidification of the cytoplasm. The suppression of the polar reaction by CO_2/HCO_3^- was relieved when the light intensity was increased. Apparently there is competition for product(s) of the photosynthetic light reactions between processes generating the polar reaction and the carbon fixation reactions. The possibility that the redox state of the cell regulates the generation of the polar reaction is discussed.

Leaves of submerged aquatic plants like *Elodea canadensis*, *E. nuttallii*, *Egeria densa*, and *Potamogeton lucens* exhibit a polar reaction in light (10). Upon illumination the medium on the lower leaf surface is acidified while the upper surface becomes more alkaline. Concurrently, an electric potential difference of a few millivolts between the upper and lower sides, the lower side positive, is established and a net cation flux from lower to upper side occurs. These polar reactions are thought to be associated with the ability of these species to use bicarbonate as a carbon source for photosynthesis. The acidification of the medium on the lower side of the leaf is due to an active proton efflux while the release of hydroxyl ions on the upper side of the leaf is a passive process (18). The polar reaction facilitates the use of bicarbonate in photosynthesis by shifting the bicarbonate-CO_2 equilibrium in the unstirred layer on the lower side of the leaf, thereby increasing the CO_2 concentration of the unstirred layer and increasing diffusion of CO_2 into the leaf (19–22). The secreted protons may also drive a H^+-HCO_3^- cotransport mechanism (13). To compensate for the loss of H^+, hydroxyl ions are released at the upper side. The polar reaction is light dependent and reversibly inhibited by low concentrations of DCMU (5). Inhibitors of plasma-membrane ATPase, DES,[2] and DCCD, inhibit the pH changes in the light (5), showing that ATPase activity is essential in the generation of the polar reaction. In aquatic plants, several light-dependent membrane transport processes are inhibited by CO_2, *e.g.* the light-stimulated Cl^- influx in leaves of *E. densa* (12). CO_2 also inhibited Rb^+ and Cl^- influx in *Vallisneria* leaves (17). Spanswick and Miller (23) found that 1 mM CO_2/HCO_3^- inhibited the Cl^- influx and induced a depolarization of the membrane potential in *Nitella translucens*. As the photosynthetic use of HCO_3^-, and thus the polar reaction, will be especially relevant under conditions of limiting DIC supply, we investigated the influence of changes in carbon concentration and light intensity on the polar reaction.

MATERIALS AND METHODS

Culturing Conditions

Elodea canadensis Michx. plants used in the ^{14}C fixation experiments were grown inside in concrete tanks on a clay substrate covered with 2 cm of washed sand to prevent perturbation of the clay particles. The tanks were filled with demineralized water. This resulted in low nutrient levels in the water and a pH between 7.5 and 8.2. The light regime was 12 h light/12 h dark. The light intensity was 40 $W \cdot m^{-2}$. For the pH measurement experiments the plants were grown in 250 mL Erlenmeyer flasks in a 5% strength Hoagland solution (11) changed twice a week. The light regime was 14 h light/10 h dark. Light intensity was 50 $W \cdot m^{-2}$ and the temperature was kept at 20°C.

pH Measurements

The leaf surface pH measurements were performed as described earlier (20). DIC concentrations were determined at the end of each experiment by titrating the solution with 0.1 N HCl to pH 4.2 (7).

[1] This study was supported by the Foundation for Fundamental Biological Research (BION), which is subsidized by the Netherlands Organization for the Advancement of Pure Research (NWO). This is an Ecotrans publication.

[2] Abbreviations: DES, diethylstilbestrol; APW, artificial pond water; DCCD, *N,N'*-dicyclohexylcarbodi-imide; DIC, dissolved inorganic carbon; DMO, 5,5-dimethyloxazolidine-2,4-dione; NEM, *N*-ethylmaleimide; PCMBS, *p*-chloromercuriphenylsulfonic acid.

