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Microscopic image instrumentation for chlorophyll *a* fluorescence from *in situ Closterium navicula*

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Introduction

The analysis of chlorophyll *a* fluorescence, which is re-emission of light energy absorbed by the antenna chlorophyll, has been useful for non-invasive and non-destructive evaluation of photosynthesis, especially analysis of electron transportation from PS II to PS I (Gobindjee 1995; Krause and Weis 1991). The chlorophyll *a* fluorescence imaging system, originated by Omasa et al. (1987) and Daley et al. (1989), has high spatial resolution, which pulse amplitude modulation (PAM) fluorimeters lack. Furthermore, it has been applied to diagnose the influence of photosynthetic activities subjected to various stresses such as water deficiency, low temperature, and air pollution (Omasa et al. 1987; 1998; Daley et al. 1989; Genty and Meyer 1995; Rolfe and Scholes 1995; Osmond et al. 1998). In recent years, study for chlorophyll *a* fluorescence imaging at the microscopic level has been demonstrated (Oxborough and Baker 1997; Osmond et al. 1999).

Concerning microalgae, chlorophyll *a* fluorescence is available for choice of mutant and measuring *in situ* natural population, and microalgae is used for barometer of environmental monitoring (Campbell et al 1998). The cellular level imaging of chlorophyll *a* fluorescence can get in-depth information about these situation.

In this study, a new system built-in optical microscope, which has resolution at the cellular level, for the chlorophyll *a* fluorescence imaging, has been developed. With this system, chlorophyll fluorescence of *in situ Closterium navicula* treated by DCMU was measured to analyse the photosynthesis reaction by the saturation pulse method.

Materials and methods

Growth conditions and treatment

Closterium navicula (strain number C-573; IAM, Japan) was cultured in C medium (List of Strains(2000); NIES-Collection, p30) at 25 °C day/20 °C night. Incubator had a photoperiod of 12h, a PPF of 75 μmol photon m^{-2} s^{-1}.

The 50μM 3-(3,4 dichlorophenyl)-1,1-dimethylurea (DCMU) was applied on the *Closterium navicula* 20 minutes after the measurement under control conditions.

Microscopic image instrumentation system of chlorophyll a fluorescence

Figure 1 shows the scheme of microscopic imaging system for chlorophyll *a* fluorescence. An optical microscope with x 50 objective was used in this system. Actinic illumination (75μmol photon m^{-2} s^{-1}) and saturating illumination (450μmol photon m^{-2} s^{-1}) were provided

by a halogen lamps, filtered with a 560-nm cut off filter. Saturating pulse was 2 s in duration. Chlorophyll fluorescence excited by these lights was detected using a chilled charge-coupled device camera (Hamamatsu Photonics, C5952-02) through an optical filter (≥ 665nm) put onto the lens-barrel. Fluorescence images were recorded by a digital video recorder (SONY DSR-V10) continuously, and converted to a resolution of 8-bit grey scale, 640x480 pixels. The relationship between the fluorescence intensity and the A/D conversion level of obtained images showed good linearity.

The Fm, F'm and F were measured as described in Takayama and Omasa (in this issue), and the fluorescence quenching parameter, NPQ was calculated by NPQ = (Fm-F'm)/F'm (Bilger and Björkman 1990).

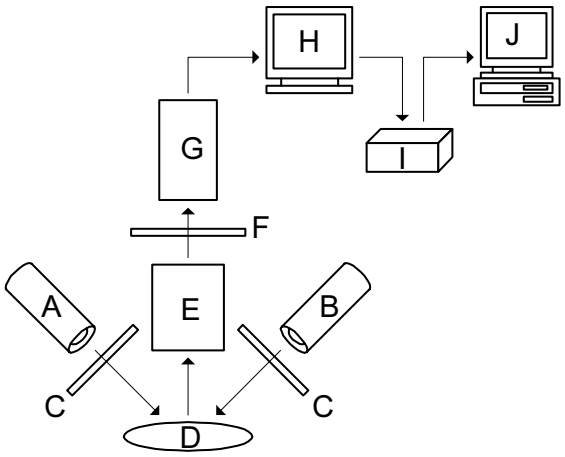

Fig. 1 Chlorophyll *a* fluorescence imaging system with optical microscope. : A, Actinic light source; B, Saturation light source; C, Short-pass filter ($\lambda \leq 560$ nm); D, Test organism; E, Objective; F, Long-pass filter ($\lambda \geq 665$ nm); G, Cooled charge-coupled device (CCD) camera; H, Monitor; I, Digital video recorder; J, Computer.

Results and discussion

Figure 2 shows fluorescence images and NPQ of *Closterium navicula* obtained with this system. The F and F'm images after the DCMU treatment demonstrated high intensity in comparison with those before the treatment (control). On the other hand, the difference between Fm images before and after the treatment was very little, because all Q_A in *Closterium* before and after the treatment were temporarily closed when saturation pulse light was irradiated on dark condition (Krause and Weis 1991). In regard to the F image, *Closterium* before the treatment was adapted to light condition due to continuous actinic light irradiation, and the fluorescence intensity was decreased by the photochemical and non-photochemical quenching. However, fluorescence intensity of *Closterium* after the DCMU treatment was kept high, since absorbed light was not used for the photosynthesis and large portion was emitted as fluorescence because DCMU inhibited electronic transportation in photosystem II.

Before the treatment, the intensity in F'm image was lower than that in Fm image because of non-photochemical quenching. Meanwhile, after the treatment, the intensity in the F'm image was about the same as that in Fm image, since photosynthesis was obstructed by DCMU and non-photochemical quenching was not formed normally.

NPQ suggested the ability of chloroplasts to generate a high intrathylakoid pH gradient, pump protons, sustain electron transport, and to waste excess excitation energy as heat (Osmond et al. 1998; Krause and Weis 1991; Siebke and Weis 1995a). The NPQ decrease

after the treatment also implied the unformation of non-photochemical quenching by the effect of DCMU.

In summary, we showed that the new microscopic image instrumentation system for chlorophyll *a* fluorescence has the ability to detect the *in situ* functional disturbance of photosynthesis by DCMU.

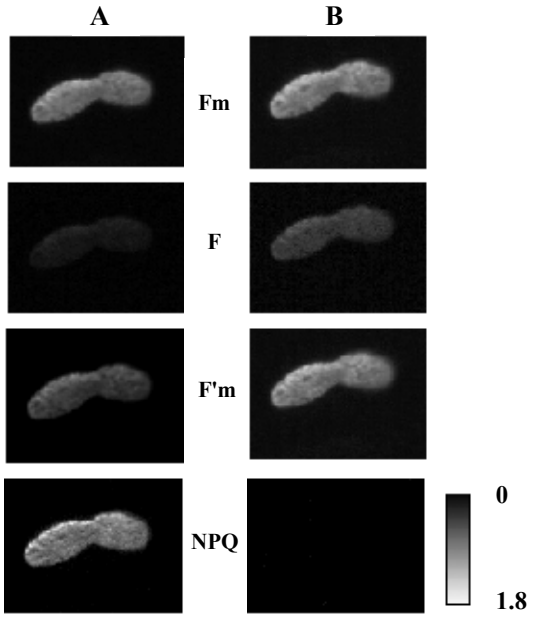

Fig. 2 Fluorescence images (Fm, F, F'm) and NPQ obtained by the saturation pulse method. A, Control; B, +DCMU 50μM.

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