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PHOTOACOUSTIC SPECTRA OF ORIENTED SYSTEMS

Photoacoustic spectroscopy permits for a direct measurement of thermal deactivation of oriented excited molecules. This method has been applied for studies of a mixture of phycoerithrin, chlorophyllin and phycocyanin and of chlorophyll a and chlorophyll b. Analysis of the obtained spectra suggests that a part of energy absorbed by chlorophyll a at the Soret band migrates to chlorophyll b. The migration probably takes place in mixed aggregates of these pigments, apparently containing LC.

Most of accessory pigments of photosynthetic organisms is in some extent oriented. These pigments can be excited directly, by light absorption or by excitation energy transfer from other molecules absorbing in shorter wavelengths region. Every one pigment molecules can emit fluorescence, transfer its excitation to other pigment or dissipate it on heat.

In organisms in result of efficient energy transfer only the fluorescence of pigment absorbing at long wavelengths region is usually observed. Three processes: emission of fluorescence, thermal deactivation and energy transfer compete with each other. The efficiency of energy transfer depends strongly on mutual orientation of donor and acceptor transition moments. This effect is specially important in oriented systems. From analysis of fluorescence spectra [1] it is not easy to establish the fate of excitation energy in a chain of excitation donors and acceptor occurring in photosynthetic organisms. Even in model system containing only part of pigments such analysis is not univocal [1].

The photoacoustic spectroscopy (PAS) [2] provides the opportunity of direct measurement of thermal deactivation of excitation in pigments. Photoacoustic spectra were measured on single beam photoacoustic spectrometer constructed in Centre de Recherche en Photobiophysique in Trois Rivières [3]. As anisotropic matrix simulating the anisotropy of lamellar system stretched polyvinyl alcohol (PVA) films or nematic liquid crystals were used. The mixture of phycoerythrin and chlorophyllin [4], phycoerythrin and phycocyanin [5], and chlorophyll a and chlorophyll b [6] were investigated.

Phycobiliproteins occur in blue green and red algae. They are transferring their excitation energy to chlorophyll predominantly in a sequence: phycoerythrin (PE) → phycocyanin (PC) → allophycocyanin (AC) → chlorophyll, but also some branching of these scheme Fig.1 is not excluded, because of strong overlapping of bands. System is even more complex, than presented in Fig. 1, because every one of biliproteins possesses more than one type of chromophores. Figure 2 presents the scheme of investigated system in PVA [4, 5].

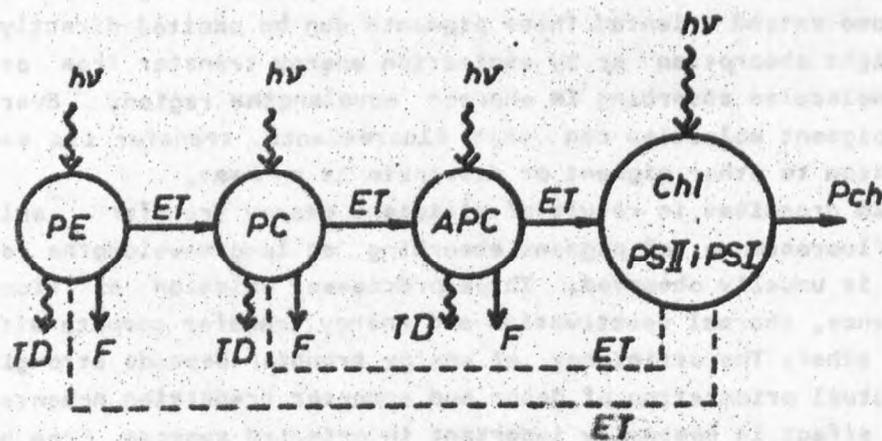


Fig. 1. Scheme of excitation energy migration in blue-green algae
PE - Phycoerythrin, PC - Phycocyanin, Chl - chlorophyll, APC - allophycocyanin, PSI - photosystem I, PCh - photochemical reaction,
ET - energy transfer, TD - thermal deactivation

Schemat migracji energii wzbudzenia w błękitnozielonych algach
PE - fikoerytryna, PC - fikocyjanina, Chl - chlorofil, APC - al-
lofikocyjanina, PSI - fotosystem I, PCh - reakcja fotochemiczna,
ET - przeniesienie energii, TD - deaktywacja termiczna