^{14}C Fixation

The ^{14}C fixation rate was determined in duplicate on whole leaves, detached from the stem, in 1.5 mL Eppendorf reaction vessels fixed to a rotation device in a thermostated water bath (25). The light intensity was measured at the surface of the water bath. The effective light intensity in the reaction vessels was somewhat lower than the indicated values and therefore shows a slight deviation from the light intensities used in the pH measurements. The leaves were incubated in the experimental solution containing 2.5 or 0.25 mM $KHCO_3$ in APW (5 mM $CaCl_2$, 1.5 mM KCl, and 1 mM NaCl) buffered at pH 7.5 with 3 mM Tris/Mes and were kept in the dark for at least 30 min. The leaves were then illuminated for 15 min at the indicated light intensity and after this period 10 μL $KH^{14}CO_3$ (7.4 10^3 Bq) was added and the leaves were illuminated for another 15 min. To stop the reaction the medium was quickly removed and the leaves were frozen, inside the cups, in liquid nitrogen. One mL 80% acetone was added and after overnight extraction the Chl content was determined according to Bruinsma (2). Four N H_2SO_4 (200 μL) was added and the extract was incubated for 2 h at 70°C to remove the acetone and any unfixed $^{14}CO_2$. The volume of the remaining extract was determined by weighing. Fixed ^{14}C was determined by counting 100 μL samples of the extract in a scintillation counter with Picofluor as the scintillation fluid.

ATP Measurements

The ATP measurements were performed using the luciferine-luceferase luminescence method according to Spanswick and Miller (23). The light intensity used was 40 $W \cdot m^{-2}$. The medium, buffered with 1 mM Mes (pH 7.0), contained 0.6 mM DIC.

RESULTS

Effect of DIC Concentration

Figure 1A shows an experiment in which the pH changes on the upper and lower side of the leaf were simultaneously measured upon illumination. The light-induced polar pH changes in a medium with low (0.9 mM) DIC concentration are shown. In the dark the pH on both sides of the leaf was 0.2 to 0.5 pH units lower than that of the medium. Upon illumination the pH on the lower side initially increased, probably due to the uptake of CO_2 (19) and then dropped to a final value about 2 pH units lower than the pH of the medium. The pH on the upper side also showed an initial rise during the first 6 min which was then followed by a further increase to a final pH value 3 units higher than the pH of the medium. When the light was turned off the pH on upper and lower side returned to the original dark value in about 30 min.

Figure 1B shows the reaction of the leaf in a medium with a high DIC concentration (3.8 mM). In this medium, the initial slow rise in pH upon the onset of illumination, again was present. However, the leaf did not exhibit a polar pH reaction; the initial rise in pH was not followed by a strong alkalinization on the upper side and an acidification on the lower side. When the lights were turned off, the pH on both the upper and lower sides returned to the original dark value.

