Atti Accademia Nazionale dei Lincei

Classe Scienze Fisiche Matematiche Naturali

Rendiconti

Maria Teresa Marrè, Francesco Albergoni, Erasmo Marrè

# Preliminary observations on the integration of photosynthesis with solute transport. II. Photosynthesis-induced activation of the vanadate-sensitive electrogenic proton pump in Elodea densa Planch leaves

*Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Serie 8*, Vol. **80** (1986), n.4, p. 233–237.

Accademia Nazionale dei Lincei

<http://www.bdim.eu/item?id=RLINA_1986_8_80_4_233_0>

Atti della Accademia Nazionale dei Lincei. Classe di Scienze Fisiche, Matematiche e Naturali. Rendiconti, Accademia Nazionale dei Lincei, 1986.

**Fisiologia vegetale.** — *Preliminary observations on the integration of photosynthesis with solute transport.* II. *Photosynthesis-induced activation of the vanadate-sensitive electrogenic proton pump in* Elodea densa *Planch leaves* (*). Nota di MARIA TERESA MARRÈ (**), FRANCESCO ALBERGONI (**) ed ERASMO MARRÈ (***), presentata (****) dal Corrisp. E. MARRÈ.

RIASSUNTO. — L'attività fotosintetica di foglie isolate di *Elodea densa* Planch risulta associata ad una iperpolarizzazione del potenziale elettrico transmembrana e ad un aumento della capacità delle foglie di acidificare il mezzo di incubazione. Tale effetto è completamente soppresso dalla presenza del DCMU, inibitore della fissazione di $CO_2$ e dell'evoluzione di $O_2$ nella fotosintesi. Esso appare inoltre fortemente dipendente dalla presenza di $K^+$ nel mezzo e completamente inibito dal vanadato, inibitore della ATPasi di trasporto di protoni del plasmalemma. I dati suggeriscono che la fotosintesi attivi la stessa pompa protonica ATP-dipendente, vanadato-sensibile e $K^+$ dipendente del plasmalemma, precedentemente dimostrata operare anche al buio in questo materiale.

## INTRODUCTION

In a previous communication we reported some results showing that excised *Elodea densa* Planch is a suitable material for the study of the relationship between photosynthesis and solute transport [1]. In the experimental conditions chosen (pH 6, agitated medium) the rate of photosynthetic $O_2$ evolution was classically dependent on light intensity and $CO_2$ (+ $HCO_3^-$) concentration and was completely inhibited by $5 \times 10^{-6}$ M dichlorophenyldimethylurea (DCMU), which, on the other hand, did not influence $O_2$ uptake in the dark. In strictly comparable experimental conditions proton extrusion in the dark appeared strongly dependent on the presence of $K^+$ in the medium, was almost completely inhibited by sodium orthovanadate, and was markedly stimulated by the toxin fusicoccin (FC); stimulation of $H^+$ extrusion by FC was associated with a significant hyperpolarization of the transmembrane (vacuole-out) electrical potential difference (PD). These results were interpreted as indicating the operation, in this material and in the dark, of an ATP-driven

(*) Ricerca eseguita con contributi del CNR e del M.P.I.
(**) Dipartimento di Biologia dell'Università di Milano.
(***) Centro Interdisciplinare Linceo.
(****) Nella seduta dell'8 marzo 1986.

FC-sensitive electrogenic proton pump, corresponding to the system already shown to operate in a number of non-photosynthetic materials from several plant species [4, 5].

In the present communication we report some data showing that the same (or a very similar) mechanism of electrogenic $H^+$ extrusion is stimulated by photosynthesis.

## Materials and methods

Treatment of the material, measurements of PD and of changes in pH of the incubation medium were carried out as described in a previous paper [1]. Experimental conditions for the single experiments are given in the figure or table legends.

## Results

*Hyperpolarization of* PD *by white light and its inhibition by* DCMU. The curve of fig. 1 shows that the induction of photosynthesis in this material and in our experimental conditions, is associated with a rapid hyperpolarization of

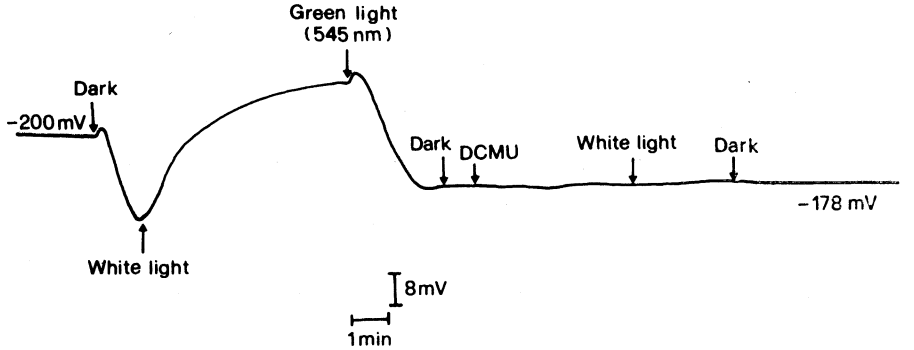


Fig. 1. – Effect of light (20.000 lux, white or green) and of $5 \times 10^{-6}$ DCMU on the transmembrane electrical potential (PD) of *E. densa* leaves. Basal medium: 0.5 mM $CaSO_4$, 0.25 mM $K_2SO_4$, MES/BTP 1 mM, pH 6. Data are means from 3 replicates

