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POLARIZED FLUORESCENCE SPECTRA OF ORIENTED CYANOBACTERIA CELLS

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The polarized absorption and fluorescence spectra of whole cells of cyanobacteria *Synechocystis* embedded in stretched polymer film were measured. The spectral properties of "native" samples and the samples in which part of chromophores of given orientation were bleached by illumination with strong polarized light with an electric vector parallel to the sample orientation axis were compared. The changes of the anisotropy of absorption and emission as well as in the paths of excitation energy transfer between antenna pigments of the cyanobacteria were observed. It was shown that in complex biological samples the photoreaction occurring as a result of illumination by polarized light provides information about interactions between various chromophores and their role in the process of excitation energy transfer.

INTRODUCTION

Excitation energy transfer in a chain of donor and acceptor of excitation in antenna systems of cyanobacteria depends strongly on the mutual orientation of the donor emission transition moment (TM) and acceptor absorption TM. In cyanobacteria giant antenna complexes occur called phycobilisomes (PBS) (Mimuro, 1990; Mac Coll & Guard-Friar, 1987). The light absorbed by PBS is transferred predominantly to pigments of photosystem 2 (PS 2), but there are some alternative ways of excitation transfer to PS 1 reaction centers (RC) *via* pigments from PS 1 (Frąckowiak & Planner, 1995). The conclusions concerning the paths of excitation energy migration and the influence of pigments orientation on these processes can be obtained from polarized fluorescence spectra of oriented cyanobacteria, using several excitation wavelengths (λ_{exc}). The absorption bands of the various photosynthetic pigments strongly overlap but by changing the excitation wavelengths the amount of energy absorbed by the various pigments can be changed. This results in modifications in the shape of the fluorescence spectra. These modifications are related to variation in the distributions of excitation energy between two photosystems (PS 1 and PS 2). In living cyanobacteria, PS 1 emission maximum at room temperature is located at about 740 nm, whereas maximum PS 2 is at 680 nm. In whole cells the PS 1 emission intensity is usually lower than that from PS 2. At low temperature the PS 1 emission intensity is higher than in room temperature (Kramer & Amesz, 1982). A similar effect is observed for a rigid matrix such as PVA films (Frąckowiak & Planner, 1995).

In the organisms investigated the PBS contain the following biliproteins: C-phycoerythrin (PE), C-phycocyanin (PC) and allophycocyanin (APC). In native PBS the excitation energy transfer (ET) from PE to PC and further to APC is very efficient (Mimuro, 1990) because of the proper structure of PBS and overlapping of emission and absorption spectra of donors and acceptors. The orientation of other antenna pigments chlorophylls (Chl), and carotenoids (Cars) may play crucial role, in transferring the excitation energy to reaction centers (RCs).

Our samples were embedded in polyvinyl alcohol PVA films. In the anisotropic stretched films the biological cells can be deformed (Frąckowiak, Skibiński, Zelent & Leblanc, 1992; Frąckowiak & Planner, 1995) which could influence the arrangement of macromolecules inside the cells. However, in stretched films the degree of cells orientation is usually higher than in unstretched films (Goc, Klecha, Waszkowiak, Miyake & Frąckowiak, 2001). In organisms, various pigment forms play different role in excitation energy transfer, due to the pigment interaction with the different surroundings. The number and spectral properties of these forms are differently described in the literature (Olson & Stanton, 1966; Litvin & Sineshchekov, 1975). We thus deconvoluted the

emission spectra on Gaussian components to a some extent arbitrary, but consequent analysis of the set of samples using the same components provide information about changes in the processes undergone in the samples. In our case, analysis of fluorescence spectra excited at various wavelengths gives information about the processes of excitation energy transfer. The polarized light application enables us to draw information about excitation energy transfer (ET) between antenna pigments.

Strong illumination of sample by polarized light can, by perturbation of pigment arrangements or by the photodestruction of the pool of dyes with their absorption transition moments (TMs) parallel to the electric vector of acting light, cause the change of the paths of excitation energy transfer towards reaction centers.