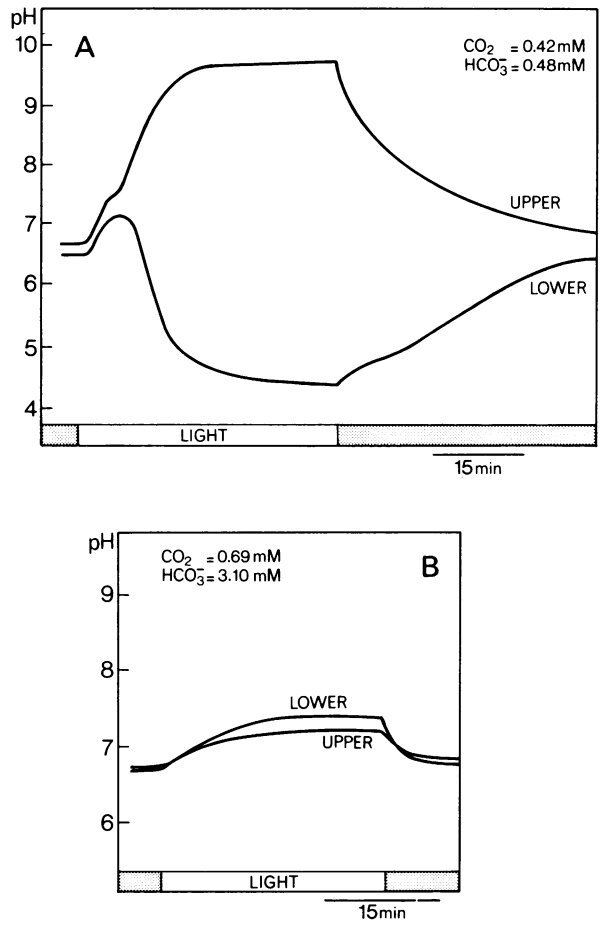

Figure 1. A, pH changes near the leaf of *E. canadensis* in a medium with low (0.9 mM) total carbon concentration. Upper curve, pH on the upper side of the leaf; lower curve, pH changes near the lower side of the leaf. The light (100 $W \cdot m^{-2}$) and dark periods are indicated at the lower side of the figure. B, pH changes in a medium with high (3.8 mM) total carbon concentration.

In Figure 2 the results of experiments in media with different carbon concentrations and pH performed at a light intensity of 100 $W \cdot m^{-2}$ are compiled. Obviously, there was no influence of the pH of the medium in the range measured on the light-induced polar pH changes. The total carbon concentration seems to determine whether a polar pH reaction was induced or not. When the carbon concentration was lower than about 1.15 mM light-induced leaf pH changes became polar, above this concentration the polarity was inhibited. As the proportion of CO_2 in the total carbon depends upon the pH of the medium and since changes in pH did not influence the polar leaf pH changes, it seems that it is not the free CO_2 concentration in the bulk medium that controls the polar leaf reaction, but rather the DIC concentration of the medium.

To test whether the effect of increasing DIC concentration was due to increased buffering capacity of the medium, we determined the pH change near the leaf surface after adding Mes (with a pK_a of 6.15, pK_a of carbonic acid is 6.1) buffer to the medium (Fig 3). The Mes-induced changes of the leaf

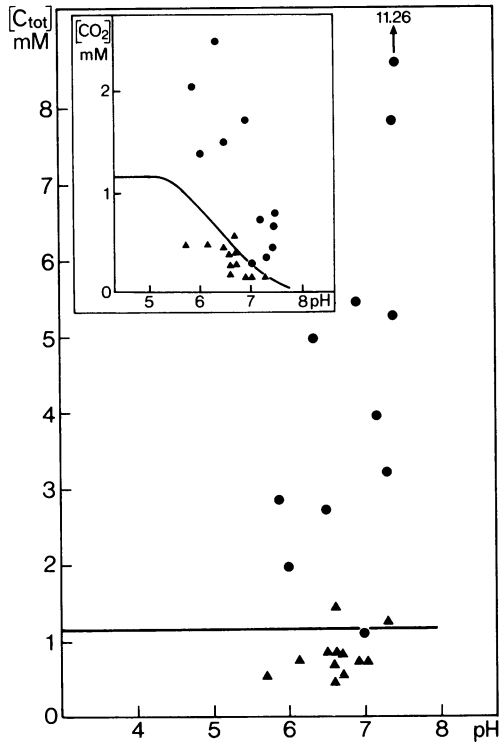

Figure 2. Reaction of leaves, polar (▲) (as in Fig. 1A) or nonpolar (●) (as in Fig. 1B), in media with different carbon concentrations and initial pH. The line drawn, dividing the polar and nonpolar reactions, is 1.15 mM total C. In the inset the same reactions are plotted against the pH and the free CO_2 concentration of the medium. The line drawn is the calculated free CO_2 concentration in a medium with 1.15 mM total C. Note that at one CO_2 concentration both polar and nonpolar reactions can occur.

pH were quantitatively less than the changes induced by increasing DIC (changes induced by Mes: pH upper side: −1.3 ± 0.2 pH units, pH lower side +0.4 ± 0.1 [n = 3]; induced by DIC: pH upper side: −3.1 ± 0.6, pH lower side +2.0 ± 0.2 [n = 3]); upon addition of Mes the leaf pH stabilized within minutes, whereas the changes induced by DIC took at least 20 min to reach a steady state.

Influence of Light Intensity

The experiments described so far were all conducted at a light intensity of 100 $W \cdot m^{-2}$. Figure 4 shows the results of an experiment in which the light intensity was varied. It is apparent that suppression of the light-induced polar leaf pH reaction by high carbon concentrations could be relieved by increasing the light intensity. Lowering the light intensity resulted in suppression of the polarity.