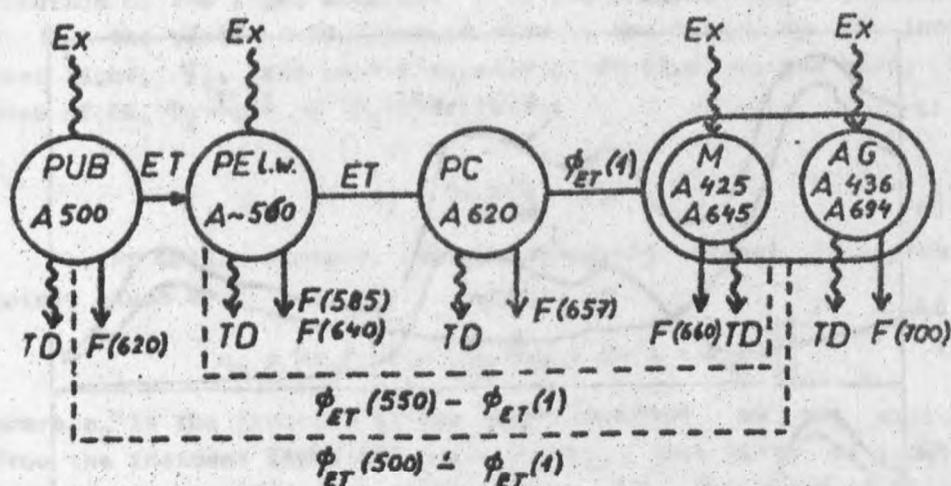


Fig. 2. Scheme of excitation energy migration in investigated model system

Schemat migracji energii wzbudzenia w badanym systemie modelowym

Chlorophyllin (chlin) is a water-soluble chlorophyll derivative which can be uniformly distributed together with biliproteins in the same PVA matrix.

Figure 3a shows the PAS of PE (curve 1), Chlin (curve 2) and their mixture (curve 3) in PVA film. In Fig. 3b the sum of PAS of PE and chlin measured in separate films (curve 4) is compared with PAS of pigment mixture (curve 3).

The difference between spectrum 3 and 4 (Fig. 3b) in a region of chlin absorption is small and can be explained by the changes in chlin aggregation in presence of PE. Similar changes have also been observed in absorption spectra. The increase of photoacoustic signal in the region of PE absorption is however very high. It is known that PE fluorescence yield, η_1 , is rather high.

For R-PE in buffer solution, η_1 is 0.56 [7]. Fluorescence quantum yield for chlorophyll in ether solution is 0.22, η_2 for Chlin is lower than that of chlorophyll. From the comparison of the fluorescence intensities of PE and Chlin in PVA excited in the region of similar absorption, the ratio $\eta_2/\eta_1 =$

