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Non-photochemical quenching in an antarctic unicellular green alga

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Introduction

The fluorescence yield of monomeric chlorophyll in solution is of the order of 20-35% (Forster and Livingston 1952, Latimer et al. 1956) and is relatively independent of the duration and intensity of the light used for fluorescence measurement. Instead, the fluorescence yield emitted under continuous illumination by a chlorophyll-containing photosynthetic organism is characterized by a complex time-course, known as the Kautsky effect or fluorescence induction (Kautsky and Hirsch 1931). In higher plants, this temporal evolution of fluorescence yield, ranges from microseconds to hours and has become one of the easiest and most frequently used experimental approaches in photosynthesis research and plant physiology.

This study presents preliminary results of the measured time-course of fluorescence induction in a microalga belonging to the Chlorophyta, isolated from samples of sea water collected at a depth of 3 m in the Ross Sea near the Italian Station of Terra Nova Bay, Antarctica. This unicellular alga, *Koliella antarctica,* was recently characterized as a new species (Andreoli et al. 1998). The unusual properties of the fluorescence emission of this organism, which is adapted to the low temperatures of the Antarctic seas (≤ 1 ¡C), were compared to those of *K. longiseta*, a phylogenetically related species adapted to the temperate zone (Andreoli et al. 2000).

The observations are interpreted in terms of a mechanism of adaptation to the unfavourable temperatures of the environment, by means of which a significant fraction of absorbed light energy is converted to heat to increase the cell temperature locally and transiently.

Materials and Methods

Koliella antarctica was isolated from samples of sea water collected at a depth of 3m near the Italian Station at Terra Nova Bay (Ross Sea, Antarctica) during the austral summer 1989-90. The algae was maintained in sea water enriched with f/2 medium (Guillard 1975), at a temperature of +2 °C and ≅ 10 E m^{-2} s^{-1} of light intensity.

The strain of the temperate alga *Koliella longiseta* (SAG 470-1), was obtained from Sammlung von Algenkulturen at the University of G ttingen. It was maintained in fresh water with Czurda medium (Aaronson, 1970) and stored in a plant incubator at temperature of 16 °C and ≅ 10 mol photons $m^{-2}s^{-1}$.

Fluorescence induction curves were obtained on a PAM fluorometer (Waltz, Germany) at a total chlorophyll concentration of 14 μg/ml.

Chlorophyll concentration was measured by a Perkin Elmer Lambda Bio 40 spectrophotometer after extraction with methanol and calculated by the Wellburn s coefficients (Wellburn 1994).

Results

The variable fluorescence induced by a saturating flash on *Koliella antarctica* and *Koliella longiseta* is shown in figure 1A. Measurements were made at the same chlorophyll concentration and with identical set-up of the PAM fluorometer. Although a higher Fo value (0.75) is observed in *K. antarctica* than in *K. longiseta* (0.42), the variable fluorescence (Fm-Fo) is approximately equal in both species. This indicates that, in the Antarctic species, besides the outfit of antenna chlorophylls which transfer excitation to the PSII reaction centre and are therefore quenched by the photochemical process of charge separation, there is a second set of antenna chlorophylls which are not excitonically connected with the reaction centre. Under continuous light (red 57 μmoles photons $m^{-2}s^{-1}$), the shape of the fluorescence induction curve is surprisingly different in the two species: *K. antarctica* shows very pronounced non-photochemical quenching, which build up in tens of seconds, bringing the fluorescence to a steady-state level of Fs = 0.39, well below its Fo value (figure 1 B). In these conditions, a saturating flash (arrows) does not increase significantly the fluorescence signal. Calculating the NP quenching parameter as :

$q_N = 1 - (Fm - F) / (Fm - Fo)$,

91% of the total quenching (Q = 64%) is of NP origin.

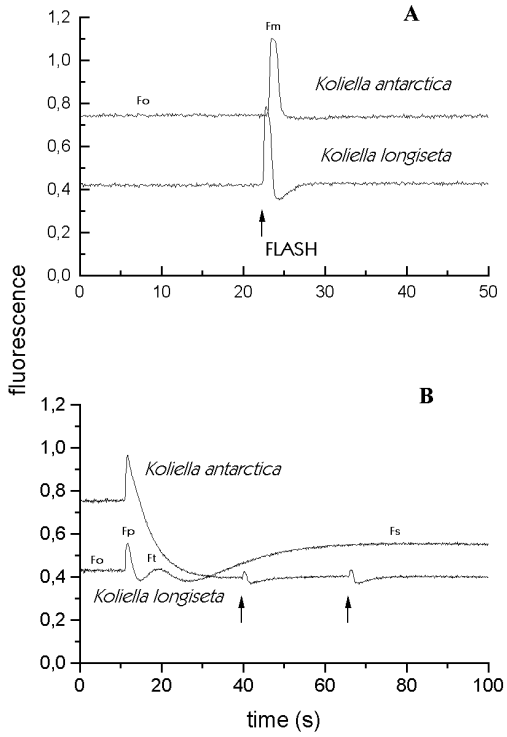

Fig. 1: Fluorescence induction. A) variable fluorescence measured by a 2s saturating flash; B) fluorescence induction in red continuous light $5.7x10^{-5}$ E m^{-2} s^{-1} . Arrows corresponds to flash firing.

