Linear dichroism, fluorescence polarization, and path of the thermal deactivation of excited cyanobacterial (*Synechococcus elongatus*) photosystem 1 immobilized and oriented in polymer films

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Abstract

Pigment-protein complexes enriched in photosystem 1 (PS1) and, for comparison, enriched in photosystem 2 (PS2) were isolated from the cyanobacterium *Synechococcus elongatus* Nag. f. *thermalis* Geitl. They were immobilized and oriented in the polyvinyl alcohol (PVA) films, and studied by linear dichroism (LD), fluorescence polarization (FP), photoacoustic spectroscopy (PAS), and polarized photoacoustic spectroscopy ($PAS_{\parallel}$ and $PAS_{\perp}$). The LD signal of β-carotene in the region with maximum at 500 nm was positive in the PS1 complex. The maximum value of fluorescence polarization (FP) in the measured photosynthetic pigment region was 1.25 and was similar to higher plant values. Carotenoids exhibited different efficiencies of thermal deactivation (max. at 500 nm) in PS1 and PS2. The thermal deactivation efficiency of carotenoids in comparison with that of chlorophyll (Chl) *a* at its red absorbance maximum was much higher in PS1 than in PS2 complexes. Cyanobacterial complexes did not contain Chl *b*, interpretation of the LD, PAS, and FP results is thus easier and can be compared with PS1 and PS2 values of higher plants, especially with Chl *b*-less mutant values.

Additional key words: carotenoids; photoacoustic and emission spectroscopy; polarized absorption; reaction centres.

Introduction

Conversion of radiant energy into chemical energy potential and energy conservation in the forms of adenosine triphosphate (ATP) and reduced nicotinamide-adenine dinucleotide phosphate (NADPH), *i.e.*, the photosynthesis process of cyanobacteria, takes place in photosystems 1 and 2. In the PS1 and PS2 complexes of cyanobacteria the main carotenoid is β-carotene, which was found in peripheral regions of the complexes as well as in close vicinity of the reaction centres of the two photosystems (core or sub-core complexes; Siefermann-Harms 1985, Lichtenthaler 1987, Brody 1988). Possible role of carotenoids in the dissipation of excitation energy in pigment-protein complexes of PS1 and PS2 is related to the following processes: (*1*) thermal deactivation, (*2*) excitation energy transfer, (*3*) photochemistry (at high irradiances), and (*4*) fluorescence.

Procaryotic organisms, *e.g.*, the thermophilic cyanobacterium *Synechococcus elongatus,* contain no Chl *b* and antenna complexes of the LHC type. Due to the missing Chl *b*, cyanobacterial PS1 and PS2 samples are very useful for the study of: (*1*) path of thermal deactivation, and (*2*) orientation of Chl *a* and carotenoids (mainly β-carotene) by means of LD method. In such samples there is no problem with overlapping of the electronic absorption spectral regions of carotenoids and Chl *b*.

In photosynthetic systems, carotenoids carry out the functions of light harvesting, photoprotecting, transfer-

Received 16 November 1999, *accepted* 25 April 2000.
Abbreviations: Chl – chlorophyll; DM - dodecyl-β,D-maltoside; FP – fluorescence polarization; LD – linear dichroism; LHC – light-harvesting complex; PAS – photoacoustic spectroscopy; PS – photosystem; PVA – polyvinyl alcohol.
Acknowledgement: This project was supported by Polish grants P.B.-62-147/99-D.S. and KBN-WO/99/38/39 and by a grant No. 206/96/1266 from the Grant Agency of Czech Republic. We thank Dr. M. Niedbalska for technical assistance.

ing, and dissipating radiant energy (Cogdell 1978, Białek-Bylka *et al.* 1982, Mathis and Schenck 1982, Cogdell and Frank 1987, Siefermann-Harms 1987, Koyama 1991, Frank and Cogdell 1993, 1996, Young and Britton 1993). Natural selection of carotenoid configurations (15-*cis* for photoprotection and all-*trans* for light-harvesting) to carry out these functions was found in purple photosynthetic bacteria (Koyama 1991), PS1 and PS2 of higher plants (Bialek-Bylka *et al.* 1995, 1996), and PS1 of green sulfur bacteria and cyanobacteria (Białek-Bylka *et al.* 1998). In oxygenic photosynthesis, carotenoids mainly desensitize the Chl triplet state, in order not to allow formation of highly reactive singlet oxygen, which is lethal to all forms of life (Frank and Cogdell 1993). In higher plants, β-carotene molecule is an acceptor of excess triplet energy thereby protecting Chl *a* from photooxidation. Carotenoids quench Chl triplets very effectively by the electron exchange mechanism (Dexter 1953).

