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Polarized photoacoustic spectra of the cyanobacterium *Synechococcus* oriented in polymer film

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Abstract

Photoacoustic spectra (PAS) were obtained for the cyanobacterium *Synechococcus* (*Anacystis nidulans*) cells embedded in isotropic and stretched polyvinyl alcohol films. The polarized radiation with the electric vector changing in 30° intervals with respect to given direction in a sample plane was used. Two cyanobacterium strains, one with very low biliprotein content, second with normal amount of biliproteins were investigated. The polarized absorption and fluorescence spectra were also measured. Conclusions were drawn about the thermal deactivation occurring in differently oriented pools of chromophores and about mutual orientation of their transition moments. Thermal deactivation in carotenoids (Cars) of both strains was different. The ratio of Car thermal deactivation to the thermal deactivation of chlorophyll (Chl) was higher in cyanobacteria with lower content of biliproteins than in the strain with normal amount of these complexes. Hence biliproteins can play the role in excitation energy transfer from Cars to Chls. For complex biological samples, polarized PAS can be a more sensitive method to investigate the directions of the absorption transition moments than the widely used polarized absorption spectra.

Additional key words: biliproteins; carotenoids; chlorophyll; polyvinyl alcohol films; transition moment orientation.

Introduction

The efficiency of thermal deactivation of pigment molecules depends strongly on the close surrounding of chromophore under investigation and on the efficiency of deexcitation of this chromophore by other means, such as excitation energy transfer to other pigments or luminescence emission. In organisms, chemically identical pigment molecules create so called "forms" by interacting with proteins, lipids, or other pigment molecules (Rabinowich and Govindjee 1969, Zuber *et al.* 1987). Absorption spectra of these "forms" are only slightly different, but the yields of fluorescence and nonradiative deactivation of excitation can be markedly different (Frąckowiak *et al.* 1986a,b,c, 1993, Dudkowiak *et al.* 1997). The radiative and thermal deactivations compete with excitation energy transfer to other chromophores and with the photochemical reaction in the investigated pigment (Frąckowiak *et al.* 1990, 1991, Dudkowiak *et al.* 1997). By changing direction of the electric vector of absorbed radiant energy and by measuring the PAS signal generated at various radiation polarizations it is possible to establish the mean direction of the group of chromophores responsible for the observed thermal deactivation. The shape of PAS is different from the absorption spectrum because of the different yields of thermal deactivation of various pigments. From the shape of PAS it is also possible to gather information about the efficiencies of non-thermal deactivation paths for the investigated pigments.

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Abbreviations: APC - allophycocyanin; Car – carotenoid; Chl – chlorophyll; C-PC - C-phycocyanin; ET - energy transfer; H – horizontal; MTEC - photoacoustic cell produced by *MTEC Photoacoustic*, USA; NAIR - National Institute for Advanced Interdisciplinary Research; PAS - photoacoustic signal and photoacoustic spectra; PBS – phycobilisome; PS – photosystem; PVA - polyvinyl alcohol; SH and SL - strains with high and low biliprotein content; TD - thermal deactivation; TM - transition moment; V - vertical.
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The cyanobacterium *Synechococcus* (*Anacystis nidulans*) contains giant antenna complexes called phycobilisomes (PBS) (Mac Coll and Guard-Friar 1987, Glazer 1989) that are built from biliproteins. The *Synechococcus* PBS contain the following biliproteins: C-phycocyanin (C-PC; with absorption maximum A_{620} and fluorescence maximum F_{647}) and allophycocyanin (APC; A_{650}, F_{660}) (Glazer 1989, Mac Coll and Guard-Friar 1989, Hirschberg and Chamovitz 1994). Thus *Synechococcus* PBS contains only two types of biliproteins. The structure of PBS for this organism is known (Glazer 1989). One part of the cyanobacteria was cultured in conditions in which the biliprotein synthesis was not efficient, the second part contained normal amounts of biliproteins. The aim of this work was to establish the influence of the amount of present biliproteins on the orientation of other pigments and pigment-protein complexes.

