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Peculiar properties of chlorophyll thermoluminescence emission of autotrophically or mixotrophically grown *Chlamydomonas reinhardtii*

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ABSTRACT

The microalgae *Chlamydomonas reinhardtii* and *Chlorella* sp. CCAP 211/84 were grown autotrophically and mixotrophically and their thermoluminescence emissions were recorded above 0 °C after excitation by 1, 2 or 3 xenon flashes or by continuous far-red light. An oscillation of the B band intensity according to the number of flashes was always observed, with a maximum after 2 flashes, accompanied by a downshift of the B band temperature maximum in mixotrophic compared to autotrophic grown cells, indicative of a dark stable pH gradient. Moreover, new flash-induced bands emerged in mixotrophic *Chlamydomonas* grown cells, at temperatures higher than that of the B band. In contrast to the afterglow band observed in higher plants, in *Chlamydomonas* these bands were not inducible by far-red light, were fully suppressed by 2 μM antimycin A, and peaked at different temperatures depending on the flash number and growth stage, with higher temperature maxima in cells at a stationary compared to an exponential growth stage. These differences are discussed according to the particular properties of cyclic electron transfer pathways in *C. reinhardtii*.

1. Introduction

Photosynthesis relies on the conversion of a quantum of light energy into a charge separation within photosystem I (PSI) and photosystem II (PSII) reaction centers, creating charge pairs stabilized on electron carriers by activation energy barriers that limit charge recombination, i.e. the wasteful back reaction of the forward charge separation [1]. In PSII centers, charge recombination, limited to a slow rate at physiological temperatures, proceeds following different pathways [2], one of these leading to the recreation at a low yield of an exciton in the chlorophyll antenna, with a probability to deactivate as fluorescence. This delayed fluorescence emitted in darkness following an illumination is also called PSII luminescence. Thermoluminescence (TL) is a technique to study luminescence emission that consists in illuminating the sample at a temperature sufficiently low to make negligibly small the recombination rate of the charge pairs under investigation, then to reveal them successively as TL bands by a progressive warming. Due to the strong temperature dependency of charge recombination, TL has a greater resolving power than luminescence multiphasic decays recorded at a constant temperature immediately after an illumination. In unstressed dark-adapted photosynthetic material, one or few flashes give rise essentially to a so-called B band, located between 25 and 40 °C depending on species and conditions, which results from a recombination of an electron stored on the secondary quinonic acceptor Q_B and a positive charge stored on the S_2 or S_3 states of the oxygen evolving complex of PSII (OEC).

Analysis of luminescence signals becomes more complex for intact photosynthetic systems, in which a delayed luminescence bounce appears superimposed to the exponential decay phases of the different types of charge pairs initially stabilized on PSII electron carriers [3]. This delayed emission called "afterglow" can be related to a dark electron back-transfer from stroma to the acceptor side of PSII [4]. The afterglow (AG) emission, highly dependent on temperature, can be optimally recorded as a sharp TL band peaking at about 45 °C at a 0.5 °C/s warming rate in unfrozen plant leaves [5]. Complex luminescence decays at constant temperatures, sometimes exhibiting one or two successive delayed luminescence bursts, have been reported in some algal species [6–8]. The kinetics observed were strongly dependent on the growth conditions, e.g. low or high CO_2, phosphate deficiency and ionic composition.

Green microalgae are considered by plant biologists as valuable unicellular model systems for photosynthesis studies. In particular, *Chlamydomonas reinhardtii* (*C. reinhardtii*), a flagellated photosynthetic protist that belongs to the class Chlorophyceae, has been widely used for biochemical, physiological and genetic studies.

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This cosmopolite microalga is able to growth in many different environments, normally deriving energy from oxygenic photosynthesis. *Chlamydomonas* exhibits a remarkable metabolic flexibility and can also thrive in total darkness with acetate as an alternative carbon source. Its adaptability and quick generation time have made it an important model organism for biological research and studies on this microalga have provided major research contributions in diverse areas of cell and molecular biology, like photosynthesis, phototaxis, cell motility, inorganic nutrient assimilation, abiotic stress, etc. [9]. Moreover, its recently sequenced nuclear genome has advanced the understanding of the ancestral eukaryotic cell of animals and plants [10].

Here we show that TL emission properties in *C. reinhardtii*, without freezing to prevent membrane disruption and uncoupling by ice crystals [5], vary widely depending on growth stage and nutritive medium and are quite different from those observed in *Chlorella* CCAP 211/84, another well known chlorophycean microalga, or in higher plants. Additional TL bands peaking at temperatures above the B band that appear in mixotrophic grown cells, can be ascribed to an "afterglow" mechanism of electron transfer with, however, noticeable discrepancies compared to what is observed in higher plants: namely, occurrence of several bands peaking at variable temperatures depending on culture growth stage, sensitivity to antimycine A, an inhibitor of the Ferredoxine-plastoQuinone-Reductase (FQR) and failure to induce these bands by a far-red illumination.

