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# Reversible reorganization of the chlorophyll-protein complexes of photosystem II in cyanobacterium cells in the dark

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A new emission band at 673 nm was detected in the low-temperature fluorescence spectrum of dark-adapted cyanobacteria *Gloeotrichia raciborski*. The excitation spectrum of this band was close to the absorbance of the isolated reaction centre of photosystem II. The relative intensities of the bands of chlorophyll and pheophytin in this spectrum showed the relative concentrations of these pigments to be about 3:1. The intensity of the band increased with darkness (half-time about 2 h). Under illumination the band rapidly disappeared (half-time about 60 s). The appearance of a 673 nm band in the dark and its disappearance in the light were accompanied by a decrease, and, respectively, an increase in the fluorescence of the PS II band at 697 nm.

Pheophytin; Reaction center; Fluorescence; Photosystem II; Cyanobacteria

## 1. INTRODUCTION

Since the detection of pheophytin for the first time in the active reaction centres of PS II [1,2], nothing is known about the mechanism of this pigment synthesis in vivo. Moreover, Pheo is a well-known product of Chl degradation both in vitro and in vivo [3–6].

The new approach to CP separation has allowed one to identify the Chl fluorescence bands at 697 and 686 nm as those belonging to 47 and 43 kDa proteins which seemed to be the internal and external antennae of PS II [7]. The isolation of the RC from this PS, which was localized in D1-D2 proteins, has been also achieved [8–10]. But for some time the RC was assumed to be incorporated in 47 kDa CP [7]. In order to solve this problem, the study of the arrangement of these proteins in the cell should be performed. Fluorescence measurements seem to be the most convenient for this purpose. The fluorescence of D1-D2 proteins in situ, however, has not been detected yet, although they have an emission maximum, in vitro, at 684 nm and a shoulder at about 675 nm [9]. Here we tried to use the difference fluorescence method, which has been applied to the study of chlorophyll biosynthesis in green leaves and chloroplasts, in the search of short wavelength RC fluorescence in living cells of a cyanobacterium.

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*Abbreviations:* ALA, 5-aminolevulinic acid; Chl, chlorophyll; CP, chlorophyll-protein complex; LHCP, light-harvesting chlorophyll-protein complexes; Pheo, pheophytin; PS, photosystem; RC, reaction centre

## 2. MATERIALS AND METHODS

Cyanobacterium *Gloeotrichia raciborski* was cultivated for 3–5 days in the nitrogenless liquid medium BG-11 [14] at 21 ± 1°C, under continuous illumination with luminescent lamps (3000 lux). The culture was collected and thoroughly homogenized at 4°C to obtain a uniform cell suspension. The suspension was spread on a membrane filter Synpor-3 (Czechoslovakia) (with pore diameter 1.5 $\mu$m) and different parts of the same filter were used in the experiments. Dark incubation of the samples thus obtained was performed in Petri's dishes with the cultural medium. In some experiments 0.5 $g \cdot l^{-1}$ chloramphenicol (Serva) or 5 mM ALA (Serva) were added: For the study of the effect of oxygen on phototransformations, the samples were transferred to vacuum vessels just before illumination and were then pumped with air or argon for 30 min.

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After that the samples were illuminated with a tungsten lamp (500 lux).

The low-temperature (77 K) emission and excitation spectra were recorded with a home-made fluorimeter equipped with two high-efficiency monochromators, a 200 W xenon lamp, and a photomultiplier with a S-20 photocathode. For correction, subtraction, and differentiation of the spectra a DCSU-2 accessory (Perkin-Elmer) was used. The optical density of the samples was about 0.05–0.1.

## 3. RESULTS AND DISCUSSION

Dark incubation of *Gloeotrichia raciborski* cells for 18–24 h caused no changes in the band composition of their low-temperature fluorescence spectrum when excited with the light absorbed by phycobilins (560 nm) (fig.1). Like cells cultivated in the light, they had intensive bands of phycocyanin (646 nm), allophycocyanin (663 nm), and phycoerythrin (578 nm, not shown) and also fluorescence bands of Chl from PS II (686 and 697 nm) and from PS I (730 nm) [15–17]. However, there was some decrease in the intensity of the 728 nm band, and an even more pronounced one in the 697 nm band in the dark. Under excitation with the light absorbed by Chl (436 nm), the emission bands 686, 697 and 730 nm are the only ones present in the spectra. The relative intensities of the bands 686 and 697 nm were independent of the excitation wavelength (436 and 560 nm, respectively) and they both seemed to belong to Chl-containing CPs. Comparison of the fluorescence spectra of the light- and dark-adapted cultures excited at 436 nm showed not only the decrease in the intensities of the 730 and 697 nm bands, but also the appearance of a new band at 673 nm for dark cultures: this is close to that previously observed under dark incubation of *Dunaliella* cells [12]. The second derivative shows this new band to have a larger half-width than other bands of PS II (fig.1), and indicates that it belongs to a monomer pigment [20]. Under excitation with 465 nm light this band disappeared, while other fluorescence bands of PS II remained. The excitation spectra of 686 and 697 nm bands for the light- and dark-adapted cultures were identical, which implies that they