As CO_2 can act as a membrane-permeable weak acid, its effect on the polar reaction could be the result of acidification of the cytoplasm. Therefore, we tested the effect of acetic acid and DMO, two other membrane permeable weak acids, on the polarity.

Potassiumacetate up to 2 mM did not inhibit the light-induced polar leaf pH reaction and even had a small stimulatory effect (Fig. 5) (changes induced by 1 mM potassium-

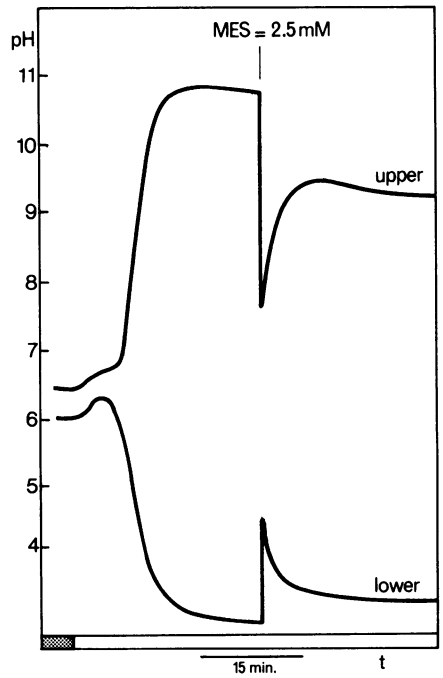

Figure 3. Influence of 2.5 mM Mes on the pH near the leaf surface of *E. canadensis*.

acetate: pH upper side +0.7 ± 0.3 pH units, pH lower side +0.1 ± 0.3 [n = 3]). Contrary to this result, DMO at relatively low concentrations (1 mM) inhibited the polarity strongly (results not shown). The inhibition by DMO could only partially be counteracted by increased light intensity.

To get an indication whether there was a direct correlation between the degree of the polar leaf pH reaction and carbon limitation of photosynthetic carbon fixation, carbon fixation was measured in relation to the light intensity at high and low carbon concentrations. From Figure 6 it can be seen that at a light intensity of 100 $W \cdot m^{-2}$ the photosynthesis with 2.5 mM DIC is much higher than with 0.25 mM DIC. With 0.25 mM DIC, photosynthesis rates must have been limited by the carbon supply to some extent. Under these conditions a polar leaf pH reaction was usually induced (Fig. 2). In the medium with a carbon concentration of 2.5 mM DIC, around 100 $W \cdot m^{-2}$ photosynthesis is linearly dependent on the light intensity and probably not limited by carbon. At this higher DIC concentration the leaf pH polarity was usually suppressed.

To check whether the polarity is directly controlled by the availability of ATP to the proton pumping ATPase, we determined the ATP concentration in light and in dark. In *Elodea nuttalli*, a species closely related to *E. canadensis* and showing the same pH polarity and the same response to increased DIC (data not shown), the ATP concentration did not change upon light/dark transitions (Fig. 7).

DISCUSSION

The DIC concentration of the ambient medium influenced the light dependent polar pH changes near the leaf surface in *Elodea canadensis*. At high DIC concentration the polarity was suppressed. A higher DIC concentration increases the