The investigated cyanobacterium contains also Cars and various forms of Chl *a*. Cars of cyanobacteria are similar to Cars which occur in higher plants but they exhibit significant differences from the Cars present in other microorganisms (Hirschberg and Chamovitz 1994). The most common Cars in cyanobacteria are β-carotene and zeaxanthin but some unique xanthophylls are also present. In our experiments Cars absorbed in a wide region of 425-510 nm (Hirschberg and Chamovitz 1994). The Cars have two main functions: firstly they serve as light-harvesting pigments in photosynthesis, secondly they protect other pigments against damage by photooxidation by quenching the triplet states of Chls and exchanging the obtained energy predominantly into heat. All Cars have a low yield of fluorescence that means they exhibit high yield of thermal deactivation (TD), when the singlet excitation is not transferred to the other pigments. This yield depends on the type of the Cars and on their surrounding. As follows from absorption spectra, the ratio of Car to Chl concentrations was higher in the strain with lower biliprotein content than in the strain with normal biliprotein amount.

Chl *a* occurs in organisms in various "forms" dependent on its state of aggregation and interactions with other molecules (Papageorgiou 1975). Some forms absorbing in the 662-692 nm region belong to both photosystems (PS1 and PS2), whereas some of them are only characteristic of one photosystem. The long wavelength forms with absorption at 695 and 705 nm (fluorescence at 715 and 735 nm, respectively) are characteristic of only PS1. The emission of Chls from PS2 is located at about 685 nm (Papageorgiou 1975). The exact positions of the absorption and fluorescence maxima for the same pigment are different in various solvents and in the organisms (Rabinowich and Govindjee 1969, Papagegorgiou 1975, Mac Coll and Guard-Friar 1987).

We compared the influence of chromophore orientation in the two strains of *Synechococcus* containing various amounts of giant phycobilisome structures on their thermal deactivation efficiency. Polarized PAS and excitation energy transfer between chromophores have previously been studied in isolated biliproteins (Romanowski *et al.* 1987), whole phycobilisomes (Frąckowiak *et al.* 1986b, 1987a, 1991, Gallant *et al.* 1991), and in model systems containing biliproteins and Chls (Frąckowiak *et al.* 1979). The previous results obtained with simpler systems help in the interpretation of PAS of whole oriented organisms. The orientation of the isolated pigment transition moments (TMs) with respect to the molecular frame or with respect to the close surrounding can be established by polarized absorption and fluorescence spectroscopy (Michl and Thulstrup 1986). The efficiency of excitation energy transfer between various groups of biological chromophores depends on their mutual orientation (Hsu 1988). But it is complicated, because of secondary perturbing effects, to establish the location of pigments in complex systems such as whole organisms or their large fragments (Breton and Vermeglio 1982, Tapie *et al.* 1986, Hsu 1988, Frąckowiak *et al.* 1989, Martyński *et al.* 1998). In the present paper we propose to use polarized PAS for establishing mutual angles between the TMs. The PAS are much less sensitive to radiation scattering than absorption and usually larger differences between the signals from the different pigment forms are observed than in the corresponding absorption spectra.

Materials and methods

One strain of *Synechococcus* (Kütz) (SL strain) was from collection of the Institute of Soil Science and Photosynthesis, Pushchino (Russia). It was grown in light conditions in which much lower amount of phycobiliproteins than reported in literature (Mac Coll and Guard-Friar 1987, Glazer 1989) was synthesised. The reason of this fact, supported by absorption spectra analysis (not shown), is not clear. Usually, a low ratio of biliproteins to Chl is observed in organisms cultivated at high irradiance (Wyman and Fay 1989) whereas the irradiance we used (3 W m^{-2}) was rather low. May be that the radiation containing a high share of blue component was responsible for cyanobacteria degeneration. After some weeks of culturing under "day light" lamps, the biliprotein content was again growing (not shown). The second strain of cyanobacterium was from the NAIR (Tsukuba) collection. This strain (SH strain) contained a normal amount of biliproteins. The longer axes of the *Synechococcus* cells vary between 3 and 11 μm (Planner *et al.* 2000).

The polyvinyl alcohol (PVA) films were produced and stretched as previously described (Frąckowiak *et al.*

1987b, Martyński *et al.* 1998). The film elongated from initial length l_0 to the final length l in a way that $(l - l_o)/l_o \times 100\ \% = 200\ \%$ is denoted as 200 % film, whereas the unstretched film as 0 %.