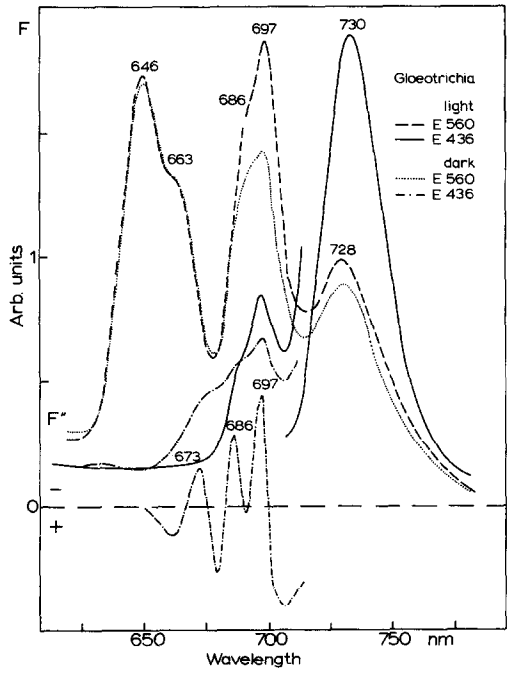


Fig.1. Low-temperature fluorescence spectra of *Gloeotrichia raciborski* cells cultivated in the light or in the dark for 20 h, at either 560 or 436 nm excitation. Under excitation at 436 nm the intensity of the long-wave band is decreased 2-fold. Bottom: the second derivative of the fluorescence spectrum of dark-adapted cells (excitation at 436 nm).

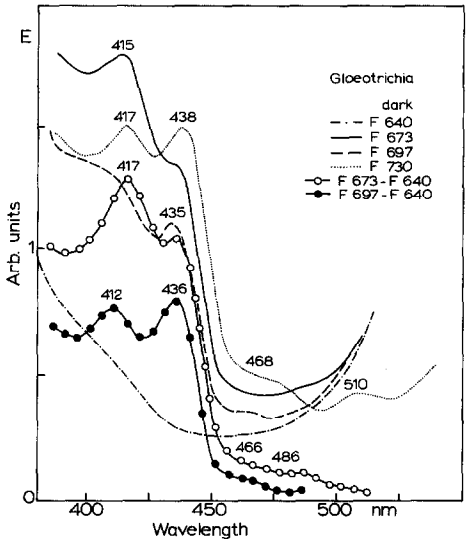


Fig.2. Low-temperature fluorescence excitation spectra of *Gloeotrichia raciborski* cells adapted to darkness for emission at 640, 673, 697 and 730 nm. The intensity of fluorescence excitation spectrum for the 730 nm emission band is decreased 5-fold, and that for the 673 nm one is increased 2-fold. Differential fluorescence excitation spectra for the 673 and 697 nm bands are obtained after subtracting the phycobilisome bands from the total fluorescence excitation spectrum. The fluorescence excitation spectrum for phycobilisomes was recorded for emission at 640 nm.

belong to complexes with an effective energy exchange. The excitation spectrum of the band at 673 nm (fig.2) has, alongside a Chl maximum at 435, an intensive band at 417 nm, similar to that of Pheo in the difference absorption spectrum of RC [1]. Thus, after subtraction of the line corresponding to phycobilins excitation, the fluorescence excitation spectrum of 673 nm band was close to the absorption spectrum of the recently isolated RC of PS II [8–10]. The rather low intensity of carotenoid bands in the excitation spectrum as compared to the absorbance seems to be due to the low efficiency of carotenoids in the energy transfer to the RC. Moreover, the relative intensities of Chl and Pheo bands in the excitation spectrum of the 673 nm band (taking into consideration the molar extinction coefficients of both pigments [18,19]) are 2.7 : 1, which is in agreement with pigment composition of the electron-transfer chain in the RC (Chl dimer, Chl monomer, Pheo monomer).

In contrast to the excitation spectrum of fluorescence of PS II, that of PS I (the band at 730 nm) contained a long-wave Chl maximum (at 438 nm instead of 436 nm) and pronounced carotenoid bands (at 468 and 510 nm). This spectrum agrees with the involvement of the long-wavelength Chl and carotenoids in the formation of a broad long-wave band of low temperature fluorescence [20–22].

Illumination of the dark-adapted cells led to a rapid disappearance of the 673 nm band, accompanied by an increase in the intensity of the 697 nm band (fig.3). The differential fluorescence spectrum showed that the 673 nm band was transformed into the 697 nm band without significant changes in other spectral regions. The kinetics of changes for both bands was the same and took about 3–5 min. The disappearance of the 673 nm band was observed in argon as well as in air, and, thus, was hardly due to photodestruction.