2. Material and methods

2.1. Algal strains and growth conditions

The unicellular green algae (Chlorophyceae) *C. reinhardtii* 21gr and *Chlorella* sp. CCAP 211/84 were grown in Sueoka liquid mineral medium (pH 7.1) under photoautotrophic conditions (continuous white light, 100 μmol m^{-2} s^{-1}) as previously described [11]. When indicated *Chlamydomonas* and *Chlorella* cultures were supplemented with 12 mM sodium acetate or 25 mM glucose, respectively, and maintained under continuous illumination (mixotrophic conditions). Growth of the algal batch cultures was monitored by measuring the chlorophyll cell content after methanol extraction. Exponential growth phase and plateau growth phase cells were collected after 2 days and 5 days culturing, respectively.

2.2. Thermoluminescence

TL emission was recorded as previously described [5,12–14]. Briefly, temperature regulation, signal recording and flash sequences were driven by a computer through a National Instrument DAQ-Pad1200 interface, using dedicated software. Temperature regulation was performed by means of a Marlow thermoelectric "Peltier" element powered by a variable (0–5 A) computer-driven power supply. Luminescence emission was detected by a H5701-50 Hamamatsu photomultiplier module. Illumination was performed through a light guide parallel to the photomultiplier, both of them being attached to the same stand sliding horizontally from the illumination to the measuring position. Single turn-over flashes were provided by a xenon white light (Walz XST-103). Far-red was provided by an Epitex LED emitting at 735 nm with negligibly small intensity below 700 nm. TL signals were analyzed with a dedicated numerical simulation software [12].

Algal suspensions were maintained in the darkness or under dim green light in Erlenmeyer vessels (about 5 mm layer thickness) that were all shaken horizontally before each TL recording. Samples were then kept at 25 °C for 2 min in complete darkness in the TL measuring cell, then cooled to 1 °C and submitted to flash or far-red illumination; the TL recording was started immediately, from 0 °C to 70 °C at a 0.5 °C/s warming rate.

3. Results

3.1. Variations of TL emission depending on autotrophic or mixotrophic growth conditions

In dark-adapted autotrophically or mixotrophically grown *Chlorella* cells at stationary phase, single turn-over flash sequences induced a single TL band (Fig. 1) reaching a maximum intensity after 2 flashes and oscillating with a period 4, as classically observed also in plant leaves for the B band [15,16]. The maximal temperature Tm(B) of the B band after 2 or 3 flashes occurred at a lower temperature in mixotrophic compared to autotrophic grown cells.

A similar unique TL band was generally observed in *Chlamydomonas* cells from autotrophic cultures in the exponential growth phase (Fig. 2A). However, when cells from a culture that reached the stationary growth phase were analyzed, 2 or 3 flashes induced a noticeable shoulder at >30 °C that is suppressed by antimycin A, in addition to the B band centered near 25 °C (see Fig. 5, Ctrl auto).

A clearly different emission pattern was observed in mixotrophically grown cells at a stationary stage (Fig. 2B). The B band peaking at 25 °C after 1 flash was downshifted to lower temperatures and broadened after 2 or 3 flashes. In addition, new bands emerged above 30 °C. A band arose at about 50 °C after 1 flash (luminescence-emitting centers are 100% $S_2Q_B^-$) and another one at about 40 °C overlapping the smaller 50 °C band after 2 flashes (mixture of $S_2Q_B^-$, $S_3Q_B^-$). Interestingly, 3 flashes (mostly $S_3Q_B^-$) induced a band at 32 °C, not at 40 °C as observed after 2 flashes, with a shoulder towards high temperatures suggesting that the 50 °C band observed after 1 flash is still present after 3 flashes, despite the low amount of S_2Q_B. As a consequence the bands observed at 50 °C, 40 °C and 32 °C cannot be simply ascribed to the proportion of S_2 and S_3 states in luminescence-emitting centers. A brief 5 s far-red illumination produced a sharp band at about 25 °C both in autotrophic (Fig. 2A) and mixotrophic (Fig. 2B) grown cells, similar to a B band, without any of the >30 °C non-B bands induced by flashes. It is worth noticing that the Tm of this band is about 3 °C lower in mixotrophic compared to autotrophic grown cells and 2 μM antimycin A decreases it by about 40%.

In mixotrophically grown *Chlamydomonas* at an exponential growth stage (Fig. 2C), the same general pattern for flash-induced TL bands was observed with, however, two differences compared to mixotrophic grown cells at a stationary phase (Fig. 2B):

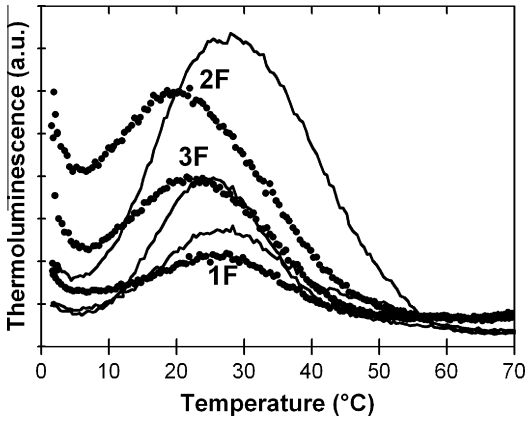

Fig. 1. Thermoluminescence emission curves of *Chlorella* sp. CCAP 211/84 cells grown autotrophically (line) or mixotrophically (dots) at the stationary phase. Cells were excited by 1, 2, or 3 flashes at 1 °C.