A more complex fluorescence induction curve is observed for the temperate species *K. longiseta*. After a rise to Fp and a fast quenching phase, a rise to a transient level Ft is observed followed by the attainment of a steady-state fluorescence level Fs, where most of the quenching is released. The shape of this curve is reminiscent of that of higher plants, reflecting the contribution of the various steps of the electron transport chain (Briantais et al. 1986).

The light- induced quenching in *K. antarctica* brings the steady state fluorescence level to a value approximately equal to the maximum quenching for *K. longiseta* (figure 1 B). This observation may be interpreted with by assuming that the set of chlorophyll molecules which are present in the antenna of the polar alga and which supposedly do not contribute to the excitation of RCII, undergo NP quenching which builds up as a consequence of PSII activity and membrane energization. This quenching is readily reversible on turning off the light (figure 2A), the time constant of the release being of the order of 5-10 seconds. Its formation depends on reduction of the plastoquinone pool, as it is strongly reduced by the addition of DCMU (figure 2B). The effect of the presence of an uncoupler such as ammonium ion is shown in figure 2C, confirming that the quenching is associated with the formation of a proton gradient. Figure 2 D shows the temperature dependence of the quenching formation, which indicates its association with an activated process.

Discussion

Koliella antarctica is an unicellular green alga which adapted to live in the Antarctic seas where the water temperature is never above 1°C. Compared with the analogous species *K. Longiseta,* adapted to temperate regions, when illuminated, the former shows characteristic, very pronounced non-photochemical quenching and anomalously high fluorescence in its dark adapted state (Fo). The quenched state, attained after a few tens of seconds of continuous illumination, corresponds to the Fo value shown by the analogous algal species which populates temperate regions (*K. longiseta*) and represents a state in which the photosynthetic electron transport is almost fully saturated (Q_A reduced). We propose that the PS II antenna apparatus of the Antarctic alga contains two sets of chlorophyll molecules: one performs in the usual way, transferring molecular excitation to the special pair of the reaction centre, the other is disconnected and highly fluorescent in the dark adapted state. When photosynthetic electron transport in the light induces membrane energization by the formation of a proton gradient, strong non-photochemical quenching is rapidly generated, which dissipates the light quanta absorbed by this specialized set of chlorophylls into thermal energy. In steady state conditions in light, part of the light energy is used to sustain the formation of the proton gradient, and part is dissipated into heat even at a relatively low light regime. The development of such a mechanism, which dissipates into heat a significant fraction of the light absorbed by the antenna moiety of PS II, as soon as the photosynthetic electron transport reaches the steady state, may be envisaged as a means of transiently increasing the local temperature of the cell. This certainly enhances the exploitation of the chemical free energy produced by the light reactions for biosynthetic work.

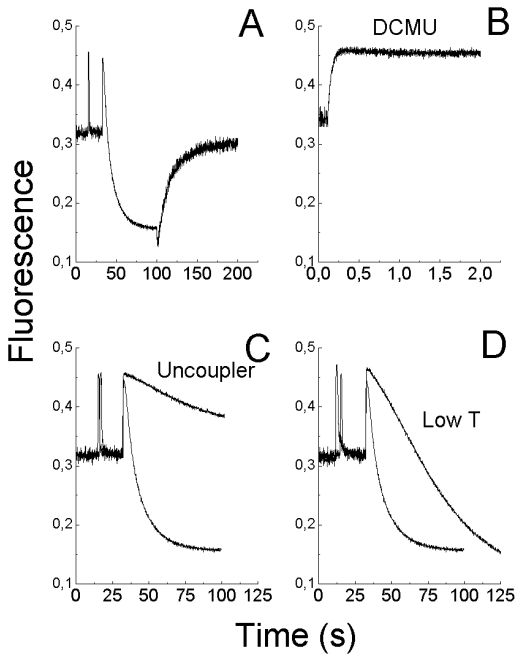

Fig. 2 Fluorescence induction curve of *K. antarctica* under continuous light in various conditions (see text). DCMU 7.5 μM; ammonium chloride 10 mM; Low T= 1 °C

It will be the aim of future work to investigate the molecular features involved in this strategy and to find out which part(s) of the complex PS II antenna apparatus are the site for this specialized set of chlorophyll molecules.

Mechanisms of energy dissipation in the light-harvesting components of PS II have evolved in higher plants and more generally in oxygen-evolving photosynthetic organisms, with the aim of protecting the photosynthetic apparatus from an excess of absorbed light (Horton et al. 1996). It may well be that a similar mechanism but with different final aim has evolved as an adaptation to the extreme environmental conditions of the polar regions.

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Acknowledgements

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