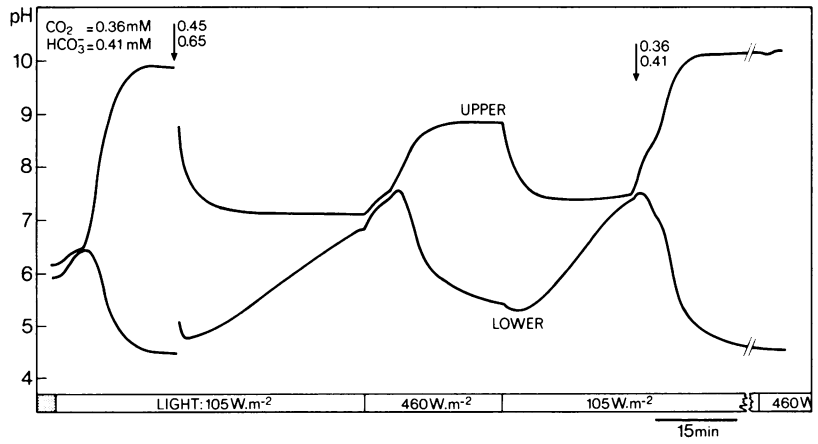

Figure 4. Effect of carbon concentration and light intensity on pH near the leaf. At the points indicated by the arrows the medium was replaced by medium with the indicated carbon concentration. Light intensity and light and dark periods are indicated at the bottom of the figure.

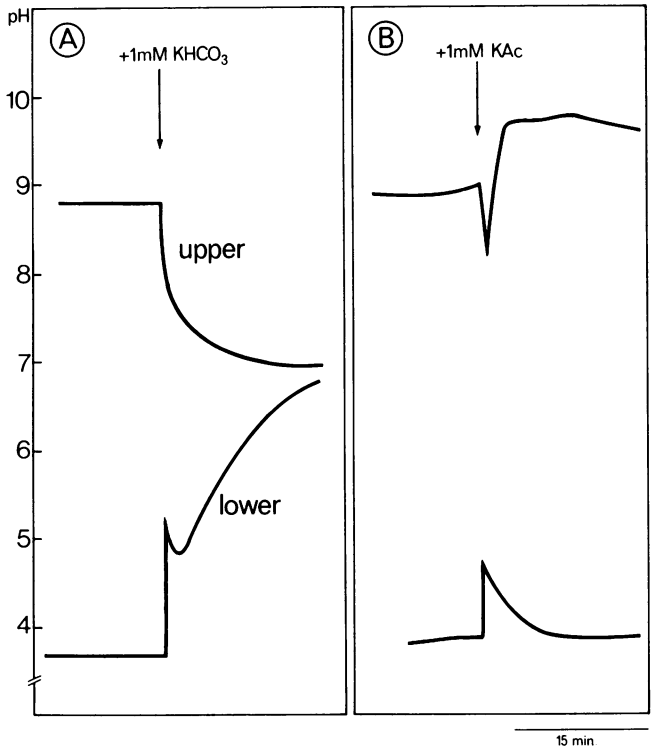

Figure 5. Different effects of (A) 1 mM $KHCO_3$ and of (B) 1 mM potassium acetate on the pH near the leaf surface of *E. canadensis*.

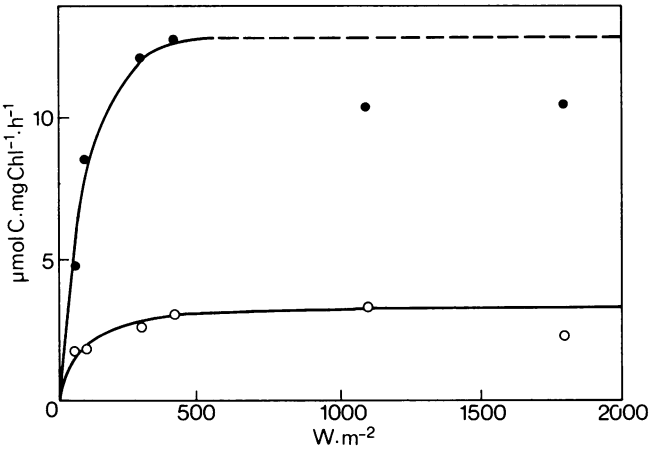

Figure 6. Influence of the light intensity on ^{14}C-fixation in media containing 0.25 (○) and 2.5 (●) mM total carbon, respectively.

buffering capacity of the medium. However, the effect of increased DIC concentration on the pH near the leaf surface could not be attributed to the increased buffering capacity. The effect of added buffering capacity could only partially explain the reduced pH changes. Using the equations given by Price and Badger (16), we calculated the effect of such an increase in buffering capacity. The change in buffering capacity by the increase in DIC concentration shown in Figure 4 could only account for a pH change of approximately 1 pH unit instead of the observed 2 pH units. Also the slow time course of the pH change on the lower side of the leaf upon increasing the DIC concentration is not in agreement with the rapid response of pH to buffering (*cf.* Fig. 3).

