Luminescence decay kinetics in relation to the relaxation of the transthylakoid ΔpH from high and low CO_2 adapted cells of *Scenedesmus obliquus*

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Luminescence decay kinetics was shown to be polyphasic with two relative maxima observed, when high CO_2 adapted cells of *Scenedesmus obliquus* were stressed by CO_2 deficiency. Low CO_2 adapted cells exhibited a single maximum. Luminescence from high CO_2 adapted cells under high CO_2 conditions decayed asymptotically without maxima. The magnitude of the transthylakoid ΔpH was shown to be related to the intensity of luminescence in an antiparallel way. The relaxation kinetics of the ΔpH was shown to be complex, with an interval of increasing or maintained ΔpH. A working hypothesis is presented in order to explain polyphasic luminescence decay kinetics in terms of luminescence quenching by the transthylakoid ΔpH.

Luminescence *CO_2 accumulation* *pH gradient* *Thylakoid membrane*

1. INTRODUCTION

Luminescence from green plants was first discovered by Strehler and Arnold [1]. It is a result of the recombination between positive charges on the donor side of PS II and negative charges on the acceptor side and can thus be considered as a true reversal of the primary photoact in photosynthesis [2]. Luminescence is affected by several treatments such as heat, acid-base transitions, electron transport inhibitors, uncouplers, etc. These treatments either influence the concentration of luminescence precursors, i.e. separated charges in PS II, or the activation energy for these charges to recombine. Treatments that result in changes of the transthylakoid ΔpH will affect luminescence in two ways. First, a ΔpH with a more acidic inside will lower the activation energy for recombinations in PS II and hence enhance the emission of luminescence [3], second, a ΔpH will also quench luminescence, through the same mechanism as it quenches prompt fluorescence, the so-called 'energy quenching' [4]. The net effect on luminescence by these two contradictory processes may differ with different conditions in the chloroplast.

Green algal cells growing in a low CO_2 environment develop the ability to transport C_i into the cell/chloroplast against a concentration gradient. The development of this C_i concentrating mechanism implies both energetic changes and alterations in the protein synthesis of the cell [5]. The energetic role of the algal chloroplast in this context was discussed by Spalding et al. [6] who concluded that photophosphorylation driven by cyclic electron transport in the chloroplast could provide the additional ATP needed for the active uptake of C_i.

The existence of a link between the activity of the chloroplast ATPase and the emission of luminescence was demonstrated by Joliot and Joliot [7] on *Chlorella* and on intact chloroplasts in [8], [9].

A correlation between luminescence decay

Abbreviation: C_i, inorganic carbon

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kinetics and the adaptation to low CO_2 conditions by *Scenedesmus obliquus* was described in detail in [10]. In [11] a working hypothesis was presented where the biophysical and biochemical background to polyphasic luminescence decay kinetics from low CO_2 adapted algal cells was discussed. In this paper the hypothesis is modified and results are presented which indicate that the difference in decay kinetics of luminescence between algal cells, adapted to different CO_2 environments, is caused by different relaxation kinetics of the transthylakoid ΔpH. It is further shown that the relaxation of the transthylakoid ΔpH includes at least two phases and that the kinetics of the relaxation are affected by the activity of the reversible chloroplast ATPase.

2. MATERIALS AND METHODS

Culturing conditions: *S. obliquus*, strain D3, was grown in an inorganic medium [12]. Light was provided by fluorescent tubes (Philips TL 20W/55) and the incident photon flux density was 90 μmol$\cdot$m$^{-2}\cdot$s^{-1} as measured with a quantum meter (Li Cor, Lincoln, NE). To obtain low CO_2 algae, air was bubbled through the culture. High CO_2 algae were generated by bubbling 2% CO_2 in air through the culture for at least 24 h prior to use in experiments.

Luminescence and fluorescence measurements were carried out in a modified Hansatech O_2 electrode (Hansatech, Norfolk, England). The white light used for luminescence excitation 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 μmol$\cdot$m$^{-2}\cdot$s^{-1} and the time of excitation 30 s. Luminescence was detected with a PM tube, selected Hamatzu R374, applied on top of the transparent lid of the electrode.