the PD, showing a first rapid phase, followed by a slower one: a behaviour already described by other authors for this material and for other acquatic higher plants [2, 3, 7]. The transition from light to dark or to green (non photosynthesis-inducing) light corresponded to a very small transient further hyperpolarization followed by a rapid depolarization which was completed in about 3 minutes. The presence of $5 \times 10^{-6}$ M DCMU (a powerful) inhibitor of photosynthetic electron transfer from system II to system I, and thus of $CO_2$ fixation and $O_2$ evolution) completely blocked the white light-induced PD hyperpolarization.

*Light-induced acidification of the medium of incubation of Elodea leaves. Effects of* $K^+$, DCMU *and vanadate.* The data of Table 1 show that white light induces a marked increase in the capacity of *Elodea densa* to acidify the incu-

TABLE I.

*Effects of light*, $K_2SO_4$, *sodium vanadate and* DCMU *on the* pH *of the medium of incubation of* Elodea densa *leaves.*

| | Δ pH from initial (units) | | Effect of light (20.000 lux) |
|---|---|---|---|
| | (*a*) light) | (*b*) dark | (*a-b*) |
| Control . . . . . . . . . | + 0.02 ± 0.01 | + 0.06 ± 0.03 | — 0.04 |
| 2.5. mM $K_2SO_4$ . . . . . | — 0.43 ± 0.06 | — 0.13 ± 0.01 | — 0.30 |
| $K_2SO_4$ + $10^{-4}$ M Vanadate. | — 0.05 ± 0.02 | 0 ± 0.03 | — 0.05 |
| $K_2SO_4$ + $5.10^{-6}$ M DCMU | — 0.12 ± 0.01 | — 0.10 ± 0.02 | — 0.02 |

Basal medium: 0.5 mM $CaSO_4$, 0.5 mM MES/BTP (MES = 2-(N-Morpholino)-ethane-sulfonic acid; BTP = 1,3 bis[tris(Hydroximethyl)Methylamino]-Propane), initial pH 6. pH of the medium (equilibrated with the atmosphere) measured after 60 minutes of incubation. White light intensity: 10.000 lux. Data are means from 3 replicates ± SE.

bation medium. This effect is strongly dependent on the presence of $K^+$, is completely inhibited by DCMU (blocking photosynthetic $CO_2$ fixation and $O_2$ evolution) and is almost completely suppressed by the plasmalemma $H^+$-transporting ATPase inhibitor vanadate. This suggests that the mechanism of electrogenic $H^+$ extrusion stimulated by light is the same operating in the dark, namely the ATP-driven $H^+$ pump. The dependence of photosynthesis-induced, as well as of the FC-induced $H^+$ secretion on the presence of $K^+$ in the medium is further documented by the data of fig. 2, showing that the presence of $K^+$ (as $K_2SO_4$) to the medium strongly and in parallel increases acid secretion by both the dark and the illuminated, photosynthetizing leaves.

Saturation of this $K^+$ effect in both conditions appears complete at the 1 mM $K_2SO_4$ concentration. The pH changes in the medium were determined after equilibration of aliquots of the medium with air, or after removal of $CO_2$ by bubbling $N_2$ (data not shown) and thus do not depend on respiration- or photosynthesis-induced changes of $CO_2$ and $HCO_3^-$ concentration. This, and the known dependence of $H^+$ extrusion on cation (particularly $K^+$) uptake in other materials [5, 6] strongly suggests that in *Elodea* leaves acidification of the medium depends on $K^+/H^+$ exchange: a point worthy of further investigation. Fig. 2 also shows the stimulating effect of FC on acid secretion, both in light and in darkness, and its dependence on $K^+$ external concentration. The stimulation of acidification by FC appears roughly additive with that by light.

## CONCLUSIONS

The data reported above indicate that illumination with white (but not green) light induces in the photosynthetic cells of *Helodea densa* leaves the activation of an electrogenic acid extruding mechanism apparently identical, for the dependence on external $K^+$ and the sensitivity to vanadate, to the FC-stimulated, presumably ATP-driven, $H^+$ pump operating, in the same material, in the dark. On the other hand, the additivity of the effect of light with that of FC suggests that these two factors activate the plasmalemma $H^+$ pump by different mechanisms of action.

The inhibition of light-induced acid secretion by DCMU indicates that this light effect depends on photosynthetic $CO_2$ fixation and $O_2$ evolution. DCMU, although blocking electron transfer from photosynthetic system II to system I, should not inhibit cyclic photophosphorylation; this suggests that the increased capacity of $H^+$ extrusion does not depend on an increase in ATP level in the illuminated leaves, but rather on the production of photosynthetic $CO_2$ fixation or, possibly, of $O_2$. Further analyses of the phenomenon are necessary for the identification of the mechanism by which photosynthesis regulates $H^+$ transport at the plasmalemma.