The conclusion concerning the orientation of various forms of pigments and their role in excitation energy transfer and in the conversion of their excitation into heat or fluorescence emission can be drawn.

MATERIAL AND METHODS

The cyanobacterium *Synechocystis* Sp. strain PCC6803 was obtained from NAIR (Tsukuba). The method of culturing was described by Erokhina (1991). It was carried out in BG11 medium (pH = 7.4) at 25°C. Cultures were aerated with 99% air plus 1% (v/v) CO_2 and illuminated with white light at 500 lux.

Methods of introducing cells into polyvinyl alcohol (PVA) film preparation and film stretching were as previously (Frąckowiak, Gantt, Hotchandani, Lipschultz & Leblanc, 1986).

Absorption spectra were measured a using Shimadzu UV-1601 UV- Visible Spectrophotometer, and fluorescence spectra with a Hitachi F 4500. Both arrangements were equipped with polarizers and sample holders. The absorption components polarized in two mutually perpendicular directions were measured: the $A_{\parallel}$ was parallel to the direction of film stretching, $A_{\perp}$ perpendicular. Anisotropy of absorption :

$$S = \frac{A_{\parallel} - A_{\perp}}{A_{\parallel} + 2A_{\perp}}, \tag{1}$$

was calculated. The same directions of light polarization were applied in measurements of fluorescence spectra. All polarized fluorescence spectra were denoted by a sequence of three letters in referring to the electric vector of exciting light, the sample axis and polarization of fluorescence beam (H-horizontal, V-vertical). The following four polarized components of stretched samples were measured: *VVV*, *VVH*, *VHV* and *VHH*.

The following fluorescence anisotropy coefficients:

$$r_a = \frac{VVV - VVH}{VVV + 2VVH}, \tag{2}$$

$$r_c = \frac{VVV - VHV}{VVV + 2VHV}, \tag{3}$$

are calculated (Martyński, Frąckowiak, Miyake, Dudkowiak & Piechowiak, 1998). Most important in our considerations are r_c describing the orientation of emission TMs in a sample, and r_a giving information about polarization of the emitted light. We calculated additionally:

$$r_e = \frac{VHH - VVH}{VHH + 2VVH}, \tag{4}$$

giving information about a pool of molecules with TMs forming a large angle relative with the axis of orientation.

The samples observed under microscope exhibited large clusters of the cells. These clusters were deformed upon stretching of the film in such a way that they were elongated in the film stretching direction.

All films were illuminated using a xenon lamp as a light source. Two types of illuminations were applied: first (I) with a filter with transmission in 400 nm-600 nm region (maximum at 459 nm, FWHM 129 nm) with an intensity of 20W/m^2, (such films are in text marked as after illumination I), or by light transmitted through a cut off filter

Table I. Absorbance anisotropy of bacterials defined by Equation (1) for unstretched (0%) and stretched (200%) PVA films. S_0 - before illumination, S_I - after first illumination, S_{I+II} - after illuminations I+II.

λ_{max} (nm)	S_0	S_I	S_{I+II}
435	0.02	0.07	0.13
622	0.08	0.14	0.17
670	0.07	0.13	0.16