Also, if the difference between polar and nonpolar reactions was caused by increased buffering alone, the acidification would be reduced but not completely suppressed. Instead, we observed that under certain conditions (low light, high DIC concentration) acidification was entirely absent.

The results thus indicate that the DIC concentration in the medium and the light intensity used had a physiological effect on the light-induced polarity change of the leaf pH. If the light intensity was relatively low and the carbon concentration relatively high, no polar reaction was observed. In contrast, when high light intensities and relatively low carbon concentrations were used, the polar reaction did occur. It seems clear that this mechanism has ecological significance: if the capacity of carbon fixation is high, at high light intensities, and the supply of carbon limited, the leaf reacts by acidifying the lower side of the leaf, a reaction thought to be beneficial in the uptake of bicarbonate.

Figure 6 shows that at a light intensity of 100 W·m^{-2} and a DIC concentration of 0.25 mM photosynthesis is carbon limited. As described in "Materials and Methods," the effective light intensities during carbon fixation were somewhat lower than the indicated values. This means that carbon limitation occurs at even lower light intensities.

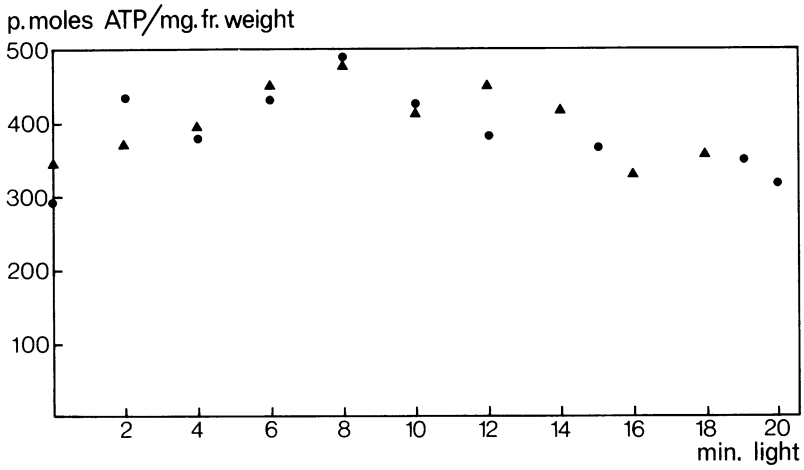

Figure 7. Effect of dark-light transitions on the ATP concentration in *E. nuttallii*.

As CO_2 is a membrane permeable weak acid, increasing the inorganic carbon concentration could have an acidifying effect on the cytoplasm. One could assume that this acidification caused the inhibition of the polar leaf pH reaction. Although there is no general agreement on the effect on the pH of the cytoplasm of illuminating photosynthesizing tissue (3, 6, 14, 15, 24, 26, 28), most studies indicate that illumination results in alkalinization of the cytoplasm (24). Illumination could thus counteract an acidification induced by a weak acid. Such a mechanism could be in agreement with the results described. Since the induction of pH polarity by light and the effect of increased light intensity when the polarity was inhibited by a high carbon concentration were similar, it is likely that they depend on the same mechanism. This would mean that in the dark, pH polarity was suppressed by the low pH of the cytoplasm. The cytoplasmic pH of plant cells in the dark is found to be about 7.0 (24). We demonstrated that the polarity is ATPase dependent (5). As the cytoplasmic pH value is higher than the optimum pH for plasmalemma bound ATPase activity (27; JTM Elzenga, M Staal, HBA Prins, unpublished data) it is unlikely that the stimulation of polar pH changes is dependent on an alkalinization of the cytoplasm; illumination would lead to less favorable cytoplasmic pH values. Although the effect of DMO supports the hypothesis that the polarity was influenced by acidification of the cytoplasm, acetic acid, which normally invokes the same reaction as DMO, did not suppress, but rather stimulated, the pH polarity. In *Vallisneria spiralis*, also a submerged aquatic member of the *Hydrocharitaceae*, acetate stimulated the light-dependent proton pump as judged from the hyperpolarization of the membrane potential (18). From these considerations, we conclude that the stimulating effect of light and the inhibiting effect of CO_2 (DIC) are not caused by a change in cytoplasmic pH.

