Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals

Publication details, including instructions for authors and subscription information:
http://www.tandfonline.com/loi/gmcl19

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Version of record first published: 04 Oct 2006.

To cite this article: David Engst , Pavel Kroh , Tomáš Polívka , Jakub Pšenčík , Ladislav Nedbal & Jan Hála (1996): Hole Burning and Low Temperature Absorption and Fluorescence Spectroscopy of Algae Affected by Uv-B Stress, Molecular Crystals and Liquid Crystals Science and Technology. Section A. Molecular Crystals and Liquid Crystals, 291:1, 103-109

To link to this article: http://dx.doi.org/10.1080/10587259608042737

Mol. Cryst. Liq. Cryst., 1996, Vol. 291, pp. 103–109
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HOLE BURNING AND LOW TEMPERATURE ABSORPTION AND FLUORESCENCE SPECTROSCOPY OF ALGAE AFFECTED BY UV-B STRESS

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Abstract: UV-B stress affects the spectral and kinetic behavior of green algae. These changes were studied by means of hole-burning and low temperature absorption and fluorescence spectroscopies. Results of this study are reported here. UV-B irradiation induces significant changes in low-temperature fluorescence spectra. An influence on persistent spectral hole-burning spectra is also reported.

INTRODUCTION

Algae and cyanobacteria are the simplest oxygen evolving photosynthetic organisms. The photosynthetic active radiation is captured and transferred from the antennae to the reaction centers where the unpaired electron is generated. Algae have the ability to protect themselves against an excess of light energy by dissipating excessive excitation energy. The capture and migration of the exciton within the antenna is reflected by the

absorption and fluorescence spectra and by the excited energy transfer rate constants (k_{FET}).

The antropogenic depletion of stratospheric ozone layer results in increasing levels of UV-B radiation (280-320 nm) affecting both the terrestrial and aquatic ecosystems[1]. The elevated UV-B radiation affects various fundamental functions of the plant cell and induces multiple protection mechanisms. However UV-B stress can damage the protecting mechanisms. This damage also affects the spectral and kinetic behavior of the algae. The fluorescence emitted from chlorophyll molecules of the photosynthetic apparatus reflects both damaging and protective mechanisms. The suppression of photosynthetic activity is accompanied by a decline of variable and, frequently, of maximal fluorescence. Constant fluorescence mostly rises during a short term UV-B exposure.

The UV-B induced changes in fluorescence emission justify the hypothesis that, in parallel to the inhibition of electron transfer activity, also the exciton transfer in photosynthetic antennae is affected by the UV-B exposure. The anticipated modification of the exciton transfer parameters are investigated here using low temperature absorption, fluorescence, and hole burning spectroscopy.

MATERIALS AND METHODS

Culture of the chlorococal alga *Scenedesmus quadricauda*[2] was grown synchronously in 14/10 hours light/dark cycle, bubbled with air enriched by 2% CO_2 at 25°C and exposed to photosynthetically active radiation of 250 $\mu E.m^{-2}.s^{-1}$ provided by tungsten filament bulbs[3]. The algae were harvested in the first half of the light period, resuspended at the chlorophyll concentration about 50 μg/ml and exposed to UV-B. Samples of the algae were taken during the exposure, centrifuged and resuspended in cultivation medium mixed with glycerine. Then the samples were placed in the cuvettes for low temperature spectroscopy and stored in liquid nitrogen.

The UV-B radiation was produced using the 200 W high pressure mercury lamp screened by UV-B filter (Carl Zeiss, Jena 1602) and cellulose acetate film. The spectrum

of the radiation incident on the sample surface is shown on Figure 1. The total UV-B power (≈ 0,3 W/m^{-2}) applied to the samples was determined using a SED 240 detector (International Light). The samples of algae were collected after 10, 20 and 40 minutes of UV-B exposure.

RESULTS AND DISCUSSION

Low temperature (77 and 4,2 K) absorption and fluorescence spectra were measured together with persistent spectral hole-burning (PSHB) on the set-up described in Ref[1]. The variable fluorescence of the algae (measured by a Walz fluorimeter) declined during the UV-B exposure approximately in parallel to the decline of photosynthetic activity measured by oxygen evolution. Typical variable fluorescence measured before, during and after UV-B irradiation is shown on Figure 2.

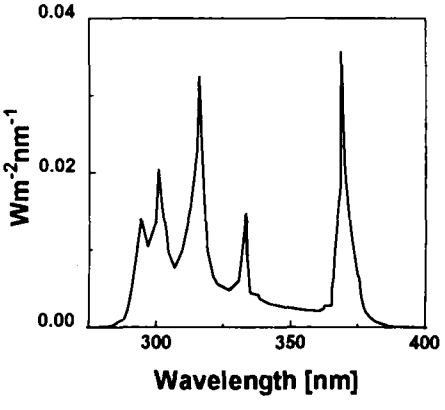

FIGURE 1. Spectrum of applied UV-B irradiation

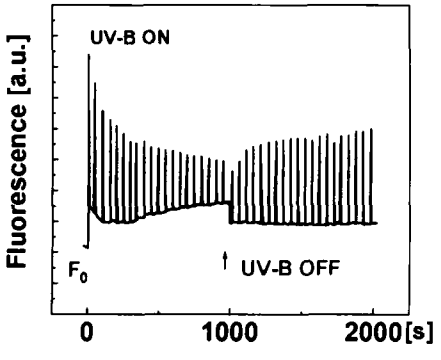

FIGURE 2. Variable fluorescence of *S. Quadricauda* measured during and afterUV-B irradiation.