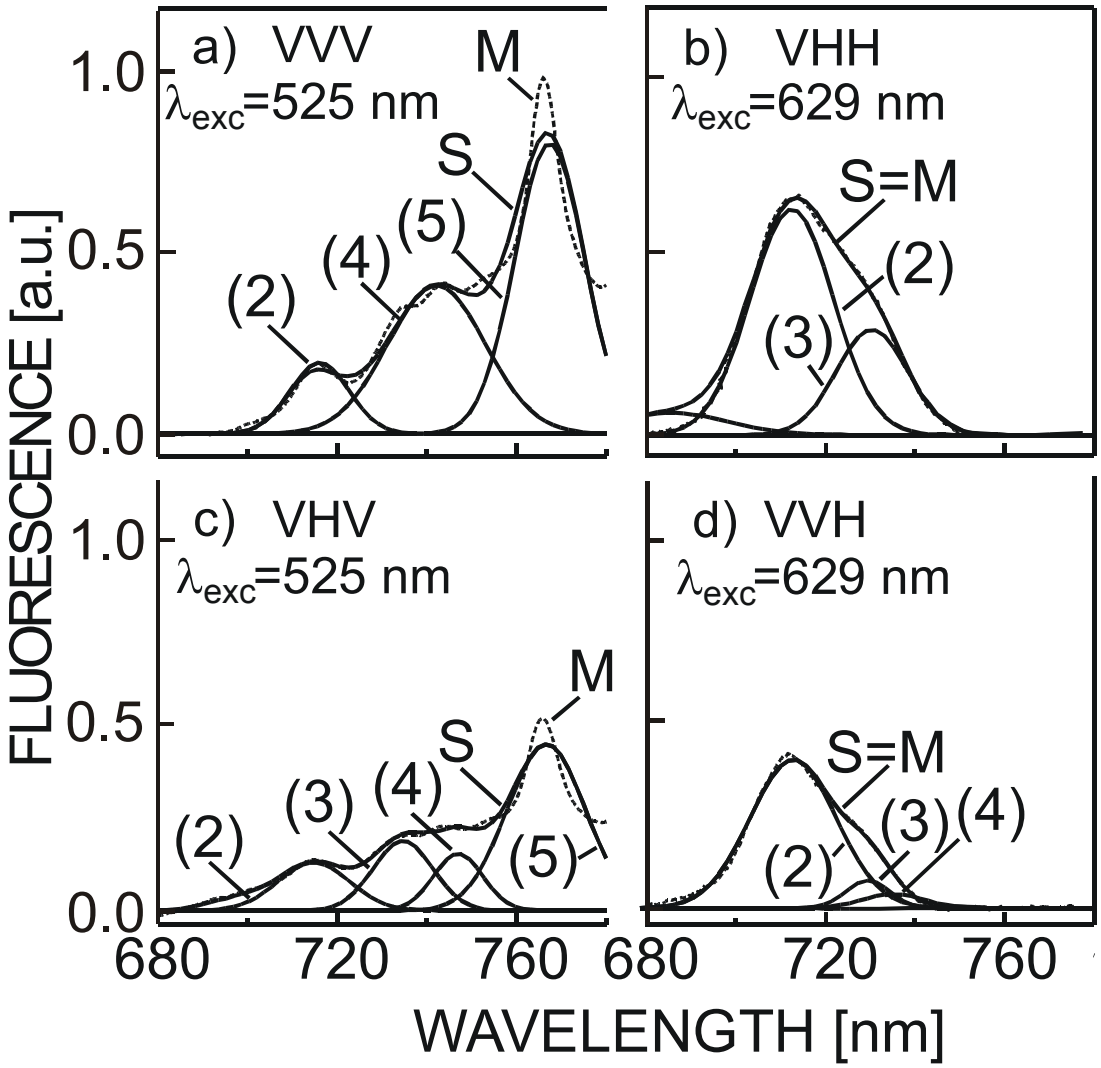

Fig.1 Polarized fluorescence spectra of cyanobacteria in dry stretched PVA films. Excitations: a) and c) 525 nm, b) and d) 629 nm; Polarized components: a) VVV, b) VHH, c) VHV, d) VVH. M - measured spectra; S - superposition of calculated components. The notation of Gaussian components (2 - 5) is explained in text.

transmitting wavelengths longer than 455 nm with an intensity 1000W/m^2 (marked as illumination II). Part of samples was illuminated sequentially by both filters (denoted as illuminations: I+II). Both illuminations were done at the same time 2.5 h using water filters additionally in order to avoid sample heating.