The purpose of this paper was to find out the orientation of β-carotene in relation to the plane of the membrane (and/or to the plane of the Chl *a* molecules in pigment-protein complexes) by linear dichroism study of oriented systems and to confirm that β-carotene in the PS1 and PS2 complexes participates in the deactivation of excitation energy.

Methods

Sample preparation, immobilization, and orientation: Thylakoid membranes from *S. elongatus* Nag. f. *thermalis* Geitl. were extracted by the detergent dodecyl-β,D-maltoside (DM) (Sofrová *et al.* 1992). The DM-extract contains Chl (absorption maxima around 430 and 670 nm), carotenoids (absorption around 500 nm), and some phycobilins (absorption around 600 nm). The step of DM-extract purification by the centrifugation in sucrose density gradient separates carotenoids and phycobilins from Chl zones.

The Chl zones, denoted upper (U) and lower (L), are enriched in PS2 and PS1, respectively, and further separation can be done by the ion exchange high performance liquid chromatography (HPLC) (Sofrová *et al.* 1992). The cyanobacterial PS1 and PS2 were embedded in films of polyvinylalcohol (PVA) (Siodmiak and Frackowiak 1972) in order to preserve the integrity and activity of isolated pigment-proteins (Białek-Bylka *et al.* 1987). Complexes were immobilized in PVA films not only for orientation but also in order to minimize the aggregation problem, because for example the PS1 complex has a distinct tendency to form supra-molecular aggregates or their degradation products (Guikema and Sherman 1981, Takahashi *et al.* 1982). The PVA content was 15 %, the Chl *a* content was 0.2 kg m^{-3}. The PVA film was drying for about 7 d, then it was stretched mechanically (Fiksiński and Frąckowiak 1980) to as much as four times of its initial length (Δl/l = 300 %). Thickness of the film was about 240 μm.

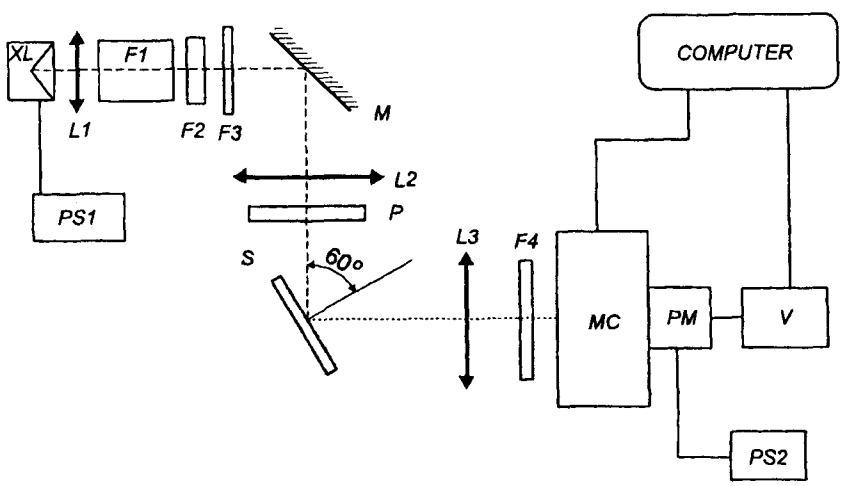

Fig. 1. Block diagram of the fluorometer for fluorescence and fluorescence polarization spectra measurements. *Abbreviations*: *F1* - water filter; *F2* - interference filter; *F3, F4* - cut-off filters; *L1, L2, L3* - lenses; *M* - mirror; *MC* - monochromator; *PM* - photomultiplier; *P* - polarizer; *PS1, PS2* - power supply stabilizers; *S* - sample; *V* – voltmeter; *XL* - light source (xenon lamp).

Spectral measurements: Absorption and LD spectra were measured with a *M40 Specord* (*Carl Zeiss*, Jena, Germany) spectrophotometer equipped with polarizers. The absorption of radiation polarized parallel ($A_{||}$) and perpendicular ($A_{\perp}$) with respect to the direction of film stretching was measured. In order to obtain $A_{||}$ and $A_{\perp}$ to the orientation axis, the polarizer was fixed in one position while the sample was rotated 90° with respect to the **E** vector of the polarized radiation. Absorption (A) of the natural (non-polarized) radiation by the unstretched sample was measured. Linear dichroism (LD) was calculated according to the formula $LD = (A_{||} - A_{\perp})/A$. The fluorescence polarization (FP) measurements were carried out using computerized home-made spectrofluorometer (Fig. 1).