The applied maximal doses of the UV-B radiation causes ≈ 50% decrease of the photosynthetic activity as determined from the variable fluorescence and oxygen evolution rates. Figure 3. shows the resonance region of low temperature (4.2 K) absorption and fluorescence spectra of UV-B treated and of control algae. All the

spectra shown in Figure 3 were normalised to the fluorescence signal of PS I (near to 715 nm).

Following UV-B irradiation effects and trends can be found in these spectra: The applied UV-B exposition was not enough intense to produce any significant changes ($\leq$ -4%) in the red part of the absorption spectra corresponding to a bleaching or a destruction of photosynthetic pigments. On the other hand the applied UV-B exposition was sufficiently intense to produce an increase of the relative fluorescence caused by a global decrease of excited energy transfer (EET) efficiency in antennae of PS II: LHC II ($\approx$ +30% near 680 nm), CP 43 and/or CP 47 ($\approx$ +16% near 688 nm). A decrease of the relative fluorescence of the RC ($\approx$ -47% near 695 nm) reflects an interruption of EET between CP 43, 47 and the RC of PS II.

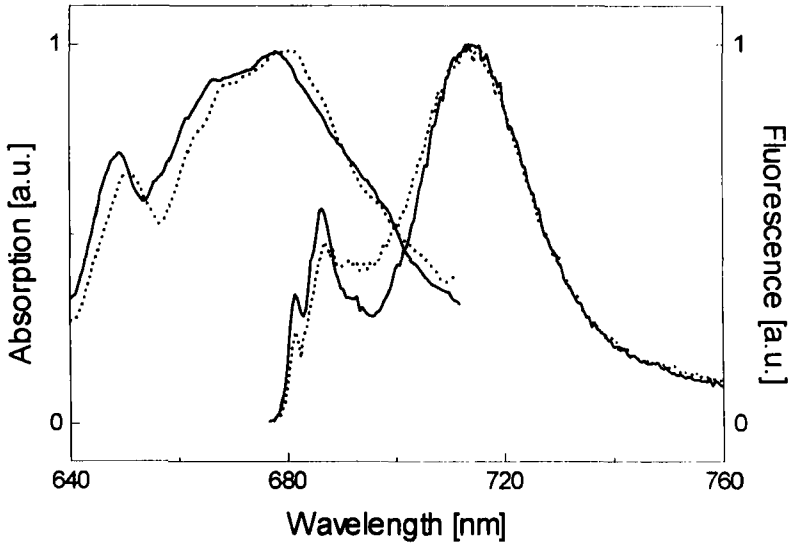

FIGURE 3. Resonance region of low temperature absorption (left) and fluorescence (right) spectra of UV-B treated (full curves) and control (dotted curves) *S. Quadricauda.*

Typical PSHB in absorption spectra are shown on Figure 5-a). The upper and lower spectrum belongs to the control (untreated) and UV-B irradiated algae, respectively. Both spectra consist of narrow zero phonon holes (ZPH) at the wavelengths of the burning laser and red shifted phonon wings (PW). Linear and logarithmic

dependencies of the ZPH widths $\delta_{HB} = \delta_{HB}$ (P.t) on the burning exposition (P.t) are shown in Figure 4. Dependencies of the ZPH widths on burning fluences following the relation $\delta_{HB} = \delta_{HB}(0) + k.(P.t)^{1/4\pm1/8}$ in the range of burning exposition densities P.t /A≈ 0.1-10 J.cm^{-2} were determined from the spectra. To remove power broadening,

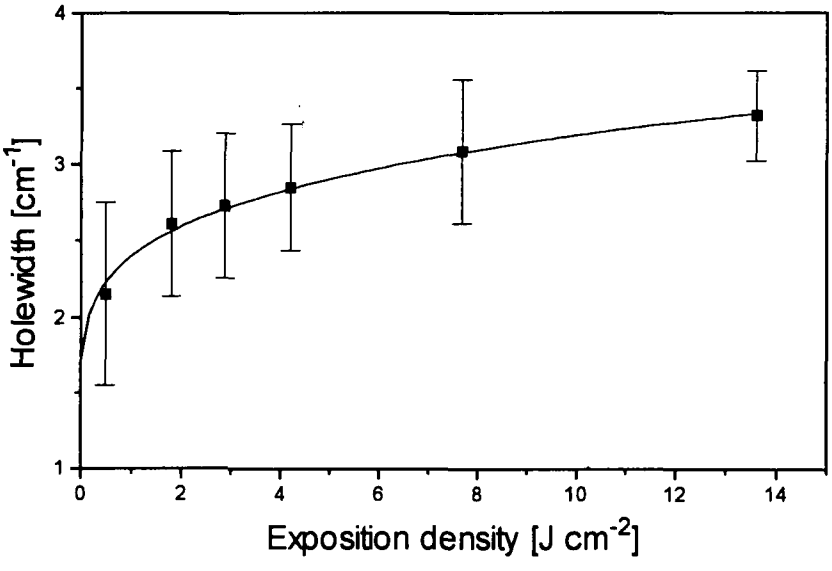

FIGURE 4. Dependence of the ZPH widths $\delta_{HB} = \delta_{HB}$ (P.t) on the burning exposition (P.t).