To estimate the kinetics of the dark decay of the transthylakoid ΔpH, pulses of saturating blue-green light were used to excite samples of algae after different times in darkness following the 30 s white light excitation. The resulting fluorescence signal was recorded and the magnitude of the maximal level was taken as an inverse indicator of the magnitude of the ΔpH according to the theory of fluorescence quenching presented in [4, 13]. The intensity of the blue-green light was about 1000 μmol$\cdot$m$^{-2}\cdot$s^{-1}. The maximal fluorescence level was reached in less than 0.5 s. It was checked by addition of 5×10^{-5} M DCMU that the excitation light was strong enough to induce complete reduction of the primary PS II acceptor Q_A [14]. A new sample was used for each measurement.

After measurement of a relaxation curve a sample was dark-adapted for 10 min with 5×10^{-5} M DCMU and thereafter excited with blue-green light. The maximal fluorescence level thus obtained was considered to be without quenching and was used to calculate per cent quenching for each measured point on the relaxation curve. For the calculations, quenching was approximated only to consist of the two major quenching components in vivo, quenching due to the transthylakoid ΔpH and quenching due to oxidized Q_A (the latter considered to be zero at the maximal fluorescence level as a consequence of the saturating actinic light).

Chlorophyll concentration was determined according to [15].

3. RESULTS AND DISCUSSION

When high CO_2 adapted algae were transferred to a low CO_2 medium, the algal cells developed luminescence decay kinetics of the type illustrated in fig.1b. When the temperature during luminescence measurements of these algae was lowered from 20°C to 16°C the asymmetry of the relative maximum dissolved into two peaks (fig.1b). Cells adapted to low CO_2 conditions exhibited a more symmetrical peak (fig.1a) which did not dissolve into a double peak as the temperature was lowered (not shown). Luminescence from cells in high CO_2 conditions decayed asymptotically (fig.1a).

The decline in energy quenching of chlorophyll *a* fluorescence, indicating the dark relaxation of the transthylakoid ΔpH from low CO_2 adapted cells, is shown together with the decay of luminescence from the same cells in fig.2. In the figure an antiparallel relationship between the magnitude of the ΔpH and the intensity of luminescence is indicated in the interval when the relative luminescence maximum appears, i.e. after 20–50 s of darkness. The antiparallel relationship between luminescence and ΔpH illustrated in fig.2 indicates that in this interval of time and magnitude the quenching effect by the ΔpH

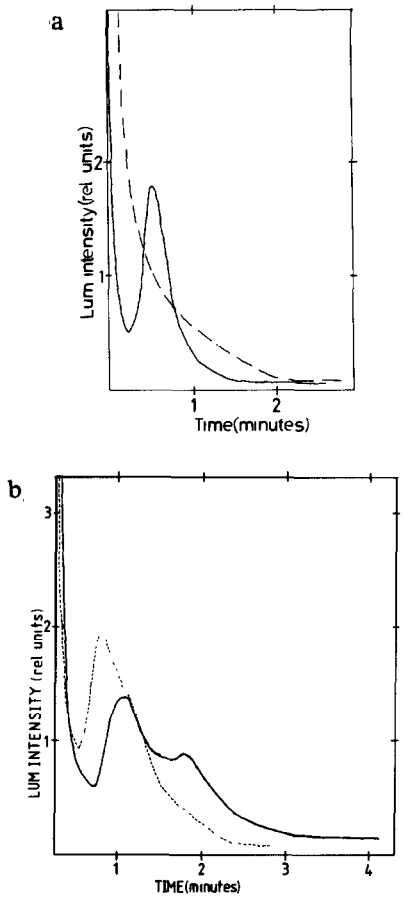

Fig.1. (a) Luminescence decay from high (– – –) and low (——) CO_2 adapted cells. Excitation time 30 s, light intensity 600 μE white light, temperature 20°C. Measurements in high and low CO_2 medium, respectively. (b) Luminescence decay from high CO_2 adapted cells transferred to a low CO_2 medium. Excitation time 30 s, light intensity 600 μE white light, temperature 20°C (-----) and 16°C (——). Measurements in low CO_2 medium.

