An energy-dependent, transient peak in the minute range decay of luminescence, present in CO_2-accumulating cells of *Scenedesmus obliquus*

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Photosynthetic O_2 evolution in the green alga, *Scenedesmus obliquus*, was shown to be more sensitive to the uncoupler FCCP when assayed in a low C_i medium than in a high C_i medium, indicating the action of an energy-dependent mechanism for C_i uptake. Low C_i adapted algae exhibited characteristic luminescence decay kinetics with a transient peak 20–60 s after excitation. This peak was abolished by addition of FCCP and HCO_3^-. The effect caused by HCO_3^- was partially reversed by methyl viologen. In view of the results obtained, a model is presented in order to discuss the origin of the transient luminescence peak.

Luminescence *CO_2 accumulation* *Oxygen evolution*

1. INTRODUCTION

Chlorophyll *a* luminescence is the result of recombination reactions in PS II and can be considered as a reversal of the primary charge separation reactions in photosynthesis [1]. Polyphasic luminescence kinetics, showing peaks or shoulders, have been reported by several authors [2–5] often after illumination with far red light [2–4]. The origin of such kinetics still remains unclear although suggestions have been made indicating involvement of ATP, cyclic electron transport and state transitions [3,4].

Algal cells growing in a low CO_2 environment (equilibrated with air) have been shown to develop an energy-dependent CO_2-accumulating mechanism [7]. It has been suggested that the extra energy needed for C_i transport into the cell/chloroplast is provided by photophosphorylation through cyclic electron transport [8].

Alterations in the degree of photophosphorylation driven by cyclic electron transport have been shown to be regulated by the redox state of the electron transport chain as a consequence of the stromal NADPH/$NADP^+$ ratio [9–11]. For instance, an inhibition of the Calvin cycle due to lack of ATP will slow down the reoxidation of NADPH leading to an accumulation of NADPH in the stroma which will trigger extra ATP formation through cyclic electron transport.

The existence of a direct link between ATPase activity in the thylakoid membrane and luminescence was demonstrated by Joliot and Joliot [12] and Schreiber [13].

In this paper we present results by which the occurrence of a transient luminescence peak, exhibited by cells of *Scenedesmus obliquus* growing in a low C_i environment, can be fitted into a model which takes into account the increased demand for energy caused by active C_i transport.

2. MATERIALS AND METHODS

Culturing conditions: *S. obliquus*, strain D3, was grown in an inorganic medium [14]. Light was provided by fluorescent tubes (Philips TL 20W/55) and the incident photon flux density was 90 $\mu mol \cdot m^{-2} \cdot s^{-1}$ as measured with a quantum meter

Abbreviations: C_i, inorganic carbon

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(LiCor, Lincoln, NE). To obtain low C_i algae, air was bubbled through the algal culture. High C_i algae were generated by bubbling 2% CO_2 in air through the culture for at least 24 h prior to use for experiments.

Oxygen evolution was measured using an oxygen electrode (Hansatech, Norfolk, England). Light was provided by a halogen projector lamp (Atlas 24 V, 250 W) and the photon flux density was 180 $\mu mol \cdot m^{-2} \cdot s^{-1}$. The signal was registered on a chart pen recorder.

Luminescence measurements were carried out in a modified Hansatech O_2 electrode. The white excitation light was provided by a halogen lamp (Narva 12 V, 55 W) and guided to the reaction vessel by an optical fiber. The photon flux density of the light was 600 $\mu mol \cdot m^{-2} \cdot s^{-1}$. Luminescence was detected with a PM tube, selected Hamatzu R374, applied on top of the transparent lid of the electrode. Manually operated shutters were used to regulate time of excitation and to protect the PM tube from the excitation light. Additions of FCCP, HCO_3^- and methyl viologen were made with a syringe into the reaction vessel. 1.5 ml samples were used throughout the luminescence experiments.