holewidths extrapolated to zero burning exposition $\delta_{HB}(0)$ were determined (1,5 cm^{-1} < $\delta_{HB}(0)$ < 2,0 cm^{-1}) [Figure 4]. All the PSHB data of untreated green algae fit well with the PSHB data obtained on related photosynthetic systems obtained from higher plants[5,6]. The corresponding EET rate constant, which represents EET between particular pigment-proteins, was 6 < $(k_{EET})^{-1}$ < 9 ps, as the dephasing contribution and spectral diffusion is in pigment protein complexes of photosynthetic systems negligible[7]. The Huang-Rhys factor S characterising electron-phonon coupling (pigment-protein interaction) was determined from the ratio of areas of ZPH and PW both from absorption and fluorescence spectra at several exposition densities. The S value was approximately 1.5 times higher in UV-B stressed algae (0.8 < S < 1.2). On the other

hand, the distance between ZPH and PW representing the mean frequency of protein vibrations Ω was ~ 24 cm^{-1} in all samples.

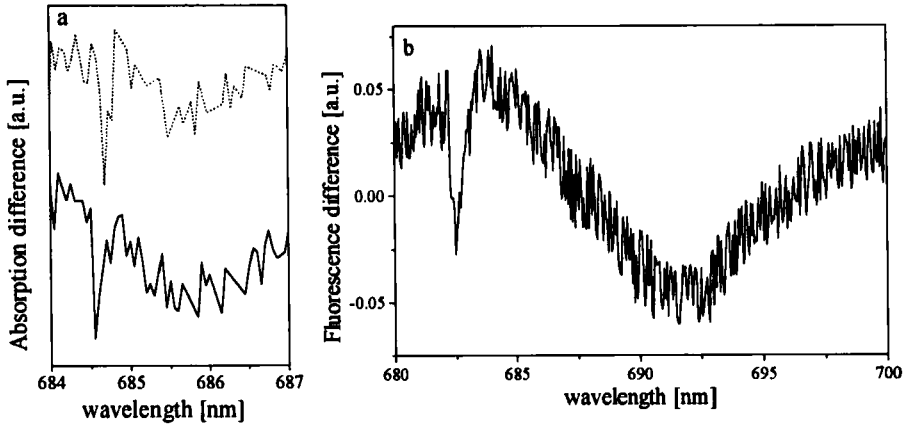

FIGURE 5-a) Zero phonon holes and phonon wings burned in the absorption spectra of *S. Quadricauda*; UV-B irradiated (top), control (bottom); -b) fluorescence spectrum with a broad-band hole

Besides ZPH and PW, broad-band holes were found in the fluorescence spectra of all samples after prolonged (≥ 10 minutes) burning using higher laser power (≥ 100 $mWcm^{-2}$) (Figure 5-b). These data fit well with the results observed in absorption of untreated systems from higher plants[8]. The hole-widths were 170 cm^{-1}, probably due to very fast (< 100 fs) exciton migration between antenna pigments in particular antenna pigment-proteins. The holewidths were not significantly affected by UV-B irradiation. From the mentioned results it can be stated, that the applied UV-B affects EET between different pigment proteins, but it doesn't affect EET inside one particular pigment-protein.

REFERENCES

1. M. Tevini, Progress in Botany, Vol. 55, Springer-Verlag Berlin Heidelberg (1994)
2. J. Komárek, B. Fott: Das Phytoplankton des Süsswassers 7, *Chlorophyceae, Chlorococcales*. In: Hüber-Pestalozzi G. ed. 1044 pp. (1983)
3. J. Knoppová, J. Masojídek, J. Pokorný, Photosynthetica 28(4) (1992), ,541-547
4. P. Kroh, T. Polívka, D. Engst, J. Pšenčík, Czech. J. Phys. A, 45, (1995) 358-361

5. M. Vacha, J. Psencik, F. Adamec, M. Ambroz, J. Dian, L. Nedbal, J. Hala, J. Lumin., 60&61, (1994), 523-526
6. N.R.S. Reddy, H. van Amerongen, S.L.S. Kwa, R. van Grondelle and G.J. Small, J. Phys. Chem., 98, (1994) 4729-4735
7. R. Jankowiak, G.J.Small, Spectral Hole Burning in: The Photosynthetic Reaction Center II, ed. J. Diesenhofer, J.R. Norris, (1993), pp. 133-170
8. Chang, R. Jankowiak, C.F. Yocum, R. Picorel, M. Alfonso, M. Seibert and G.J. Small, J. Phys. Chem., 98, (1994) 7717-7724