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HOLE-BURNING STUDY OF ENERGY TRANSFER IN ANTENNA PROTEINS OF *Dunaliella tertiolecta* AFFECTED BY IRON-LIMITATION

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Abstract. Results of persistent spectral hole burning (PSHB) experiments in fluorescence spectra of Fe-limited and Fe-replete cells of *D. tertiolecta* are reported. Low temperature fluorescence spectra exhibit an increase of PS II fluorescence of Fe-limited cells due to a decrease of an efficiency of excitation energy transfer (EET) in PS II. A 1.5 nm blue shift of PS II fluorescence maximum observed for Fe-limited cells is the result of excessive light harvesting complexes LHCII in thylakoid membrane. The PSHB experiments revealed an influence of Fe stress on EET from and between core antenna proteins CP43 and CP47. EET within core antenna proteins CP43 and CP47 is not affected by iron limitation.

INTRODUCTION

One of the main goals of the present biophysical research is complete understanding of primary events of light energy harvesting and charge separation in all photosynthetic systems. An influence of iron limitation on excitation energy transfer (EET) in PS II of unicellular marine alga *D. tertiolecta* was studied recently[1]. All results of the studied

effects suggest that Fe-limitation alters the efficiency of EET from the antenna to the reaction center of PS II in this organism. These ideas were examined by the application of PSHB in fluorescence spectra. PSHB offers an independent method for determination of excited state lifetimes T_1 of pigment molecules in particular pigment-protein complexes. Furthermore, the Huang-Rhys factor S and the mean frequency of protein phonons Ω characterizing the pigment-protein interaction could be determined[2,3]. PSHB in fluorescence spectra has been successfully used for the study of the antenna complexes: CP43 and CP47 of *Synechococcus sp.*[4,5], CP47 of wild type and mutant of *Synechocystis sp.* PCC 6803[6]. The aim of this paper is to compare the EET rates and pigment-protein coupling in PS II antennae of Fe-limited and Fe-replete whole cells of *D. tertiolecta.*

MATERIALS AND METHODS

Spectroscopic measurements of iron-induced changes in light harvesting complexes of PS II were performed on whole cells of *D. tertiolecta.* Growth conditions and sample preparation are described in Vassiliev et al.[1]. All prepared samples were diluted to a final optical density ≈ 0.2 OD and stored in the dark at liquid nitrogen temperature. The low temperature fluorescence was obtained by exciting the sample into the Soret absorption band by the 488 nm line of *cw* Carl Zeiss Jena ILA 190 Ar^+ laser. The fluorescence spectra were analyzed by a Jobin Yvon HRD 1 double grating monochromator and a cooled RCA C34031A photomultiplier. The signal was further processed by a home-built photon-counter together with a personal computer. The persistent holes were burnt by means of a *cw* dye laser Spectra Physics 375B with a pyridine dye, equipped with a three-plate birefringent filter and a thin etalon (bandwidth less than 0.3 cm^{-1}). Used burning doses were in range of 1 - 100 $mWcm^{-2}$. By a control measurement of fluorescence spectra after each burning it was established that no sample degradation occured during the burning process.

RESULTS AND DISCUSSION

The low temperature (4.2 K) fluorescence spectra of both Fe-limited and Fe-replete cells are shown in Figure 1. Both spectra consist of the PSII fluorescence band with maximum at 683.5 nm (Fe-replete) or 682 nm (Fe-limited). The fluorescence yield of the PSII of Fe-limited cells is higher due to a decrease of the EET efficiency in antenna complexes of the PSII: The iron depletion limits the amount of reaction centers and core antenna proteins of the PSII in the thylakoid membrane, while the amount of outer antenna complexes LHCII remains at the same level[1]. Consequence of this is a blue shift of the maximum of the PSII fluorescence band of Fe-limited cells due to excessive LHCII protein complex in thylakoid membrane emitting around 680 nm. A detailed low temperature (4.2 K) PSII fluorescence of Fe-limited cells is presented in Figure 2.

