Regular paper

Luminescence decay kinetics in relation to quenching and stimulation of dark fluorescence from high and low CO₂ adapted cells of *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*

Lars-Göran Sundblad, Göran Samuelsson, Bosse Wigge & Per Gardeström
Dept. of Plant Physiol., Univ. of Umeå, S-901 87 Umeå, Sweden

Received 21 November 1988; accepted 26 July 1989

**Key words:** luminescence, fluorescence, CO₂-accumulation, *Chlamydomonas reinhardtii, Scenedesmus obliquus*

**Abstract**

Two green algal species, *Chlamydomonas reinhardtii* and *Scenedesmus obliquus*, exhibited a relative maximum during the decay of luminescence, when adapted to low CO₂ conditions that was not observed in high CO₂ adapted cells.

From the kinetics of transient changes in the level of dark fluorescence, after illumination and parallel to the luminescence maxima, it was concluded that the maximum in Scenedesmus was mainly related to a decrease in nonphotochemical quenching, whereas in Chlamydomonas the maximum was mainly related to a dark reduction of the primary PS II acceptor QA.

ATP/ADP ratios from low CO₂ adapted Scenedesmus showed transient high levels after a dark/light transition that was not observed in high CO₂ adapted cells. After 30 s of illumination the ATP/ADP ratios however stabilized at the same steady state level as in high CO₂ adapted cells.

Dark addition of HCO₃⁻ to low CO₂ adapted cells of Chlamydomonas resulted in a rapid transient quenching of luminescence that was not observed in low CO₂ adapted cells of neither species.

It is concluded that the luminescence maxima present in both low CO₂ adapted Scenedesmus and Chlamydomonas reflect adaptation of the cells to low CO₂ conditions. It is further suggested that the difference in mechanistic origin of luminescence maxima in the two species reflects differences in adaptation.

**Abbreviations:** ADP – adenosine-diphosphate, ATP – adenosine-triphosphate, Ci – inorganic carbon, F₀ – dark fluorescence recorded under dark adapted conditions, F⇌ – fluorescence with all reaction centers open, FV – variable fluorescence, PS I – photosystem I, PS II – photosystem II, QA – the first quinone acceptor of PS II

**Introduction**

Chlorophyll a luminescence is the result of the recombination between electrons on the acceptor side of photosystem II (PS II) with positive charges on the donor side (Lavorel 1975). Several factors can affect the intensity, decay kinetics and yield of luminescence. These factors can be classified as affecting one or several of 4 parameters:

- The concentration of electrons on the acceptor side of PS II (Lavorel 1975).
- The presence of positive charges on the donor side of PS II (Lavorel 1975).
- The activation energy for electrons and positive charges to recombine (Crofts et al. 1971).
- The competition from pathways other than light emission for dissipation of excitation energy from charge recombination (Sundblad et al. 1986).
Luminescence normally decays asymptotically with the most light emitted during the first few seconds of the decay (Malkin 1977). Secondary kinetics with peaks and shoulders have however been reported by several authors (Bertsch and Azzi 1965, Rubin et al. 1966, Desai et al. 1983, Björn 1971, Palmqvist et al. 1986, Mellvig and Tillberg 1986, Schmidt and Senger 1987a, b).

Green algal cells subjected to low CO₂ conditions develop a CO₂ concentrating mechanism. This adaptation involves changes both in protein synthesis and in energetics of the cells (Badger et al. 1980). The energetic role of algal chloroplasts was discussed by Spalding (Spalding et al. 1984). In *Scenedesmus obliquus* a correlation between a transient peak in the minute range decay of luminescence and the ability for active uptake in inorganic carbon (Cᵢ) was shown to be related to the energetic state of the algal chloroplast (Palmqvist et al. 1986, Sundblad et al. 1986a, b).

Chlorophyll a fluorescence has been widely used as a probe for the function, induction, intactness and photochemical potential of the photosynthetic apparatus (Krause and Weis 1984, Schreiber et al. 1986). The use of weak, modulated excitation light together with stronger photosynthetically active light and pulses of saturating light in combination with lock in amplifiers have made it possible to discriminate between the two major quenching components in vivo; nonphotochemical quenching (mainly related to the transthylakoid ΔpH) and photochemical quenching, determined by the redox state of the primary PS II acceptor (Qₓ)(Quick and Horton 1984).

The term 'dark fluorescence' was used for the fluorescence resulting from excitation with weak (photosynthetically inactive) light (Schreiber 1986), since it is believed to closely mimic conditions in the dark. Although resulting from excitation incapable of driving photosynthesis, dark fluorescence can work as a probe for changes in photochemical and nonphotochemical quenching, induced by a second stronger and photosynthetically active light, provided that fluorescence is excited with modulated light and the resulting signal is amplified with a lock in amplifier tuned to the frequency of the exciting light. The procedure is based on the assumption that fluorescence unrelated to the function of PS II photochemistry (F₀ fluorescence) is not affected by those factors that cause non-photochemical quenching of variable fluorescence. As shown by Bilger and Schreiber (1986) this assumption does not hold true under conditions of extreme energization of the thylakoid membrane. It was concluded that nonphotochemical quenching of F₀ under such conditions (i.e. strong light and low CO₂) was a result of state quenching (i.e. quenching due to spillover of excitation energy from PS II to PS I). However, it has also been proposed that nonphotochemical quenching of F₀ is caused by the transthylakoid ΔpH through a decrease in the ratio of, rate of transfer of excitation-energy to open reaction centers/ rate of backtransfer, at high ΔpH (Weis and Berry 1987).

In this paper dark fluorescence was measured in parallel with luminescence from high and low CO₂ adapted cells of *Scenedesmus obliquus* and *Chlamydomonas reinhardtii*, in order to reveal the mechanistic background to the luminescence maxima observed in low CO₂ adapted cells.

**Material and methods**

**Algal material and culturing conditions**

The unicellular green algae *Scenedesmus obliquus*, strain WT D3 and *Chlamydomonas reinhardtii*, strain 137 c⁺ were grown in continuous light (80 μmol m⁻² s⁻¹) in inorganic media (Bishop 1971) at 25°C. Low CO₂ adapted cells were obtained by bubbling the growth medium with air for at least 48 h prior to use in experiments. High CO₂ adapted cells were obtained by bubbling with air containing 2% CO₂. For the experiments illustrated in Fig. 11, high CO₂ adapted *Chlamydomonas* were harvested and centrifuged at 800 g for 5 min, whereafter the cells were resuspended in low CO₂ medium.

**Luminescence measurements**

The measurements were carried out in a modified Hansatech O₂ electrode. White excitation light was provided by a metal halogen lamp (Atlas 24 V, 250 W). The photon flux density of the exciting light was 600 μmol m⁻² s⁻¹ (except for experiments shown in Fig. 9) and the time of excitation 30 s. Luminescence was detected by a selected Hamamatsu R 374 photomultiplier and the signal ampli-