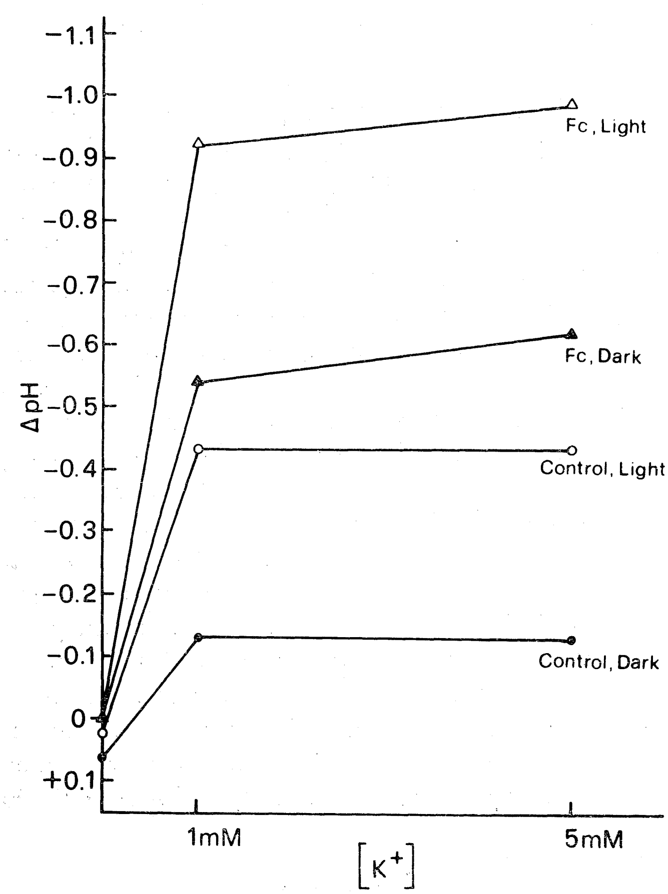


Fig. 2. – Effect of light, $K_2SO_4$ and $10^{-4}$ M FC on the capacity of *E. densa* to acidify the incubation medium leaves. Basal medium: 0.5 mM $CaSO_4$, MES/BTP 0.5 mM, initial pH 6; 25 leaves in 7 ml of medium per flask, agitation 60 strikes per min. pH changes measured at the end of 60 min of incubation.

The causal relationship suggested by these data between the activation of photosynthesis by light and the stimulation of the proton pump is open to interesting speculation from the point of view of its physiological meaning. The building up of an electrochemical $H^+$ gradient by the proton pump is thought to represent the main source of energy for the uptake of a number of solutes. Moreover, the $H^+$ pump-depending acidification of the unstirred liquid layer at the external plasmalemma surface might influence diffusional $CO_2$ influx in the photosynthetizing cells, by locally increasing the $CO_2/HCO_3^-$ ratio (see 8). Proton extrusion-induced changes of cytosolic pH might also be of importance. Work is presently in progress in this laboratory aimed at the further analysis of these, as well as of other possible alternatives.

## References

[1] F. Albergoni, M.T. Marré and E. Marré (1986) – *Preliminary observations on the integration photosynthesis with solute transport.* I. Elodea densa *Planch as a suitable experimental material,* « Rend. Acc. Naz. Lincei », *80,* 149-155.

(2) W.D. Jeschke (1969) – *Lichtabhängige veränderungen des membranpotentials bei blattzellen von* Elodea densa, « Z. Pflanzenphysiol. », *62,* 158-172.

[3] W.D. Jeschke (1972) – *Über den lichtgeförderten influx von ionen in blättern von* Elodea densa. *Vergleich der influxe von* $K^+$*—und* $Cl^-$*—ionen,* « Planta », *6,* 499-512.

[4] E. Marrè (1985) – *Fusicoccin- and hormone-induced changes of* $H^+$ *extrusion: physiological implications.* In: *Frontiers of Membrane Research in Agriculture,* Rowman and Allanheld, New Jersey, pp. 439-460.

[5] E. Marré and A. Ballarin-Denti (1985) – *The proton pumps of the plasmalemma and the tonoplast of higher plants,* « J. of Bioenerg. and Biomemb. », *17,* 1-21.

[6] M.T. Marrè, G. Romani, M. Cocucci, M.M. Moloney and E. Marrè (1982) – *Divalent cation influx, depolarization of the transmembrane electric potential and proton extrusion in maize root segments.* In: *Plasmalemma and Tonoplast their Functions in the Plant Cell,* D. Marmé, E. Marrè and R. Hertel, eds., Elsevier Biomedical Press, pp. 3-13.

[7] A. Novacky, C.I. Ullrich–Eberius and U. Lüttge (1980) – pH *and membrane potential changes during glucose uptake in* Lemna gibba *Gl and their response to light,* « Planta », *149,* 321-326.

[8] H.B.A. Prins and R.J. Helder (1980) – In: *Plant Membrane Transport: Current Conceptual Issues,* R.M. Spanswick, W.J. Lucas and J. Dainty, eds., Elsevier/North-Holland Biomedical Press, pp. 625-626.