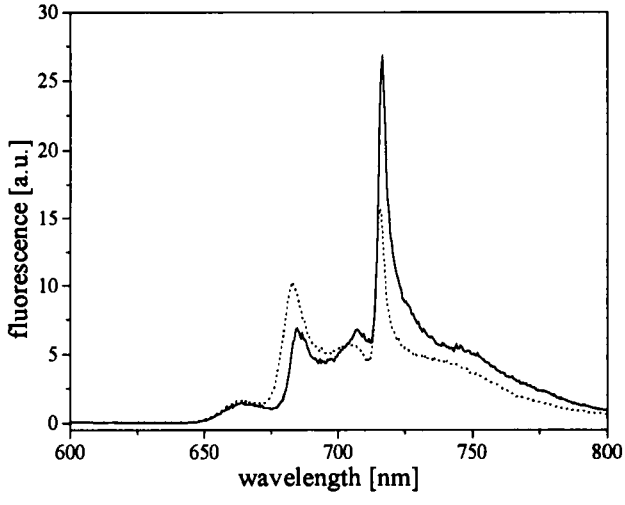

FIGURE 1. Low temperature (4.2 K) fluorescence spectra of Fe-limited (---) and Fe-replete (—) cells of *D. tertiolecta*.

In the spectral region above 700 nm at least four fluorescence bands with maxima 707 nm, 716 nm, 730 nm and 750 nm can be distinguished. The bands 707 nm and 716 nm are generally ascribed to the PSI core complex (716 nm) and light harvesting complex LHCI (730 nm), while the origin of the 707 and 750 nm bands is still unknown[7].

The PSHB experiments were focused on the 680 nm PSII fluorescence band. Typical PSHB fluorescence spectra at 4.2 K of Fe-replete and Fe-limited cells are shown in Figure 3. The spectra consist of resonant zero-phonon holes (ZPH) and red shifted phonon side-band holes (PSBH). From the ratio of the integral intensities of ZPH and PSBH the related Huang-Rhys factor S was determined. At 4.2 K the S values are equal (within experimental accuracy) for both studied systems ($S \approx 0.9$) at all burning wavelengths. This indicates a relatively weak electron-phonon coupling typical for antenna proteins. Also the related mean phonon energy of the protein phonons was the same for both Fe-replete and Fe-limited cells, $\Omega \approx 30$ cm^{-1}. These results show no changes in the parameters of the pigment-protein interaction under iron-limited conditions.

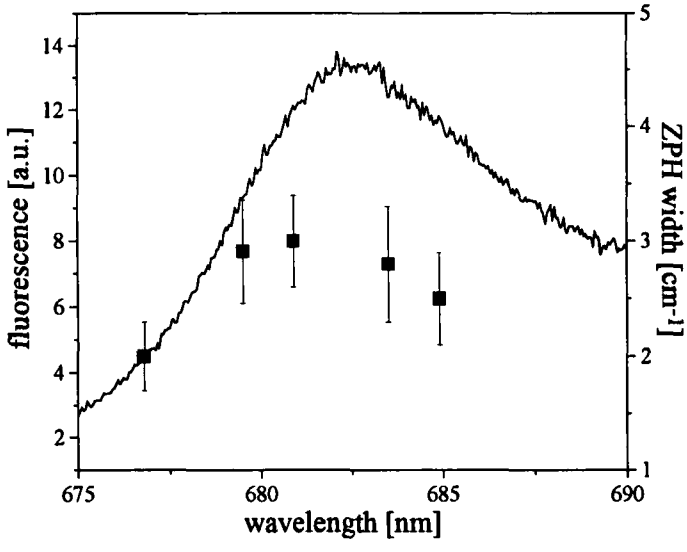

FIGURE 2. Detailed low temperature (4.2 K) fluorescence spectra of Fe-limited cells and wavelength dependence of holewidths (■) for Fe-limited cells.

Furthermore, the wavelength dependencies of the holewidths in these samples were determined in the spectral range 675-688 nm (Figure 2, Fe-replete not shown). From the ZPH widths the lifetimes of excited states T_1 were calculated. To eliminate saturation effects we measured the holewidths as a function of burning fluences P.t (P - burning

power, t - burning time) and extrapolated the obtained dependencies to zero P.t values. The EET rate constants were found about 2-fold slower for Fe-limited cells in the spectral region above 683 nm. The corresponding values of the excited state lifetimes $T_1 \approx 4$ ps (Fe-limited) and $T_1 \approx 8$ ps (Fe-replete) document a slower EET in Fe-limited cells. The affected spectral region of fluorescence (above 683 nm) can be ascribed mainly to the core antenna proteins of PSII[5,8].