Absorption spectra were collected using a *Shimadzu UV-V-1601* spectrophotometer whilst the fluorescence spectra were obtained using *Hitachi F4500*. Both arrangements were equipped with polarizers. For the stretched samples the following four polarized components of fluorescence were measured: VVV, VVH, VHV, and VHH (V - vertical, H - horizontal). The first and last letters refer to the direction of the electric vector of the excitation and the emission radiation, respectively, and the middle letter to the orientation of the axis of the PVA film. In unstretched samples, two components (VOV and VOH) were measured (the middle 0 means that the film was unstretched).

The PAS were measured using a one-beam photoacoustic spectrometer (Frąckowiak *et al.* 1986c, 1992a) that contains an *MTEC 300* photoacoustic cell (produced by *MTEC Photoacoustic*, Ames, Iowa, USA) and a spherical light reflecting mirror or with a home made cell similar to that constructed at the Université du Québec, Trois-Riviéres (Frąckowiak *et al.* 1986b, 1993). The *MTEC* cell needs smaller amount of material than the home made one but both cells are similarly sensitive because in the *MTEC* cell the sample is located in a helium atmosphere which has better thermo-elastic properties than air. For most measurements of polarized PAS the home made cell was used because it easier enabled to precisely arrange the direction of electric vector of radiation and stretched sample axes.

The shape and accuracy of PAS spectra of the same sample measured using both types of photoacoustic cells were compared. For the same sample the amplitudes of PAS for home made cell were usually slightly higher. The amplitudes of PAS and absorbance of various films with organisms were different because of slightly different amount of material embedded. In such situation all PAS and absorption spectra were normalized and only the shapes of spectra can be used for discussion. From the shapes of absorption and PAS spectra the ratios of thermal deactivation of various pigments in both cyanobacteria strains can be evaluated.

Both the measured and reference carbon black PAS spectra were taken using in acting beam polarizer. For the stretched samples the electric vector of the acting radiation was first aligned along the film stretching axis and then rotated at 30° intervals to 180°. For the unstretched samples the initial direction of the radiation polarization was arbitrary, but these samples also exhibited some PAS spectra anisotropy. The polarization angle was varied at 30° intervals between 0° and 180° as for stretched samples. The dependence of PAS signal amplitude on frequency of radiation modulation $(\nu)^{-1/2}$, measured from 5 to 40 Hz, was for the investigated samples linear; this shows that the thermal properties of samples on various levels below sample surface were similar.

Results

***Synechoccocus* with low content of phycobili-proteins (SL)**: Fig. 1(*left*) shows the absorption spectra of whole cyanobacteria cells with low biliprotein content (SL) in unstretched and stretched films. The shape for the unstretched SL sample was similar to that of the stretched sample. Microscopic observation of stretched samples confirmed that cyanobacteria in such films were uni-axially oriented. All spectra presented in Fig. 1(*left*) show that the ratio of biliprotein to Chl concentrations in investigated samples was much lower than usual (Mac Coll and Guard-Friar 1987, Glazer 1989). This non-typical, low content of biliproteins is a proof that such low concentration of pigments can be responsible for measurable PAS signal and give the opportunity to compare spectral properties of biliprotein-deficient and normal strains of the same cyanobacterium.

In the SL samples, stretching caused an increase of absorption anisotropy in the 670 nm region (Table 1). At a uniaxial orientation of pigment transition moments (TMs) inside cells and a high degree of cell orientation one would expect a much higher absorption anisotropy.

Fig. 1(*right*) shows the polarized fluorescence spectra of SL samples. The PAS of unstretched SL samples

Table 1. Anisotropy of absorption and fluorescence coefficients. Coefficients of emission anisotropy: $r_a = (VVV - VVH)/(VVV + 2\ VVH)$ or $r_a = (VOV - VOH)/(VOV + 2\ VOH)$; $r_c = (VVV - VHV)/(VVV + 2\ VHV)$; $r_d = (VVH - VHH)/(VVH + 2\ VHH)$. Orientation factor $S = (A_{II} - A_{\perp})/(A_{II} - 2\ A_{\perp})$, where A_{II} and $A_{\perp}$ are absorption polarized components.