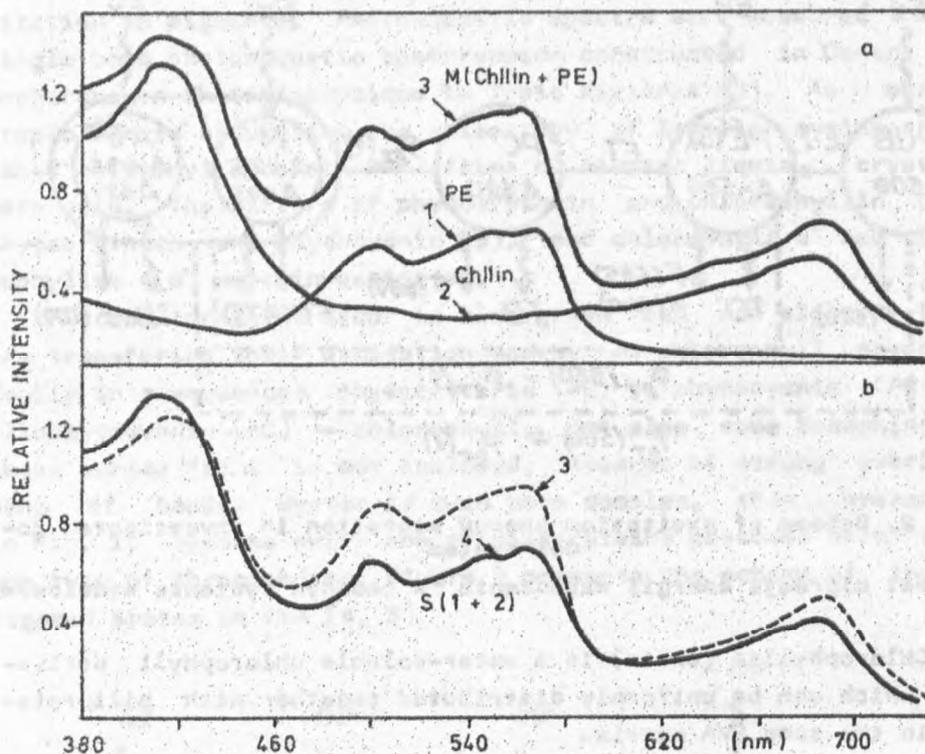


Fig. 3. Photoacoustic spectra of pigments in PVA

a) 1 - PE $c_1 = 3.2 \cdot 10^{-6}$ M, 2 - Chllin $c_2 = 15.7 \cdot 10^{-5}$ M, 3 - mixture of PE c_1 and Chllin c_2 in the same film; b) 4 - Sum of curves 1 and 2 from Fig. 3a compared to spectrum 3

Widma fotoakustyczne barwników w PVA

a) 1 - PE $c_1 = 3,2 \cdot 10^{-6}$ mole, 2 - chllin $c_2 = 15,7 \cdot 10^{-5}$ mole, 3 - mieszanina PE c_1 i chllin c_2 w tym samym filmie; b) 4 - suma krzywych 1 i 2 z rys. 3a porównane do widma 3

$\eta = 0.21$ is found. Therefore the quantum yield of Chllin fluorescence (η_2) in PVA is about 0.12 supposing that η_1 in PVA is similar to that in buffer.

Photoacoustic signal q_1 of PE alone can be expressed approximately by the following formula:

$$q_1 = S a_1 P \left(1 - \Phi_1 + \Phi_1 \frac{\gamma - \bar{\gamma}_f}{\gamma} \right) \quad (1)$$

where S is a constant depending on apparatus sensitivity; a_1 , the fraction of the light absorbed from the incident light intensity P ; Φ_1 , the yield of PE fluorescence; γ , the frequency of incident light, $\bar{\gamma}_f$, the mean frequency of PE fluorescence band. In case of PE, $\Phi_1 \frac{\gamma - \bar{\gamma}_f}{\gamma} \ll \Phi_1$ therefore:

$$q_1 \approx Sa_1 P(1 - \Phi_1) \quad (2)$$

For PE Chllin mixture, the photoacoustic signal is approximately given by:

$$q_2 = Sa_2 P \left[1 - \left\{ \Phi_2 \Phi_{ET} + \Phi_1(1 - \Phi_{ET}) \right\} \right] \quad (3)$$

where a_2 is the fraction of the light absorbed by the mixture from the incident light intensity P ; Φ_{ET} , the yield of excitation energy transfer from PE to chllin; Φ_2 , the yield of Chllin fluorescence.

At $\Phi_2 < \Phi_1$:

$$q_2 = Sa_2 P(1 - \Phi_1 + \Phi_1 \Phi_{ET}) \quad (3')$$

and $q_1 > q_2$ is expected as it is found (Fig. 1).

From equations (2) and (3):

$$\frac{q_1 a_2}{q_2 a_1} = \frac{1 - \Phi_1}{1 - \Phi_1 + \Phi_1 \Phi_{ET} - \Phi_2 \Phi_{ET}} \quad (4)$$

is obtained.

From equation (4), using experimental values and previously evaluated fluorescence yields, the yield of excitation energy transfer Φ_{ET} for two absorption bands $\Phi_{ET}(500) = 0.14$ and $\Phi(560) = 0.33$ is obtained. Figure 4 shows absorption and photoacoustic spectra of PE in isotropic and stretched PVA. It is known from linear dichroism spectra B that 500 nm absorbing PE chromophores have tendency to be oriented parallel to the direction of film stretching, whereas long wavelength absorbing chromophores are oriented rather perpendicular to this direction. From Fig. 4, it is seen, that after normalization of all spectra at 500 nm, the 560 nm absorption is much higher than PSA (curves 1 and 2), i.e. PAS to absorption ratio is small.