An alternative mechanism could be competition for products of the photosynthetic light reaction between carbon fixation processes and the reactions involved in the generation of the polar reaction. Spanswick and Miller (23) postulated that the effect of CO_2 on the membrane pump reflects the operation of a control system. The simplest model for a control system would be regulation of the proton pumps by the products of photosynthesis. We did not find any indication that the pump was directly controlled by the availability of ATP (Fig. 7). Hampp *et al.* (9) showed that the energy state of the cytosol is kept at a constant value in dark and light by an effective collaboration of photosynthetic and oxidative phosphorylation. Intermediates of the reductive pentose phosphate cycle are not likely candidates since the signal for the onset of the polar reaction would be a reduced concentration of such an intermediate (caused by reduced carbon fixation), a situation that would also occur in the dark. A number of observations indicate that the mechanism(s) involved in regulating carbon fixation cycle enzymes by light are redox reactions, mediated by thioredoxin or ferredoxin systems and possibly by NADPH (1). Possibly the regulation of the polarity in *Elodea* is also mediated by changes in the concentration of reducing equivalents produced in the light reaction. DCMU, an inhibitor of the photosynthetic electron transport chain, preventing the reduction of $NADP^+$ inhibits the polar reaction (5). For tonoplast ATPase, Hager and Biber (8) showed that light mediated oxidation of —SH groups influenced the activity. Using the SH blocking reagents NEM and PCMBS, we demonstrated that in *Elodea* —SH groups are also essential for the polar reaction (5). And we demonstrated that reductase activity in the plasmalemma is associated with the polar reaction (4).

Whether the ATPase activity is directly regulated by reduction-oxidation by a membrane bound reductase is subject of further study.

LITERATURE CITED

1. **Anderson LE** (1979) Interaction between photochemistry and activity of enzymes. *In* M Gibbs, E Latzko, eds, Encyclopedia of Plant Physiology (New Series), Photosynthesis II. Springer-Verlag, Berlin, pp 271–281
2. **Bruinsma J** (1963) The quantitative analysis of chlorophylls a and b in plant extracts. Photochem Photobiol **2**: 241–249
3. **Davis RF** (1974) Photoinduced changes in electrical potentials and H^+ activities of the chloroplast, cytoplasm and vacuole of *Phaeoceros levis*. *In* U Zimmermann, J Dainty, eds, Membrane Transport in Plants. Springer-Verlag, Heidelberg, pp 197–201
4. **Elzenga JTM, Prins HBA** (1987) Light induced polarity of redox reactions in leaves of *Elodea canadensis* Michx. Plant Physiol **85**: 239–242