The photoacoustic and photoacoustic polarized spectra were measured by means of a single-beam photoacoustic spectrometer, built at the University of Gdańsk (Tukaj and Szurkowski 1993), at a modulation frequency 20 Hz, and incident irradiance of about 5 000 mW m^{-2}. The signal to noise ratio was better than 1/500. The preamplified acoustic signal was transmitted to a phase lock-in amplifier. Taking into account the maximum of the amplifier output signal, the phase shift was provided by mechanical chopper with respect to reference signal. Spectral bandwidth of the incident radiation did not exceed 10 nm. The samples were optically transparent and the acoustic signal was proportional to the absorption (Rosencweig 1980). Carbon black was used as a reference sample.

All measurements were done at room temperature (293 K), and at equipment wavelength resolution of 1 nm.

Results and discussion

In a PS1 complex, fluorescence excited in the carotenoid region is emitted by the long-wavelength forms of Chl *a*. The interaction of β-carotene and Chl *a* forms can be detected by fluorescence emission from the long-wavelength Chl *a* forms (Brown 1983), because of the very low carotenoid fluorescence yield (Van Grondelle 1985, Cosgrove *et al.* 1990, Katoh *et al.* 1991, Mimuro and Katoh 1991, Andersson *et al.* 1992, De Coster *et al.* 1992). The fluorescence quantum yield is around 4×10^{-6} according to Wasielewski *et al.* (1986). The emission yield can be expressed as the ratio of the lifetime and the natural radiative lifetime of the molecular state. Considering estimated natural lifetime to be around 10^{-9} s for carotenoids such as β-carotene (Gillbro and Cogdell 1989, Shreve *et al.* 1991), the lifetime of the emission from the S_2 state of β-carotene is around 200 fs. Direct kinetic measurements of Shreve *et al.* (1991) showed this value to be around 200-250 fs at room temperature. However, the lifetime of the emission from the S_1 state of β-carotene is in the range of 3-10 ps (Wasielewski and Kispert 1986, Shreve *et al.* 1991). Values for the $2A_g$ state of carotenoids, measured by Raman spectroscopy, are discussed by Noguchi *et al.* (1989) and Hashimoto and Koyama (1990).

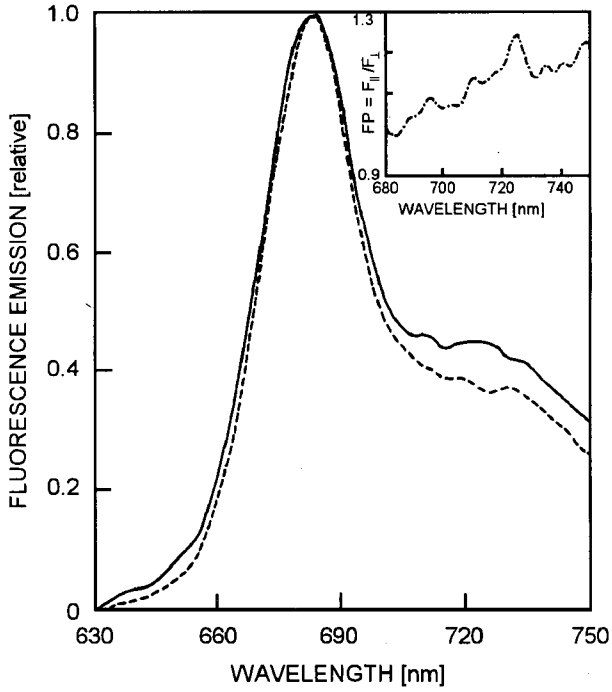

Fig. 2. Fluorescence polarization (FP) spectra of photosystem 1 complex in PVA. Component $F_{||}$ (—) and $F_{\perp}$ (- - -); excitation at 500 nm (300 % stretched). *Insert*: spectrum of FP = $F_{||} / F_{\perp}$.

In the cyanobacterial PS1 complex, homologue of PS1 of higher plants, some amount of the excitation energy directed to the longer-wavelength Chl *a* forms can be released as fluorescence, similarly as in higher plants (Brown 1983). According to Białek-Bylka *et al.* (1982), about 30 % of energy is transfered from β-carotene to Chl *a* and the rest of excitation energy is used for the photochemistry and thermal deactivation of molecules. The yield of excitation energy transfer is not influenced by the incorporation of the complex in isotropic PVA films (Białek-Bylka and Brown 1986). In the cyanobacterial (*Synechococcus elongatus*) L complex (enriched in PS1) a very efficient energy transfer from β-carotene to the 717 nm form of Chl *a* is observed (Białek-Bylka *et al.* 1997).