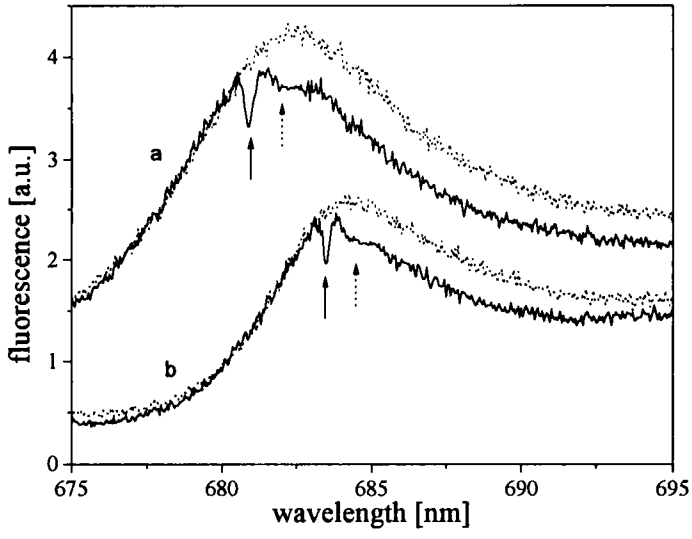

FIGURE 3. Typical hole-burning spectra of Fe-limited (a) and Fe-replete (b) cells; burning wavelength 681 nm (limited) and 683.5 nm (replete). ZPH (solid arrow) and PSBH (dashed arrow) are marked. The dotted lines correspond to the spectra before burning.

A higher burning expositions (above 150 $mWcm^{-2}$) result in very broad holes situated at ≈ 684 nm (Figure 4). These broad holes were of the same width and spectral location of the minimum for both Fe-limited and Fe-replete cells at all burning wavelengths. The widths of these holes were 180 cm^{-1} what is in a good agreement with the broad holes observed in absorption spectra[9]. These very broad holes correspond to a very fast excitonic coupling within particular pigment-protein complexes, while the EET rates determined from resonant ZPHs correspond to the EET between particular pigment-protein complexes.

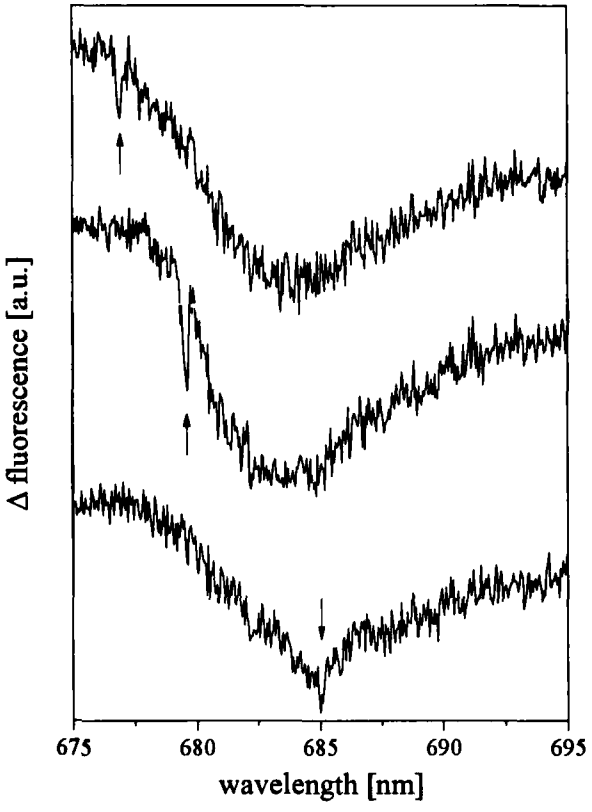

FIGURE 4. Difference hole-burning spectrum of Fe-limited cells obtained for higher burning exposition (6 min, 180 $mWcm^{-2}$). Burning wavelengths (677 nm, 679.5 nm, 685 nm) are marked by arrows.

The results indicate that the lack of iron has no effect on the ultrafast energy transfer between chlorophyll molecules within pigment-protein complexes. The slower EET between particular pigment-protein complexes can be explained by the missing core proteins of PSII. The path of EET from the outer antenna to the core antenna and subsequently to the P680 is broken in some of PSII. This leads to a decrease of EET efficiency between antenna proteins and to an increase of the fluorescence intensity in the spectral region of PSII.

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