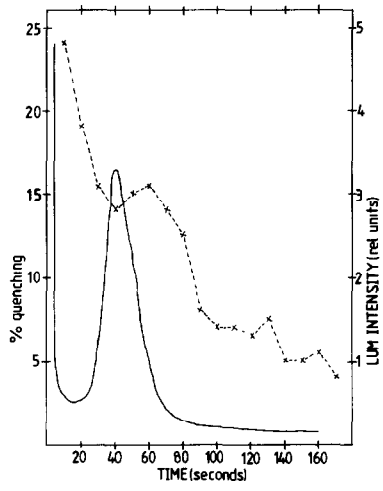

Fig.2. Luminescence decay (——) and relaxation of quenching of chlorophyll *a* fluorescence (×---×) from low CO_2 adapted cells. Initial excitation time 30 s, light intensity 600 μE white light. Fluorescence excitation, 1000 μE blue-green light. Chlorophyll concentration 15 $\mu g \cdot ml^{-1}$. Measurements in low CO_2 medium.

dominates compared to the enhancing effect.

From fig.2 it can also be seen that the dark relaxation of the ΔpH (more precisely the dark relaxation of energy quenching) contains at least two phases separated by an interval of increasing ΔpH after 40–60 s in darkness, resulting in a peak or shoulder in the relaxation. To explain a build-up of the transthylakoid ΔpH in the dark, H^+ pumping due to hydrolysis of ATP by the reversible chloroplast ATPase has to be considered. ATP hydrolysis will occur when the thylakoid membrane is de-energized after the light is turned off, since the ATPase in the presence of ATP will switch from an active energized form (capable of ATP synthesis) to an active de-energized form (capable of ATP hydrolysis [16]). As ATP will be produced in light and during the first phase of the ΔpH relaxation (as long as ΔpH is above the threshold for ATP synthesis) the occurrence of ATP hydrolysis during later stages is expected.

It can hence be concluded that H^+ conduction in both directions by active ATPases in the thylakoid membrane affects the kinetics of the dark relaxation of the transthylakoid ΔpH and therefore also the decay kinetics of luminescence.

In fig.3 the kinetics of the dark relaxation of the transthylakoid ΔpH (as indicated by change in energy quenching) from high and low CO_2 adapted cells are compared. In this experiment a plateau instead of a relative maximum in the relaxation kinetics was observed from low CO_2 adapted cells. Low CO_2 adapted cells showed a faster relaxation of the ΔpH than did high CO_2 adapted cells. The difference in quenching was most prominent after about 30 s in darkness.

Taken together the results presented in figs 2 and 3 provide a possibility to explain the differences in luminescence kinetics illustrated in fig.1a and b:

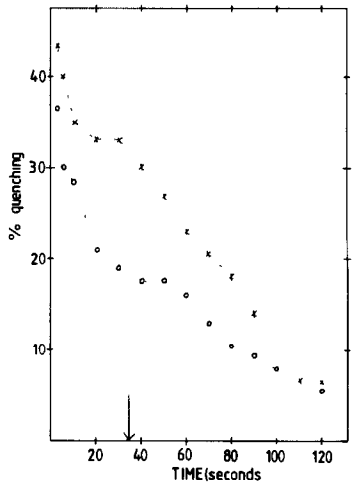

Fig.3. Relaxation of quenching of chlorophyll *a* fluorescence from high (×----×) and low ((○----○)) CO_2 adapted cells. Initial excitation time 30 s, light intensity 600 μE white light. Fluorescence excitation 1000 μE blue-green light. Chlorophyll concentration 15 $\mu g \cdot ml^{-1}$. The time for the relative luminescence maximum from low CO_2 adapted cells is indicated with an arrow. Measurements in high and low CO_2 medium, respectively.