Fig. 2 shows FP spectra of the oriented L complex (PS1-enriched sample) measured with excitation at 500 nm. Long wavelength forms of Chl *a* are highly oriented ($F_\perp < F_\parallel$) and the FP values are shown in the insert of Fig. 2. The FP value was highest for 725 nm (1.25), followed by 710 nm (with shoulder at 700 nm), and finally by 695 nm. Different Chl *a* spectral forms existing *in vivo* in the PS1-complex (Brown 1983) have different FP values between 1.00 and 1.25. These results agree with those of the higher plant PS1 preparations (Chl *b*-less mutant) which were depleted of antenna pigments (Burton *et al.* 1984). In higher plants, Chl *b* (missing in cyanobacterial PS1 preparation) and β-carotene affect the state of the longer wavelength-absorbing forms of Chl *a* (Mullet *et al.* 1980, Białek-Bylka and Brown 1986). The long-wavelength emission maximum is blue-shifted in the Chl *b*-less barley mutant compared with wild types of barley, similarly as observed in cyanobacterial PS1. Such effect was also observed for the PS1 model system (Białek-Bylka 1992).

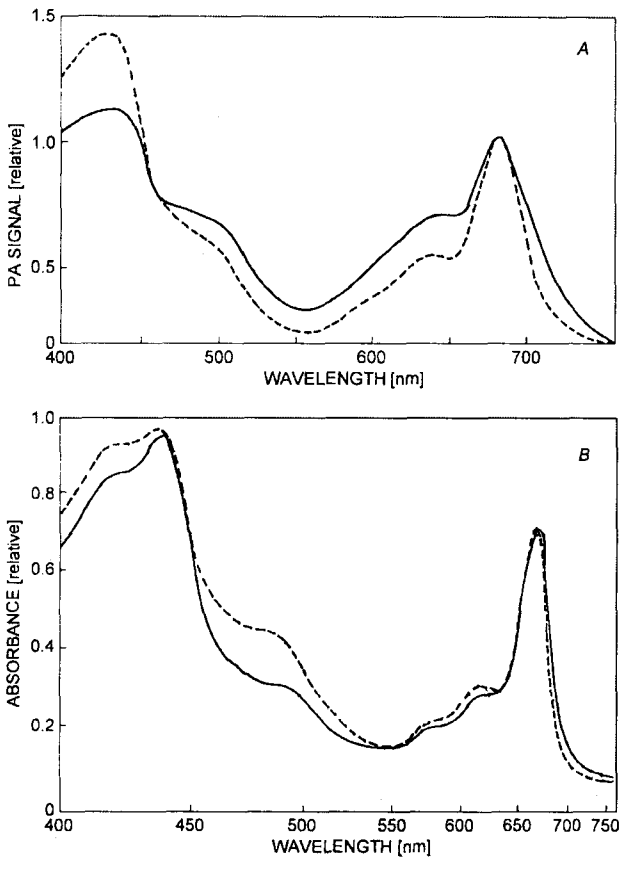

Fig. 3. Photoacoustic (*A*) and absorption (*B*) spectra of photosystems 1 (—) and 2 (- - -) in PVA films (normalized at 680 nm in *A* and at red maximum-Q_y transition of Chl *a* in *B*).

Comparing the absorption (Fig. 3*B*) and photoacoustic spectra (Fig. 3*A*) of PS1 and PS2 complexes in PVA isotropic film we found a difference in the energy dissipated as heat by photosynthetic pigments. The amplitude of PAS in the red region was lower than that in the blue part (Soret band) of the spectrum, because there exists a very efficient internal conversion between states S_2 and S_1 of the excited Chl *a* molecules. This effect is more distinct in the PS2 sample than in the PS1 sample. In the PS1 complex, very high thermal deactivation in the carotenoid spectral region is related to a very low fluorescence yield of carotenoids. The carotenoid absorption maximum of PS1 is shifted approx. 10 nm towards longer wavelength compared with the PS2 sample (Fig. 3*B*).