Sample	λ [nm]	r_a	r_c	r_d	S 436 nm	S 671 nm
200 %	684	0.29	-0.24	-0.33	0.009	0.011
Cells	737	0.27	-0.24	-0.31		

(Fig. 2) show the Car contributions in the region 450-500 nm, in which Car contributions are not superimposed with the Soret band of Chl. The contents of Cars in the SL sample were higher than in the SH sample as follows from the comparison of absorption spectra (Figs. 1, *left* and 4). The ratio of TD of Cars/Chl *a* for SL cyano-bacteria in the stretched film, calculated on the basis of Figs. 1(*right*) and 2, is about 0.8 (accuracy of this evaluation is about 0.2). This suggests a rather low efficiency of excitation ET from Cars to Chl. The red

band of Chl *a*, the C-PC band (629 nm), and the APC (650-680 nm) bands are evident. The ratio of TD of C-PC/Chl *a* in stretched film was about 1.5±0.1, in unstretched film about 1.7±0.1. Thus in both cases a large part of excitation of C-PC was converted into heat. The Chl *a* red band (670-680 nm) was partially hidden in the APC region, therefore the conclusions concerning Chl TD are not univocal.

The dependence of PAS intensities on radiation polarization for SL samples is shown in Fig. 3. The orientations of the Car and biliprotein chromophores were dependent on sample stretching. In the stretched SL cells sample the angle between the TMs of Car and APC was about 30°. Of course, only the average angles of the orientation of the TMs for all pigments absorbing in the given spectral range were evaluated.

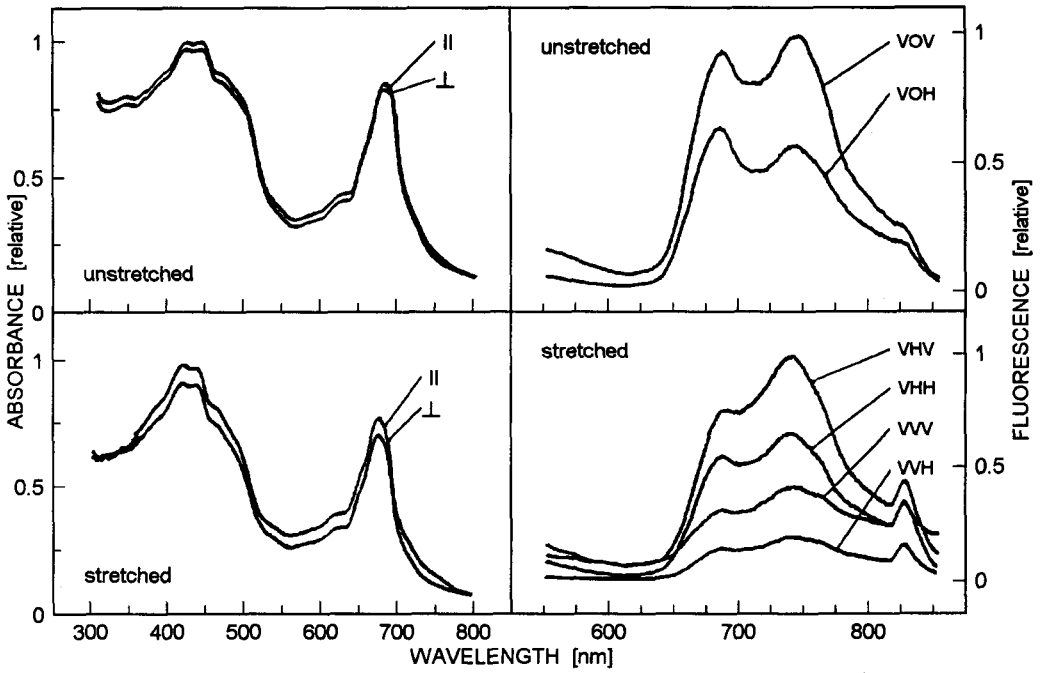

Fig. 1. Polarized absorption (*left*) and fluorescence (*right*) spectra of *Synechococcus* with low biliprotein content (SL) cells in unstretched and stretched films. Polarized components' notation is given in text.

***Synechococcus* with normal amount of phycobiliproteins (SH)**: Fig. 4 shows absorption of SH cells embedded in fluid medium (PVA solution in water), in unstretched and stretched films. The sample in fluid medium contained a high amount of biliproteins. The ratio of C-PC (at 624 nm) and Chl *a* (at 670 nm) absorption maxima in cells embedded in unstretched PVA film was lower than that in fluid PVA. Film stretching caused a further decrease of this ratio. Both polarized absorption components of the stretched sample were, similarly as in the SL cells, very close one to other. The SH cells were, as follows from microscopic observations, uniaxially ordered in a high degree.