The frequency of PS II recombinations that results in luminescence decreases with time in darkness as the concentration of separated charges, which are the substrate for recombinations, decreases. Simultaneously the transthylakoid ΔpH will relax through two pathways, the basal flux across the membrane and through the active ATPases. The decreasing frequency of PS II recombinations in darkness will reduce the emission of luminescence. On the other hand the relaxation of the ΔpH will tend to increase the emission of luminescence since the quenching effect by the ΔpH (fig.2) gradually disappears. If the enhancing effect on luminescence caused by a decreased quenching exceeds the decreasing effect, caused by the continuous decrease in PS II recombinations, an interval of increasing luminescence will occur. Thus, the difference in luminescence decay kinetics between high and low CO_2 adapted cells, i.e. with and without a single maximum, could be explained by the difference in both the magnitude and the relaxation rate of the ΔpH in the time interval preceding the relative luminescence maximum, i.e. during the period of increasing luminescence (fig.3, 10–35 s).

A working hypothesis to explain the three types of decay curves in fig.1 could thus be formulated:

When cells are cultured under low CO_2 conditions for a prolonged time they adapt to the low CO_2 environment by synthesis of polypeptides, i.e. carbonic anhydrase and C_i pumping proteins. The active C_i transport requires energy which is produced, partially or fully, through photophosphorylation driven by cyclic electron transport in the algal chloroplast [6]. Cyclic electron transport is triggered by the increased redox state of the electron transport chain and the PQ pool as a consequence of an increased NADPH/$NADP^+$ ratio in the stroma [17], when the Calvin cycle is slowed down due to partial substrate inhibition. The increased rate of cyclic electron transport results in a higher membrane energization that activates a large fraction of the chloroplast ATPases [18]. The resulting increase in ATP production could be utilized for active C_i uptake. If cells during such conditions are subjected to sudden darkness, the high H^+ conducting capacity of the large fraction of active chloroplast ATPases will to a large extent contribute to the relaxation of the transthylakoid ΔpH, resulting in a fast ΔpH relaxation and a luminescence decay with a relative maximum.

When cells adapted to a high CO_2 environment are transferred to a low CO_2 environment, a more severe CO_2 deficiency could be predicted since adaptation to low CO_2 conditions by synthesis of polypeptides will not occur immediately. A more complete inhibition of the Calvin cycle could therefore also be predicted, with an even further increased redox state of the electron transport chain, cyclic electron transport, membrane energization and ATPase activity as a result. The probability for a second time interval with increasing luminescence, i.e. fast disappearance of the quenching after the interval of increasing ΔpH, will therefore be higher since the period when the ΔpH is maintained by ATP hydrolysis will be more shortlived with higher ATPase activity.

When cells are cultured under high CO_2 conditions, no substrate inhibition of the Calvin cycle will occur with a lower ATPase activity as a consequence. The relaxation of the transthylakoid ΔpH will therefore be slower due to the lower H^+ conducting capacity of the relatively smaller fraction

of active ATPases. The resulting luminescence will therefore decay without a relative maximum.

This hypothesis is based on the assumption that the ATP synthesizing capacity of the chloroplast ATPase in the light is proportional to the ATP hydrolyzing capacity in the dark. The assumption seems reasonable since the presence of the enzyme in its active de-energized form (capable of ATP hydrolysis) is dependent on a preceding active energized form (capable of ATP synthesis) [19].

In fig.3 tendencies towards irregularities in the relaxation kinetics of the ΔpH, other than the plateau observed from low CO_2 adapted cells, are seen. These tendencies are observed after 20–30 s in the dark with high CO_2 adapted cells and after 5–10 s with low CO_2 adapted cells. Similar tendencies are indicated in fig.2 after 120 s in the dark. These observations might indicate that dampening oscillations are superimposed on the general relaxation of the ΔpH. The interval of increasing ΔpH from low CO_2 adapted cells (fig.2) could in this way be interpreted as the most marked oscillation.

It should also be noted that there is a variation in the kinetics of ΔpH relaxation and luminescence decay between different experiments. The difference in ΔpH relaxation kinetics is exemplified by the difference in kinetics from low CO_2 adapted cells in the two experiments illustrated in figs 2 and 3. The reason for this variation is not known. The characteristic pattern for the relaxation kinetics described in this paper is however reproducible.

The relevance of the tendencies to oscillations in ΔpH relaxation and the reason for the variation in kinetics are currently under investigation.

At present, the experimental system does not allow measurements of the dark relaxation of the ΔpH from cells transferred from high to low CO_2 environments since the cells gradually adapt to the new environment during the time course of the experiment.

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