RESULTS

Fig.1 shows some examples of polarized fluorescence spectra of samples analyzed on Gaussian components. According to our previous data obtained for unstretched films (Goc et al. 2001) we attempted the fluorescence analysis with five Gaussian components. The components of fluorescence spectra are marked only by numbers from 1 till 5 and are sometimes referred to as bands. Table I presents the absorption anisotropy of samples and the influence of illumination on anisotropy value. The limiting values of positions of component maxima are given below in brackets, but usually they are located in the middle of these wavelengths regions: 1 (686 nm-710 nm), 2 (712-718 nm), 3 (725 nm-736 nm), 4 (737-751 nm) and 5 (764-766 nm). On the basis of literature (Mac Call & Guard –Friar, 1987; Frąckowiak & Planner, 1995) one can assume that band 1 belongs predominantly to PS 2, whereas bands 2, 3, 4 and 5 belong to PS 1.

In Fig.1 of the stretched samples band 1 is probably hidden in the background. This emission band was previously (Goc *et al.*, 2001) measurable and ascribed to arise from Chls of PS2. In our case this band could be hidden as a result of film

stretching by the change of Chls TM direction to the perpendicular orientation to film surface, or by the shift of these Chls bands towards the long wavelength side. It is very probable that band 2 also contribute emission of some Chls belonging to PS2.

From the comparison of fluorescence excited at Cars region (525 nm) with emission excited at PBS absorption (629 nm) it is clear that the ratios of fluorescence intensities of Chls emitting in short wavelength and long wavelength regions are dependent on wavelength of excitation. Excitation energy transfer from Cars to Chls have been reported by Peterman et al. (Peterman, Monshouwer, van Stokkum, van Grondelle & van Amerongen, 1997) that is very efficient and ultra fast in green plants. The immobilization of the cells in PVA usually diminishes the PS 2 emission and causes an increase in PS 1 fluorescence. It was found (Białek-Bylka, Sofrova, Szurkowski, Skwarek, Sopko & Manikowski, 2000) that complexes enriched in PS 1 embedded with Cars in dry PVA film excited at 500 nm, exhibit emission to at least 750 nm. Our samples exhibit also efficient ET from excited Cars to various long wavelengths forms of Chls belonging to PS1, and less efficient excitation of forms emitting in shorter wavelengths range (Fig.1 a and Fig.1c).

Excitation in the PBS absorption region (at 629 nm) is followed by strong emission from bands 2 and 3, but band 3 can be diminished by proper choice of light polarization (Fig.1d).

The stretching of polymer film with embedded biological objects usually deformed the shapes of embedded objects, therefore the arrangement of some pigment-protein complexes can be changed. For PBS in PVA (Frąckowiak, Erokhina, Picard & Leblanc, 1987) it was shown that excitation energy transfer between biliprotein inside PBS changes as a result of film stretching. This change depends on the shape of PBS and on the strength of the interaction between biliproteins. Generally taking the elongated biological samples are not only better than an isotropic sample oriented in anisotropic polymer, but also are less deformed than almost spherical cells such as *Synechocystis*.

The stretching in most cases improves the uniaxial orientation of cells or molecules (Lorrain, Frąckowiak, Romanowski & Leblanc, 1987) and as a result causes the increase of absorption anisotropy. In a case of our sample, as a result of stretching the absorption anisotropy changes differently for various spectral range, as it follows from comparison of Table I with our previous results obtained for unstretched films (Goc *et al.*, 2001). For our samples the absorption anisotropy is rather low before the illumination and increases as a result of strong irradiation by polarized light with the electric vector parallel to film stretching axis. It is an unexpected result suggesting conformational changes rather than photobleaching of chromophores with TM parallel to electric vector of light. Also, emission anisotropy (Table II) is in many cases lower than that of unstretched samples (Goc *et al.*, 2001), but some effects can be more clearly seen using such stretched slightly perturbed samples.