Fluorescence spectra of both SL and SH samples were similar (not shown). Anisotropy of fluorescence was also, as for SL, much higher than anisotropy of absorption.

Fig. 5 presents the PAS measured in non-polarized radiation for unstretched PVA films for SH cells. The ratio of thermal deactivation yields of C-PC (at 624 nm) to that in a red Chl band (at 670 nm) in unstretched film for whole SH cells was 0.9±0.1, whereas for SH cell fragments (spectra not shown) it was 1.2±0.1. In stretched films this ratio was 1.4, hence only slightly lower than that for the stretched SL sample (1.5) and much higher than in SH cells in unstretched film. It suggests that for C-PC the TD yield was increasing as a result of phycobiliprotein denaturation due to cell fragmentation or film stretching. The denaturation can cause the change in efficiency of excitation energy transfer to Chl pigments and as a result the increase in TD of C-PC.

The ratio of Car TD to that of Chl measured for stretched sample with SH cyanobacteria was 0.4±0.1 showing that ET from Cars to Chl was more efficient for SH than SL sample in which this ratio was 0.8±0.1.

Fig. 6 shows the set of polarized PAS of SH cells: the shapes of PAS of the SH samples depended on the direction of radiation polarization. Results in the short wavelengths region (400-420 nm) were perturbed by the contributions of anisotropic PVA film absorbing in this region and giving different signal at different radiation polarization. Fig. 6 also shows that various Cars were differently oriented with respect to the PVA stretching axis.

Fig. 7 presents the dependence of PAS amplitude of

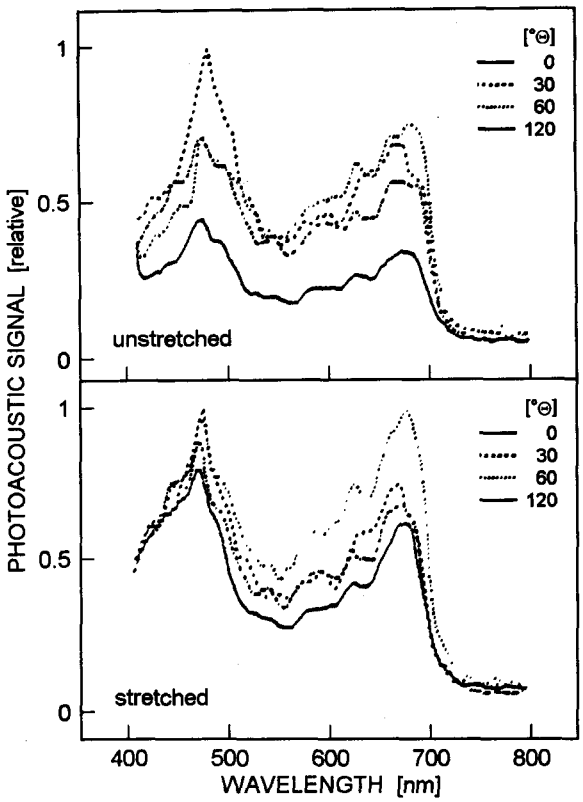

Fig. 2. Polarized photoacoustic spectra of *Synechococcus* (SL) samples: whole cells in unstretched and in the stretched films taken using *MTEC* photoacoustic cell. Angles Θ between film axis and electric vector of radiation are given on the graphs.