The kinetics of the 673 nm band increase in the

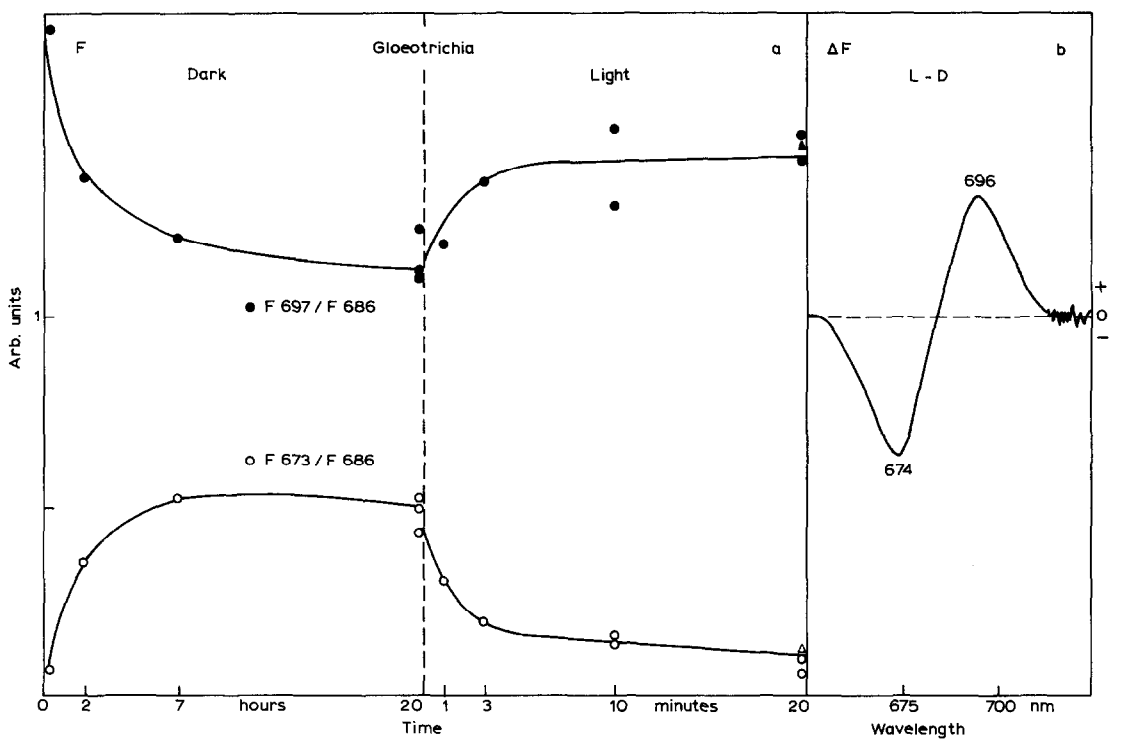


Fig.3. Kinetics of changes in the intensities of 673 nm (—○—) and 697 nm (—●—) bands in the low-temperature fluorescence spectrum of *Gloeotrichia raciborski* cells under cultivation in darkness (left) or in the light (centre). The spectra were normalized by the band 687 nm. Mind the difference in time scales for cultivation in darkness and in the light. Intensities of the 673 (△ ,○) and 697 nm (▲ ,●) bands after illumination of dark-adapted cells in air (○,●) and in argon (△ , ▲). (Right) The difference in the low-temperature fluorescence spectra of cells adapted to darkness for 7 h and of cells after illumination for 30 min. Excitation at 436 nm.

dark was rather slow (transformation half-time was about 2 h), it took 7 h to complete, which is close to the time of D1 protein formation [13–25]. Then, there was a slow decrease in the intensities of both the 673 and 697 nm bands which may be related to their dark destruction [5,6]. The presence of chloramphenicol, an inhibitor of protein biosynthesis, considerably lowered the intensity of the 673 nm band, making it no more than 50% of that observed without inhibitor. On the other hand, the intensity of this band was much higher in the presence of ALA, a known substrate for porphyrin biosynthesis.

It should be noted that dark incubation of cells (even in the absence of ALA) leads to the accumulation of various Chl precursors, such as protoporphyrin IX and Mg-protoporphyrin IX, which could be revealed by measuring both fluorescence emission and excitation spectra of intact cells. However, this pigment accumulation could be recorded only 6–7 h after the start of the dark incubation, i.e. when the formation of the complex fluorescing at 673 nm was saturated.

Thus, the data obtained show that the fluorescence band at 673 nm observed for the intact cells of cyanobacteria in the dark may belong to RC of PS II which is separated from the internal antenna. In this case, the mechanism of PS II assembly is to be studied directly in the cell.

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