As it follows from Fig.1a, c and Table II in the fluorescence spectrum excited at 525 nm are strong long wavelengths components 4 and 5, exhibiting rather high anisotropy r_c showing orientation of emitting TMs. The strong illumination (I+II) does not change the situation dramatically (Table II). Band 5 is still high. It is known from literature the (Frąckowiak, Cegielski & Abdurakhmanov, 1991) that Cars can transfer their excitation to Chls, but this transfer depends strongly on Cars conformation (Grudziński, Matuła, Sielewiesiuk, Kernen, Krupa & Gruszecki, 2001). In our system conformation of Cars and orientation with respect to the long wavelength forms of Chls from PS 1 have to be suitable for efficient ET from Cars to these forms (bands 5 and 4). Similar effects were observed previously (Goc *et al.*, 2001) for unstretched samples where natural arrangement of complexes is probably preserved and for actually investigated stretched films, which suggests that the film elongation is not perturbing mutual orientation of Cars and PS 1 Chls molecules.

Table II. Fluorescence anisotropy (Equation (3)) for Gaussian area components (2 - 5) for stretched samples

λ_{exc} (nm)	Illuminations	r_c		
		(2)	(3) + (4)	(5)
525	0	0.19	0.25	0.25
525	I + II	0.36	0.31	0.27

The spectra of stretched films excited at 629 nm (Fig.1b) differ strongly from the spectra excited at 525 nm (Fig.1a). Only short wave length components probably partially belonging to PS 2 are large. A similar conclusion follows from the area of components for various wavelengths of excitation and light polarizations measured for the stretched samples (not shown). Fig.1b presents the polarized component *VHH* and in Fig.1d is shown component *VVH* at 629 nm excitation. Emission component *VHH* is higher than *VVH* which shows that excitation of chromophores of PBS forming a large angle with the orientation axis is followed by the emission of similarly oriented Chl molecules belonging probably partially to PS 2. For band 2 (712 nm) $r_e = 0.15$, for band 3 (729 nm) it equals 0.48. This shows different arrangements of the chromophores belonging to these two bands. The strong illumination I+II causes the increase of r_c anisotropy of short wavelengths component (band 2) of fluorescence excited at 525 nm from 0.19 before illumination to 0.36 after illumination. The light emitted with 629 nm excitation is also in polarized to a higher degree after I+II illumination: for example band 2 exhibits $r_a = 0.36$ after illumination whereas before illumination it is only $r_a = 0.09$, for band 3 the values of r_a coefficient are 0.13 before and 0.40 after illumination. The different changes in emission and absorption anisotropy due to the same illumination shows that the strong illumination by polarized light changes the excitation energy transfer between chromophores.

SUMMARY

Excitation of the Cars region is followed by strong emission of Chl from PS 1, especially in the 765 region (band 5). The emission from short wavelengths Chls is much lower. The strong irradiation of samples by polarized light has a low influence on anisotropy of the observed emission due to excitation ET from Cars to PS 1 Chl.

Excitation in the PBS absorption region is followed predominantly by the short wavelengths emission of Chl. By proper choice of electric vector direction of exciting light (perpendicular with respect to orientation axis) it is possible to eliminate the ET from PBS to the longer wavelength forms of PSI Chls. It suggests that TMs of molecules (Cars and maybe also some biliproteins) transferring excitation to PS 1 Chls have their TMs located under the low angle with respect to the polymer axis, whereas molecules with TMs under high angle are able to transfer excitation to shorter wavelengths emitting antenna.

It is still an open question if one can extrapolate results obtained on cyanobacteria embedded in artificial polymer matrix, on the living organisms. It is observed that the ratio of PS 1 to PS 2 intensities of emission in PVA is higher than in physiologically accepted conditions, but which are similar to effects at low temperature. Observed effects can be due to the change in the fluorescence yields of PS 1 Chls or/and by the perturbation of some paths of excitation energy transfer.

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Abbreviations:

APC - allophycocyanin, Chl - chlorophyll, Cars - carotenoides, ET - energy transfer, LHC - light harvesting complex, PBS - phycobilisomes, PAS - photoacoustic spectra, PC - C-phycocyanin, PE - C-phycoerythrin, PS - Photosystem, PVA - polyvinyl alcohol, RC - reaction centre, TMs - transition moments.

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