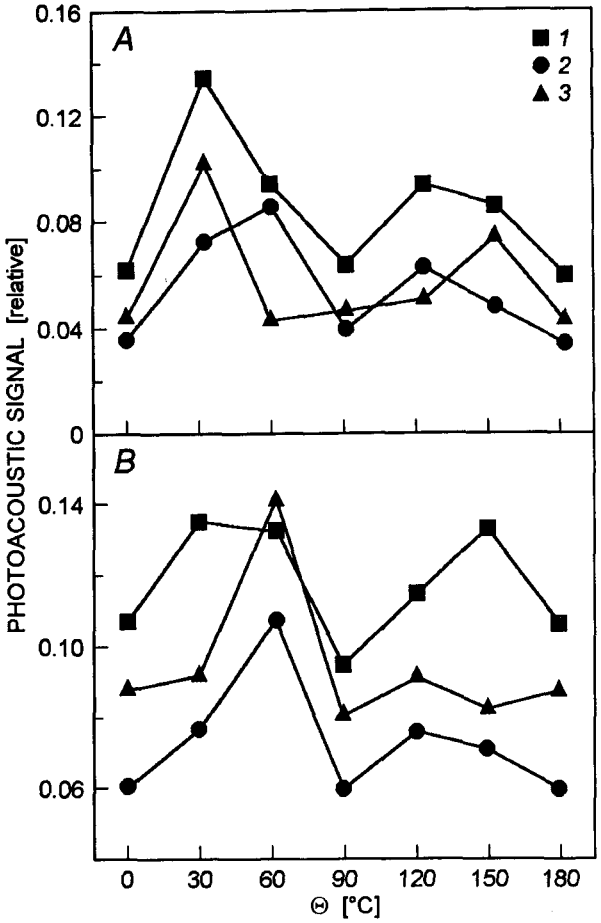

Fig. 3. Amplitudes of PAS signal (taken from spectra of SL cells in Fig. 2) at various polarizations of acting radiation for different spectral regions characteristic for absorption of the following pigments: 475 nm - carotenoids; 620 nm - C-Pc; 670 nm - Chl *a*. Θ-angle between film axis and electric vector of light. *A*: unstretched film, *B*: stretched film.

SH cells (from Fig. 6) at given wavelength of acting radiation *versus* the angle Θ between film axis and electric vector of polarized radiation. The wavelengths of acting radiation were chosen in regions of absorption of various pigments. These wavelength values and description of the pigments predominantly absorbing in given spectral regions are gathered in the legend of Fig. 7.

For SH cells the most frequently observed angle Θ of TMs of Chl *a* and Cars orientation in the region of 430 nm was not far from 0°, but the second maximum was located at about 90°. The red band of Chl *a* (670 nm) exhibited maximal PAS amplitude in the region of 0-30° and minimum at 90°. The TMs of Chl red and Soret bands were mutually almost perpendicular, therefore the orientation in the 430 nm region was probably due to high extent of Car contributions whereas the maximum at 90° could belong to TMs of the Soret band of Chl *a*. The TMs of C-PC macromolecules were oriented at low angles with respect to the PVA axis, but because this was also the orientation of long axis of cyanobacteria they were directed also along axes of the cyanobacteria. Cars absorbing in the 476 nm region were for the whole cells oriented in a low degree. Average angle of orientation of PS1 Chl *a* (at about 700 nm) was rather similar to that of Chl *a* absorbing at 670 nm. Because of the low PAS amplitude in the 700 nm region, the accuracy of this evaluation was low.

Discussion

Anisotropy of fluorescence of emitting chromophores is higher than absorption anisotropy. This is apparent from a comparison of the polarized absorption and fluorescence spectra (Fig. 1) and absorption and emission anisotropy coefficients (Table 1). It shows that various forms of pigment with rather similar absorption and