This ratio is strongly increasing as a result of film stret-

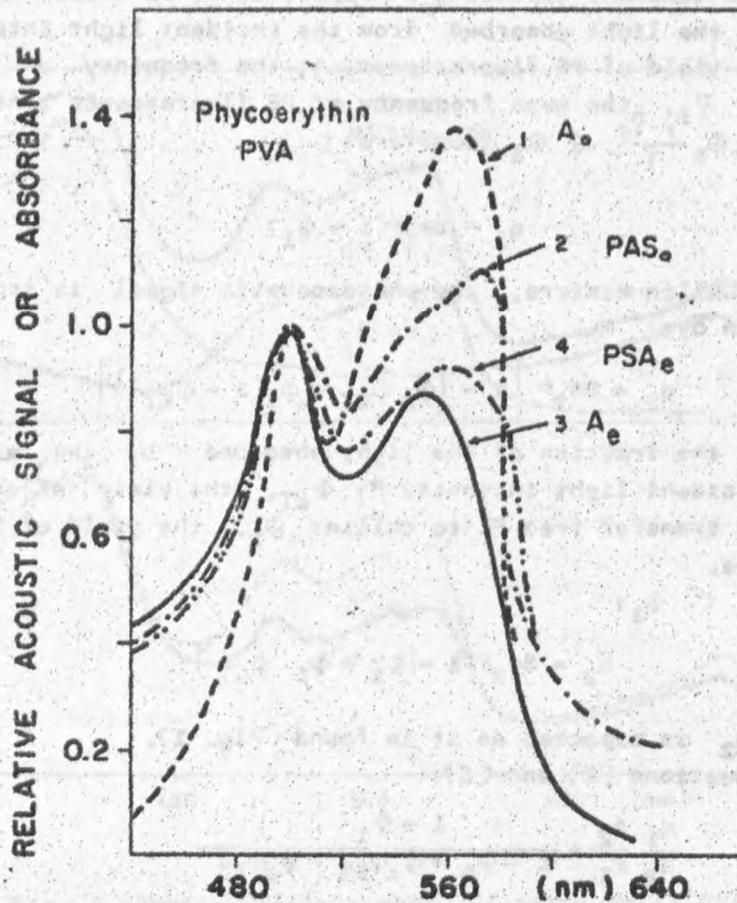


Fig. 4. Absorption (curve 1 and 2) and PAS (2 and 4) of PE in isotropic (1 and 2) and deformed (3 and 4) PVA films
 Absorpcja (krzywa 1 i 2) i PAS (2 i 4) fikoerytryny w izotropowych (1 i 2) i zniekształconych (3 i 4) PVA filmach

ching (curves 3 and 4). It means that thermal deactivation in chromophores differently oriented with respect to the anisotropic matrix is different. There are two possible explanations of this effect. The film stretching causes not only the chromophores reorientation but also some deformations of protein part of PE. Therefore, the chromophores surroundings are modified and pigment-protein interaction can be changed. The PE reorientation can also influence the interaction between chromophores and oriented polymer molecules.

The result presented show that two types (absorbing at 500 nm and at 560 nm) PE chromophores are to some extent energetically separated - because they have different thermal deactivations of excitation. It is in agreement with the previous observations on their different sensitivities on fluorescence quenchers [7] and efficiencies of excitation energy transfer to other pigments [9]. ET from these two types of chromophores is also changed as a result of polymer deformation [1].

This example [4] shows, that from photoacoustic spectra yield of excitation energy transfer between chromophores of strongly different yields of fluorescence can be obtained. Similar method was independently proposed by Schneider and C a u f a l [10].

Photoacoustic spectra of PE and PC in PVA were also measured [5]. In the film containing the mixture of both biliproteins, the photoacoustic spectra suggest the formation of mixed aggregates for which the thermal deactivation of excitation has been found to be more efficient compared to that for PE and PC alone.

The presence of such aggregates was supposed previously [11] in order to explain the yield of energy transfer between these pigments. Fluorescence yield of PC in PVA was found to be equal 0.45, it means lower, then in solution (0.56).

Figure 5 shows normalized at 670 nm measured and calculated photoacoustic spectra of chl a, chl b and their mixture dissolved in nematic liquid crystal (MBBA + EBBA). Calculations were done supposing that in mixture excitation energy is not transferred between chl a and chl b molecules. In calculation it was taken into account that contribution to PAS of mixture of every one pigment is proportional to

$$\epsilon c(1 - \Phi)$$

where:

ϵ - molar extinction coefficient;

c - molar concentration of pigment in mixture;

Φ - yield of fluorescence of pigment calculated from measured lifetime of fluorescence.