3. RESULTS AND DISCUSSION

The results shown in fig.1 illustrate the energy dependence for C_i accumulation. The uncoupler FCCP, which makes energy-transducing membranes permeable to H^+, inhibits photosynthetic O_2 evolution more efficiently in cells assayed in a low C_i medium than in a high C_i medium with 7 mM HCO_3^- added. This is an indication that the decreased ATP production caused by FCCP limits the ability of the algae to concentrate C_i inside the cells. This would cause CO_2 deficiency at the carboxylating enzyme.

The energy dependence of C_i accumulation has been discussed by several authors [7,8,15–18]. Spalding et al. [8] concluded from fluorescence-induction studies that photophosphorylation driven by cyclic electron transport in chloroplasts was the source of the extra ATP needed for C_i transport. On the other hand, Kramer and Findenegg [19] observed that mitochondria in low CO_2 grown cells of *S. obliquus* seemed to gather adjacent to the plasmalemma indicating a high rate of energy consumption there. It has also been argued [7] that the

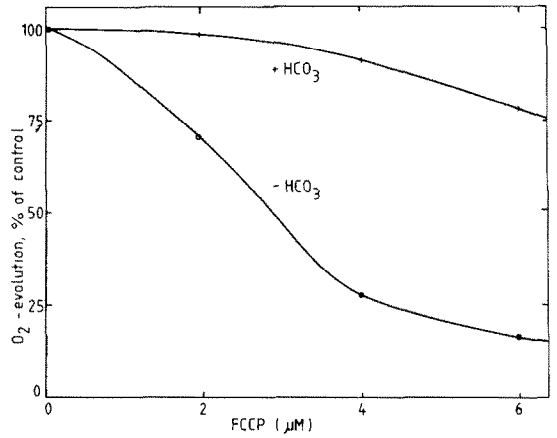

Fig.1. The effect of FCCP on photosynthetic oxygen evolution of *S. obliquus*. The algae were grown under air conditions. The concentration of $NaHCO_3$, when used, was 7 mM.

increased demand for ATP in cells actively transporting C_i could be met by both photophosphorylation in the chloroplast and phosphorylation in general.

In [20] we have shown that a transient peak in the decay of luminescence is correlated to an active C_i uptake in *Scenedesmus* cells. In fig.2, the effect of FCCP on this luminescence peak is shown. Already very low concentrations of FCCP decreased the peak and an almost complete inhibition was found at 1.2 μM, indicating a sensitive dependence of the peak on the trans-thylakoid H^+ gradient.

An H^+ gradient over the thylakoid membrane, with a more acidic value inside, affects luminescence by reducing the activation energy for recombination reactions in PS II [21]. This is explained by the fact that protons on the thylakoid exterior are in equilibrium with protons associated with reduced PQ, while protons on the inside are in equilibrium with protons generated in the water-splitting reaction. Therefore a proton gradient, with a more acidic value inside, will push electrons on the acceptor side of PS II to recombine with positive charges on the donor side, resulting in an increased probability of emission of luminescence. This enhancement effect on luminescence by the trans-thylakoid ΔpH is complicated by the quenching effect that the ΔpH also has on luminescence, as has been shown in studies of chlorophyll *a* fluorescence [22]. The relative importance of

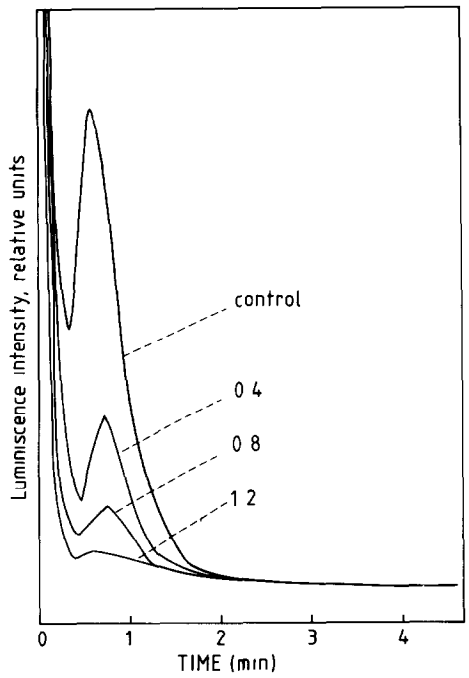

Fig.2. The effect of FCCP on the luminescence decay kinetics from air-grown *S. obliquus*. Inserted numbers refer to the concentration of FCCP used, in μM. FCCP was dissolved in ethanol. The control contained the maximal concentration ethanol used with FCCP. Time of exciatation, 30 s.