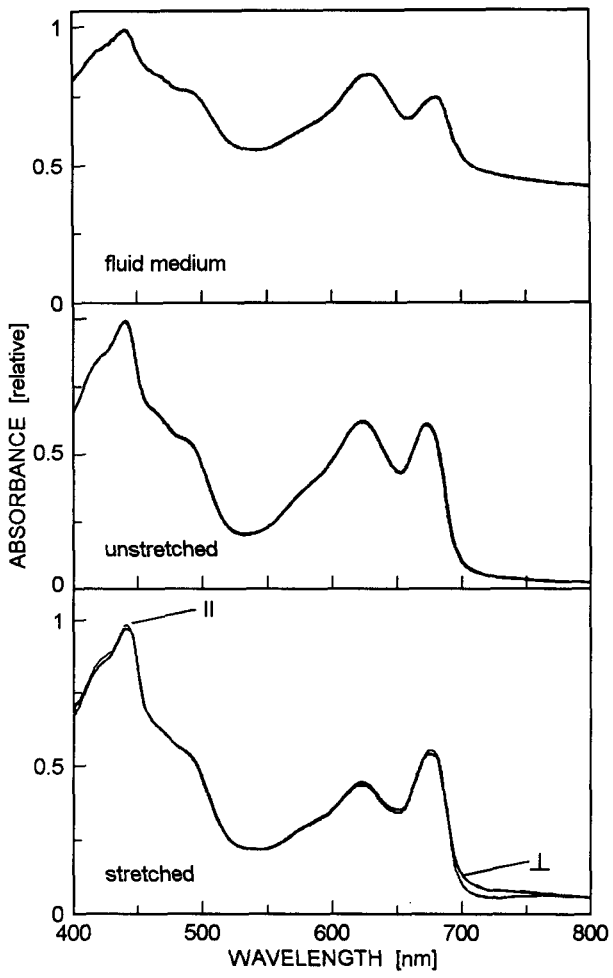

Fig. 4. Absorption spectra of SH *Synechococcus* whole cells: in fluid medium PVA +water; in unstretched (0 %) PVA film, and the polarized absorption of cells in stretched (200 %) PVA film.

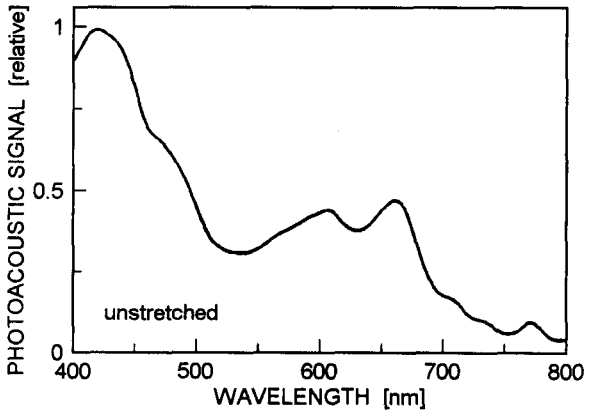

Fig. 5. PAS spectra of SH cells in unstretched film.

different yields of fluorescence are differently oriented. For isolated PBS, low values of anisotropy of absorption spectra and PAS were observed (Frąckowiak *et al.* 1987a). The dissociation of the PBS causes an increase in absorption and PAS anisotropy (Frąckowiak *et al.* 1986a,b). The rather high PAS anisotropy of biliproteins observed in the investigated cyanobacteria may suggest a partial denaturation of giant PBS complexes in both SL and SH samples. Nevertheless, Skibiński *et al.* (1990)

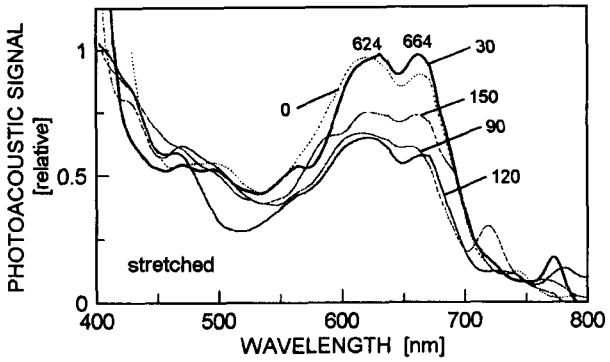

Fig. 6. Polarized PAS spectra of SH cells in stretched PVA. Angles Θ between film axis and electric vector of radiation are marked.