As it follows from Fig. 4 measured PAS exceed calculated in a region of predominant chl b absorption (470 nm and 660 nm). Chl b

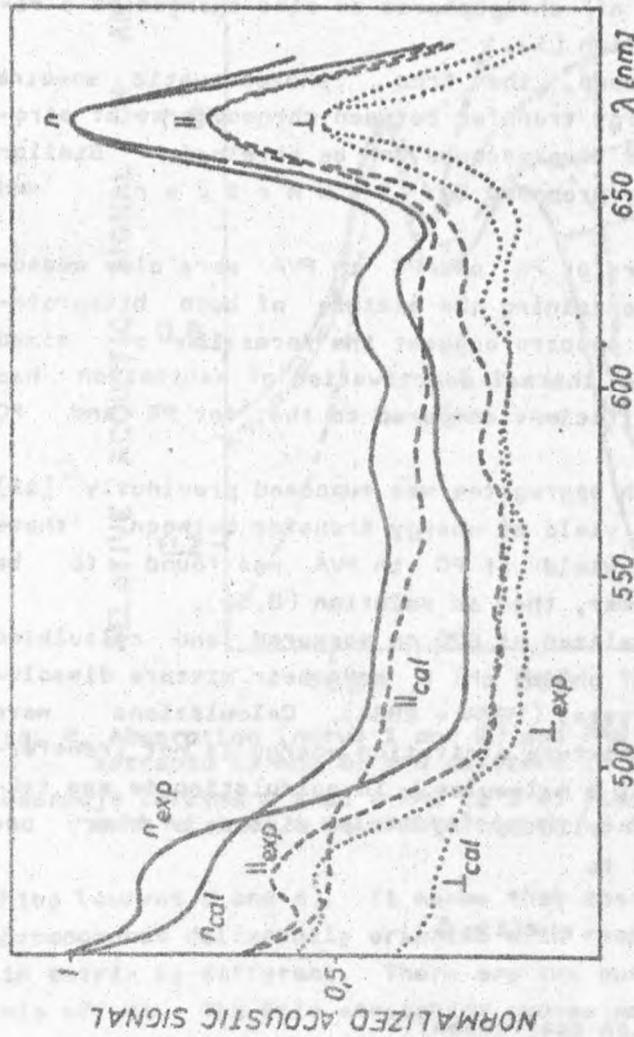


Fig. 5. Experimental (exp) and calculated (cal), explanation in text, photoacoustic spectra of chl a + chl b mixture

n - natural light. || and ⊥ parallel and perpendicular components of light. Every pair of exp and cal spectra are normalized at 670 nm

Eksperymentalne (exp) i wyliczone (cal), objaśnienie w tekście, widma fotoakustyczne mieszaniny chl a + chl b

n - światło naturalne. || i ⊥ równoległe i prostopadłe składowe światła. Każda para exp i cal widma była normalizowana do 670 nm

gives higher contribution to PAS, than chl a, because of its lower yield of fluorescence. Both spectra are normalized at red maximum of chl a PAS and absorption spectra. Results obtained suggest efficient excitation energy transfer from chl a to chl b ("back transfer"). From the difference between both curves at 470 nm divided by "calculated" value of signal at the same wavelength the yield of excitation energy transfer from chl a to chl b is obtained

$$\Phi_{\text{chl a} \rightarrow \text{chl b}} = 0.16$$

From the comparison of PAS obtained with illumination of sample with two polarized components of light it follows that this ET effect is more pronounced for perpendicular component. It is an evidence, that ET is differently efficient in differently oriented fractions of molecules.

Strong back transfer (from chl a to chl b) is unexpected result. But from fluorescence spectra of very high concentration of chl a and chl b in LC it follows that a new maximum appears located between chl a and chl b emission bands.

PAS spectra, as well as the analysis of fluorescence lifetimes of the same samples suggest that part of energy absorbed by chl a Soret band is migrating to chl b. From fluorescence spectra follows, that this migration occurs in mixture aggregate formed from both pigment probably with LC participation.

The usage of polarized light to generation of photoacoustic signal provides the opportunity to show, that these aggregates have to be differently located in anisotropic matrix, then separated pigments. It seems that the polarized PAS can be very useful in separate investigation of differently oriented groups of chromophores in vivo.

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