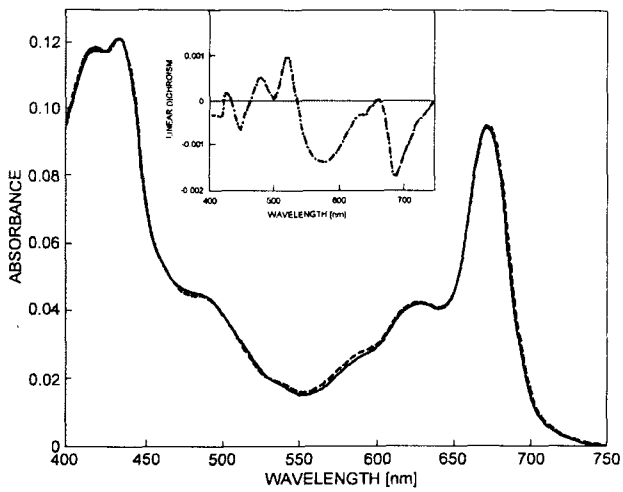

Fig. 4. Polarized absorption spectra of photosystem 1 (PS1) complex; components: $A_\parallel$ (—) and $A_\perp$ (- - -). *Insert*: LD spectrum of PS1 complex oriented in PVA film (300 % stretched).

Linear dichroism and FP studies of PS1 isolated from higher plants (high homology with cyanobacterial PS1) show that most of the Chl-protein complexes are ellipsoidal in shape and their longest dimension in the plane of thylakoid membrane (Biggins and Svejkovsky 1980, Tapie *et al.* 1982, Szitó *et al.* 1985). The Q_y transition of the longer wavelength absorbing Chl *a* is more highly oriented than that of Chl *a* absorbing at shorter wavelengths, and the β-carotene molecules are oriented parallel to these long-wavelength forms (Junge *et al.* 1977). In a photoselection technique an excitation beam of linearly polarized radiation is used to create an anisotropy in a sample (photosynthetic complex). The complexes, which in nature are oriented random, are macroscopically oriented prior to the photoselection effect (Breton and Vermeglio 1982). In a sample oriented by some techniques (in our case by mechanical stretching of the PVA film) most complexes are oriented parallel to the axis of orientation (direction of the film

stretching), and the unstretched (0 %) film serves as non-oriented sample. Information of the absorption transition moments of the photosynthetic pigment molecules, with respect to the geometrical longest axis of the complex, can be obtained from polarized absorption

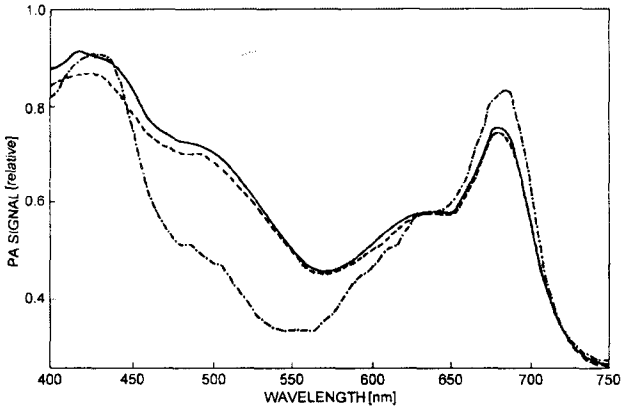

Fig. 5. Polarized photoacoustic spectra of photosystem 1 complex oriented in PVA film: $PAS_{||}$ (—) and $PAS_{\perp}$ (- - -) (both components 300 % stretched), and PAS (- · - · -) (0 %-non-oriented).

spectroscopy of oriented samples. The LD data of PS1 (Fig. 4) show that the LD signal of the PS1 complex is distinct and positive ($A_{||} > A_{\perp}$) in the entire carotenoid spectral region (around 500 nm). These values are similar to those for PS1 complexes of higher plants (Junge *et al.* 1977). Polarized PAS is a good tool for measuring deactivation of oriented chromophores. Fig. 5 shows polarized PAS ($PAS_{||}$ and $PAS_{\perp}$ components) of PS1 complex oriented in PVA film (300 % stretched) and non-oriented sample (0 %). In photoacoustic spectrum of non-oriented sample, two forms (max. at 500 nm and around 480 nm) of carotenoids can be distinguished as shoulders. In the polarized PAS (components parallel and perpendicular) a very broad band exists with maximum between 480 and 500 nm. Thus the parallel component of the photoacoustic signal is higher than the perpendicular one in the entire spectral region measured.

Concluding, carotenoids in the PS1 reaction centres of cyanobacteria (*Synechococcus elongatus*) behave like β-carotene in higher plants (Junge *et al.* 1977, Biggins and Svejkovsky 1980, Tapie *et al.* 1982, Szitó *et al.* 1985). In particular, there is a strong analogy with a Chl *b*-less mutant (Burton *et al.* 1984) of higher plants.

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