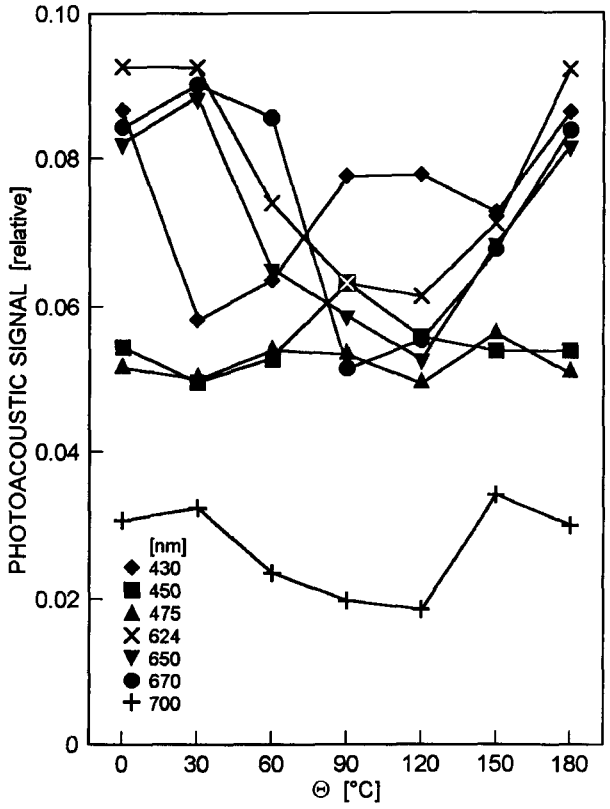

Fig. 7. Amplitudes of PAS signal (taken from PAS spectra of SH cells from Fig. 7) *versus* polarization of acting radiation (various Θ) for the following wavelengths in spectral regions characteristic for given pigment absorption: 430 nm - carotenoids +Chl *a*; 450 and 475 nm - carotenoids; 624 nm - C-PC; 650 nm - APC; 670 nm - Chl *a* from PS1 and PS2; 700 nm - Chl *a* from PS1.

and Frąckowiak *et al.* (1992) observed an anisotropy of absorption for whole cells and cell fragments of the same cyanobacterium that was even higher than in the present work. The shapes of PAS spectra for SH (Figs. 5 and 6) and SL (Fig. 2) were different. The contributions to PAS signal from low amount of C-PC and APC in SL samples were high, showing that in such biliprotein deficient organisms the excitation energy transfer from biliproteins to Chl molecules was not efficient. But even in SH cells the TD yield of C-PC was high because the ratios of TD values of C-PC/Chl *a* for SH cells in unstretched film were 1.2. Such efficient TD of biliproteins suggests their partial denaturation in PVA film. It shows that cell orientation in PVA film causes a change in the ratio of biliprotein and Chl contributions to TD. It is possible because in stretched films the cells can be deformed. The reorientation of TMs influences the yield of excitation energy transfer and in result also of TD. Comparison of the biliprotein orientation in SL and SH samples suggests that in the SL sample C-PC exhibits lower alignment along the film axis than in the SH sample. Also the directions of the TMs of Cars and of the red band of Chl *a* are more parallel to film axis in SH than observed for SL samples. It shows that the low content of biliproteins influences the orientation of other pigments and pigment-protein complexes.

Frąckowiak *et al.* (1992) found that the change in biliprotein content due to chromatic photoadaptation of cyanobacteria influences the excitation ET from Cars to the fluorescent form of Chl (Chl 740). It may suggest that the ET from Cars to Chl can occur *via* biliproteins. Our results showing that the ratio of Car TD to Chl TD was higher in SL than SH samples may support such possibility. But the interpretation of observed effects is not so univocal, because chromatic adaptation influences also the ratio of Chl molecules belonging to PS1 and PS2 (Frąckowiak *et al.* 1992). Our results show that the change in biliprotein content has a strong influence on the excitation ET between various pigments.

The yield of TD of Cars in SL cyanobacteria was much higher than in the SH sample. The TD of Cars engaged in ET of excitation to reaction centre has to be lower than that engaged in the quenching of Chl triplets.

Conclusions: (*1*) It is possible to evaluate the angles between the transition moments of various chromophores based on the polarized PAS.
(*2*) These angles vary as a result of stretching of the polymer film with cyanobacteria.
(*3*) The methods of polarized fluorescence and polarized PAS are more sensitive for investigating the location of TMs of various pigment forms than polarized absorption spectra because there is a larger difference in the yields of fluorescence as well as thermal deactivation than in the absorption of these forms. For all investigated samples with isolated PBS (Frąckowiak *et al.* 1991) and for whole cells a much lower anisotropy of absorption than emission was observed. This is in good agreement with the present results. It shows that not all absorbing pigment molecules are responsible for emission.
(*4*) It is not easy to compare the present results with known PBS structures, because these results only show average location of the pigments with high yield of thermal deactivation. It is the difference between the directions of Car and biliprotein TMs in SL and SH cells.
(*5*) The high PAS maxima observed in the biliprotein absorption region for SL samples cannot be due to the small Chl maxima, such as Q_x or Q_y (0.1); because it follows from the thermal deactivation in the Soret and red bands, the contributions of Chl to TD are much lower that the contributions from Cars and biliproteins.
(*6*) The ratio of TD of Cars to that of Chl is higher for the SL than SH sample. The ratio of TD of C-PC/Chl is higher for the SL than SH sample. Thus ET between various pigments is different in the investigated strains.

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