5. **Elzenga JTM, Prins HBA** (1989) ATPase activity and redox reactions in polar leaves of the submerged macrophyte *Elodea canadensis. In* NA Walker, ed, Proceedings of the 7th International Workshop on Plant Membrane Transport, Sydney (in press)
6. **Felle H, Bertl A** (1986) Light induced cytoplasmic pH changes and their interrelation to the activity of the electrogenic proton pump in *Riccia fluitans.* Biochim Biophys Acta **848:** 176–182
7. **Golterman HL, Clymo RS** (1969) Methods for chemical analysis of fresh waters. IBP Handbook No 8. Blackwell, Oxford
8. **Hager A, Biber W** (1984) Functional and regulatory properties of H^+ pumps at the tonoplast and plasma membranes of *Zea mays* coleoptiles. Z Naturforsch **39c:** 927–937
9. **Hampp R, Goller M, Ziegler H** (1982) Adenylate levels, energy charge and phosphorylation potential during dark-light and light dark transition in chlorplasts, mitochondria and cytosol of mesophyl protoplasts from *Avena sativa* L. Plant Physiol **69:** 448–455
10. **Helder RJ, Boerma J** (1973) Exchange and polar transport of rubidium ions across the leaves of *Potamogeton.* Acta Bot Neerl **22:** 686–693
11. **Hoagland DR, Snijder WC** (1933) Nutrition of strawberry plants under controlled conditions. Proc Am Soc Hortic Sci **30:** 288–296
12. **Jeschke WD, Simonis W** (1969) Uber die Wirkung von CO_2 auf die lichtabhangige Cl^- Aufname bei *Elodea densa*: Regulation zwischen nicht cyclisher und cyclischer photophosphorylierung. Planta **88:** 157–171
13. **Lucas WJ** (1983) Photosynthetic assimilation of exogenous HCO_3^- by aquatic plants. Annu Rev Plant Physiol **34:** 71–104
14. **Mimura T, Kirino Y** (1984) Changes in cytoplasmic pH measured by P-31 NMR in cells of *Nitellopsis obtusa.* Plant Cell Physiol **25:** 813–820
15. **Mitsumori F, Ito O** (1984) Phosphorus-31 nuclear magnetic resonance studies of photosynthesizing *Chlorella.* FEBS Lett **174:** 248–252
16. **Price GD, Badger MR** (1985) Inhibition by proton buffers of photosynthetic utilization of bicarbonate in *Chara corallina.* Aust J Plant Physiol **12:** 257–267
17. **Prins HBA** (1974) Photosynthesis and ion uptake in leaves of *Vallisneria spiralis* L. Thesis. University of Groningen, The Netherlands
18. **Prins HBA, Harper RJ, Higinbotham N** (1980) Membrane potentials of *Vallisneria* leaf cells and their relation to photosynthesis. Plant Physiol **65:** 1–5
19. **Prins HBA, Snel JFH, Helder RJ, Zanstra PE** (1980) Photosynthetic HCO_3^- utilization and OH^- excretion in aquatic angiosperms. Light induced pH changes at the leaf surface. Plant Physiol **66:** 818–822
20. **Prins HBA, Snel JFH, Zanstra PE, Helder RJ** (1982) The mechanism of bicarbonate assimilation by the polar leaves of *Potamogeton* and *Elodea.* CO_2 concentrations at the leaf surface. Plant Cell Environ **5:** 207–214
21. **Prins HBA, Snel JFH, Zanstra PE** (1982) The mechanism of photosynthetic bicarbonate utilization. *In* JJ Symoens, SS Hooper, P Compere, eds, Studies On Aquatic Vascular Plants. Royal Botanical Society of Belgium, Brussels, pp 120–126
22. **Prins HBA, O'Brien J, Zanstra PE** (1982) Bicarbonate utilization in aquatic angiosperms. pH and CO_2 concentration at the leaf surface. *In* JJ Symoens, SS Hooper, P Compere, eds, Studies On Aquatic Vascular Plants. Royal Botanical Society of Belgium, Brussels, pp 112–119
23. **Spanswick RM, Miller AG** (1977) The effect of CO_2 on the Cl^--influx and electrogenic pump in *Nitella translucens.* Colloque du CNRS **258:** 239–245
24. **Sianoudis J, Kusel AC, Mayer A, Grimme LH, Leibfritz D** (1987) The cytoplasmic pH in photosynthesizing cells of the alga *Chlorella fusca*, measured by P-31 NMR spectroscopy. Arch Micro biol **147:** 25–29
25. **Staal M, Prins HBA, van Harmelen M, Helder RJ** (1988) The isolation of leaf protoplasts from the submerged aquatic angiosperm Potamogeton lucens L. Plant Cell Environ **11:** 715–719
26. **Steigner W, Kohler K, Simonis W, Urbach W** (1989) Signal transduction from chloroplasts to potassium channels in *Eremosphaera viridis. In* NA Walker, ed, Proceedings of the 7th International Workshop on Plant Membrane Transport, Sydney (in press)
27. **Sze H** (1985) H^+-translocating ATPases: advances using membrane vesicles. Annu Rev Plant Physiol **75:** 175–208
28. **Walker NA, Smith FA** (1975) Intracellular pH in *Chara corallina* measured by DMO distribution. Plant Sci Lett **4:** 125–132