these two competing processes on luminescence has not been evaluated in detail. However, from the results of Mayne [23], we can conclude that a trans-thylakoid ΔpH gradient can cause a net increase in luminescence. Therefore, in view of the FCCP effects on both C_i uptake and luminescence kinetics, we suggest that the correlation between the two results from their responses to the energetic state of the chloroplast at a given C_i access.

It is however an oversimplification to discuss the energetic state of the chloroplast only in terms of the ΔpH, since the ΔpH is linked to the ATP/ADP ratio of the stroma by the reversible chloroplast ATPase. In fact, we suggest that the results illustrated in fig.3 are best explained in terms of ATP consumption/production.

Schreiber [13] showed that artificially increased ATP levels in the stroma of preilluminated, intact chloroplasts resulted in ATP hydrolysis, H^+ pumping into the thylakoid lumen, reversed electron flow leading to a reduced Q and a burst of luminescence. Another example of such reverse coupling reactions was demonstrated by Joliot and Joliot [12] in *Chlorella*. They showed that the kinetics of luminescence was dependent on ATP hydrolysis in the dark. From the time scale of the phenomenon they concluded that the hydrolysis was driven by ATP from the mitochondria.

If we combine what is known of an energetic influence on luminescence as discussed above with findings indicating that C_i-transporting algal cells produce at least part of the energy needed for C_i transport in the chloroplast [8], it might be argued that the chloroplast in a C_i-transporting cell is likely to have more ATP available for possible hydrolysis after a light/dark transition than a chloroplast in a high C_i adapted cell. The fast inhibitory effect of HCO_3^- and the reversing effect of methyl viologen (fig.3) could then be explained if photophosphorylation driven by cyclic electron transport is the mechanism for production of at least part of the extra ATP needed for C_i uptake.

Photophosphorylation driven by cyclic electron transport has been shown to function as a regulator of the ATP/NADPH ratio in the stroma during the changing demand for ATP vs reductive power from the Calvin cycle [24]. When the cycle is slowed down because of lack of ATP the reoxidation of NADPH will also slow down, leading to an accumulation of NADPH in the stroma and a consequent increase in the redox state of the electron transport chain. The increased redox state directs electrons through the cyclic pathway and thereby causes an increase in the ATP/NADPH ratio. CO_2 deficiency will in a similar way have an inhibitory effect on the Calvin cycle reactions and the reoxidation of NADPH. It is therefore tempting to assume that the regulatory mechanism for production of ATP vs NADPH can function not only to overcome a temporary ATP deficiency, but also to work against C_i deficiency by production of ATP that could be utilized in active C_i transport.

In our experiments, addition of excess HCO_3^- to air-grown cells (fig.3b) would increase the Calvin cycle turnover and lead to reoxidation of NADPH, a decreased redox state of the electron transport chain, less cyclic electron transport and a lowered ATP/NADPH ratio. Less ATP could therefore be assumed to be available for possible hydrolysis after a light/dark transition. The Calvin cycle will

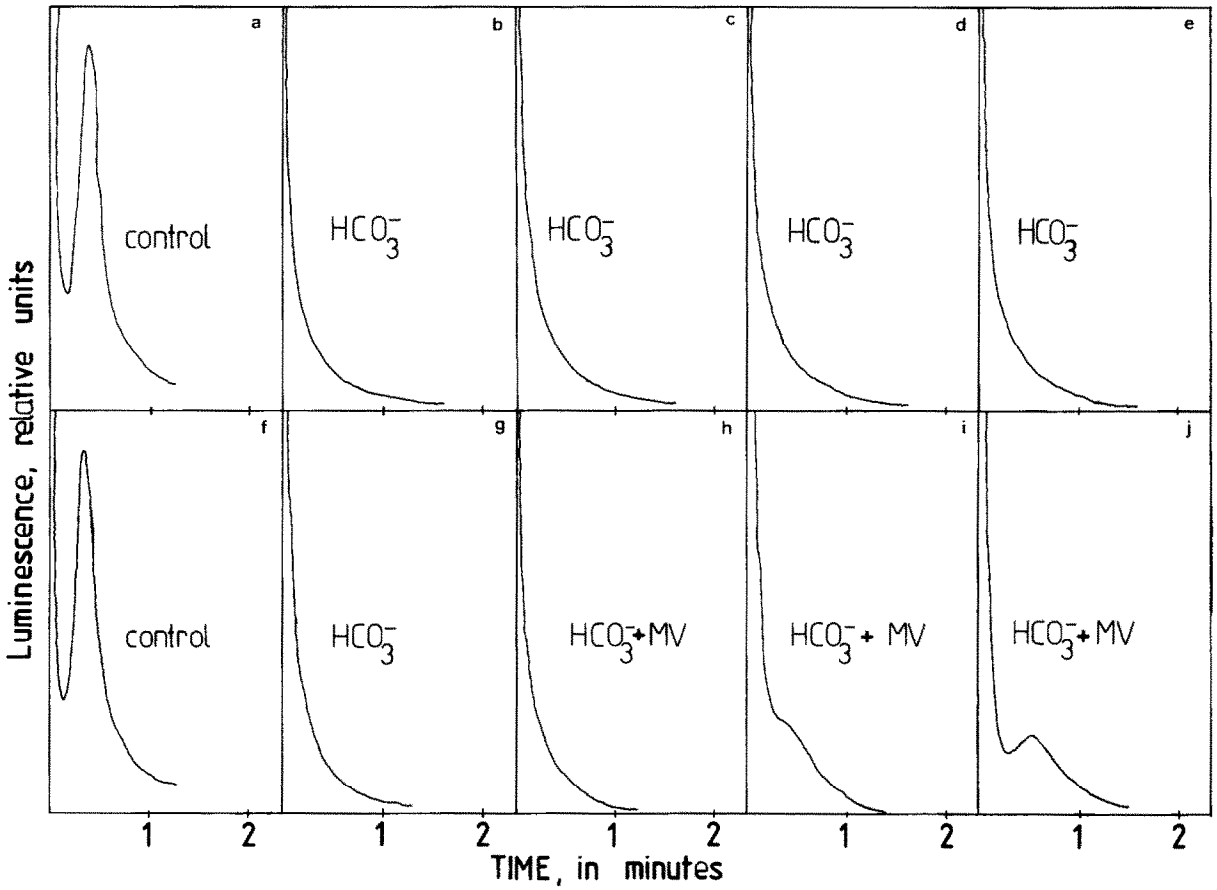

Fig.3. The effect of HCO_3^- and methyl viologen (MV) on the luminescence decay kinetics of air-grown cells of *S. obliquus*. (a–e) Decays from the same sample following 5 subsequent illuminations. HCO_3^- (7 mM) was added after recording of the first control decay. (f–j) Similar sequence but MV (200 mM) was added after recording of the luminescence decay shown in g. Time of each excitation, 30 s; total time in darkness (including luminescence measurements) between illuminations, 120 s.

also act as a stronger ATP sink after addition of HCO_3^- and thus further reduce the amount of ATP available for ATP hydrolysis.

Addition of methyl viologen partly restored the luminescence peak (fig.3h–j). Methyl viologen is an electron acceptor that accepts electrons in PS I before $NADP^+$ and hence inhibits its reduction. Using an O_2 electrode, we checked that the methyl viologen concentration used was high enough to induce O_2 consumption (not shown). Addition of methyl viologen therefore induces a condition where the coregulation between ATP and NADPH production no longer works. As methyl viologen does not inhibit linear electron flow, photophosphorylation would continue while no NADPH could be formed. The lack of reducing power will inhibit the Calvin cycle and its consumption of ATP. Under these conditions, produced ATP could be viewed as being in excess and thus be available to drive reverse coupling reactions after illumination.

In conclusion, conditions in the chloroplast where production of ATP is expected to be high and/or consumption low seem to be well correlated to the appearance of a transient peak in the decay of luminescence. It is suggested that ATP under these conditions could be hydrolysed and drive reverse coupling reactions manifested as a transient luminescence peak. It is tempting to assume that the transient luminescence peak

observed after far-red illumination [2–4] is due to a similar mechanism. This is currently under investigation.

This model does not take into account the possibility that energy is stored in reversible forms other than ATP. Phosphorylated compounds which in darkness could be dephosphorylated and produce ATP exist in both the stroma and cytoplasm. Furthermore, the model does not consider whether ATP hydrolysis itself causes the build up of the luminescence peak by an increasing ΔpH during the time interval when the peak appears, or whether ATP hydrolysis only produces the conditions necessary for reverse reactions that otherwise would not occur. Such reactions could, for instance, utilize electrons reversing from specific reduced components in the electron transport system as long as the ΔpH is maintained above a critical value by ATP hydrolysis.

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REFERENCES

[1] Lavorel, J. (1975) in: Bioenergetics of Photosynthesis (Govindjee ed.) pp. 223–317, Academic Press, New York.
[2] Bertsch, W.F. and Azzi, J.R. (1965) Biochim. Biophys. Acta 94, 15–26.
[3] Björn, L.O. (1971) Physiol. Plant. 25, 316–323.
[4] Desai, T.S., Rane, S.S., Tatake, V.G. and Sane, P.V. (1983) Biochim. Biophys. Acta 724, 485–489.
[5] Rubin, A.B., Fokht, A.S. and Venediktov, P.S. (1966) Biofizika 11, 299–305.
[6] Björn, L.O. (1971) Photochem. Photobiol. 13, 5–20.
[7] Badger, M.R., Kaplan, A. and Berry, J.A. (1980) Plant Physiol. 66, 407–413.
[8] Spalding, M.H., Critchley, C., Govindjee and Ogren, W.L. (1984) Photosynth. Res. 5, 169–176.
[9] Arnon, D.I. and Chain, R.K. (1977) Plant Cell Physiol. Spec. Issue 3, 129–147.
[10] Arnon, D.I. and Chain, R.K. (1979) FEBS Lett. 102, 133–138.
[11] Tsujimoto, H.J., Hiyama, T. and Arnon, D.I. (1980) Biochem. Biophys. Res. Commun. 93, 215–222.
[12] Joliot, P. and Joliot, A. (1980) Plant Physiol. 65, 691–696.
[13] Schreiber, U. (1980) FEBS Lett. 122, 121–124.
[14] Bishop, N.I. (1971) Methods Enzymol. 23, 372–408.
[15] Miller, A.G. and Colman, B. (1980) J. Bacteriol. 143, 1253–1259.
[16] Kaplan, A., Zenvirth, D., Reinhold, L. and Berry, J.A. (1982) Plant Physiol. 69, 978–982.
[17] Raven, J.A. and Glidewell, S.M. (1978) Plant Cell Environ. 1, 185–197.
[18] Zenvirth, D. and Kaplan, A. (1981) Planta 152, 8–12.
[19] Kramer, D. and Findenegg, G.R. (1978) Z. Pflanzenphysiol. 89, 407–410.
[20] Palmkvist, K., Sundblad, L.G., Samuelsson, G. and Sundbom, E. (1985) Photosynth. Res., in press.
[21] Crofts, A.R., Wraight, C.A. and Fleischmann, D.E. (1971) FEBS Lett. 15, 89–100.
[22] Krause, G.H., Vernotte, C. and Briantais, J. (1982) Biochim. Biophys. Acta 679, 116–124.
[23] Mayne, B.C. (1968) Photochem. Photobiol. 8, 107–113.
[24] Slovacek, R.E., Crowther, D. and Hind, G. (1980) Biochim. Biophys. Acta 592, 495–505.