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Polarized absorption, fluorescence and photoacoustic spectra of phycobilisomes embedded in poly(vinyl alcohol) films

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Polarized absorption, fluorescence and photoacoustic spectra as well as fluorescence lifetimes of phycobilisomes from *Porphyridium cruentum* located in poly(vinyl alcohol) films were measured. Reorientations of various biliproteins in phycobilisomes was caused by stretching of the film. The phycobilisomes in various degrees of dissociation, and in some extent deuterated, were also investigated. It was found that allophycocyanin occurs in *Porphyridium cruentum* in various forms having different lifetimes of fluorescence and exhibiting various reorientations in stretched polymer. Also B-phycoerythrin and b-phycoerythrin exhibit different reorientation. Mutual orientation of biliproteins strongly influences their lifetime of fluorescence, thermal deactivation of excitation, and a process of excitation energy transfer between the various complexes constituting the phycobilisomes.

Introduction

Phycobilisomes are supermolecular pigment protein aggregates which serve as the light-harvesting antenna in red algae and cyanobacteria [1]. The composition and structure of phycobilisome enable very efficient excitation energy transfer to reaction centers [2]. For this reason we have been using a deformed polymer matrix, to estimate the influence of mutual orientation and interaction of the biliproteins constituting phycobilisomes on excitation energy transfer [3]. Biliproteins in native phycobilisomes are strongly bonded, therefore stretching of the polymer matrix reorients them only slightly, as suggested by small differences between polarized components of polarized absorption and photoacoustic spectra [3]. Strong deformation of samples causes the increase in phycoerythrin emission showing that samples become partially at least dissociated [3]. Zilinskas and Glick [4] have found that deuterium at exchangeable sites on biliproteins decreases the rate of phycobilisome dissociation, because hydrophobic interactions responsible at least partially for phycobiliproteins aggregation became stronger [5]. A similar effect can be expected for phycobilisomes introduced into poly(vinyl alcohol) dissolved in ^2H_2O. Therefore such samples would be more resistant to dissociation in a result of film stretching.

In this paper the spectral properties of native

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Abbreviations: A, absorption; LD, linear dichroism.

and partially dissociated phycobilisome from *Porphyridium cruentum* in deuterated and undeuterated polyvinyl alcohol film are reported. Phycobilisome from this red algae consists largely of B-phycoerythrin, *b*-phycoerythrin, some R-phycocyanin and small amount of allophycocyanin [6]. In the literature [2–9] it has been suggested that allophycocyanin occurs in more than one form. Using polarized spectroscopy it is possible to distinguish between pigments having strongly overlapped absorption spectra when they exhibit different orientations. The aim of this paper is to gather information about allophycocyanin forms occurring in *P. cruentum*.

Material and Methods

Phycobilisomes were isolated from *Porphyridium cruentum* according to Gantt et al. [10] and as modified by Erokhina et al. [11]. Various degrees of dissociation of phycobilisomes were obtained by different times of dialyses undertaken before introducing phycobilisome into poly(vinyl alcohol) solution. Methods of poly(vinyl alcohol) film preparation and stretching were described previously [12]. Samples prepared using poly(vinyl alcohol) dissolved in heavy water, are referred to as deuterated samples. Measurements of polarized absorption and emission spectra have been described previously [13]. The decay of fluorescence was investigated using phasemodulation fluorescence spectroscopy [14], which provides information not only about the mean emission lifetime, but also about the character of decay curves. In case of the multiexponential decay the ratio of the demodulation factor, m, to the cosine of the phaseshift angle, $\cos \phi$, is lower than 1 [15]. The measurement of the photoacoustic spectra of oriented samples, using the illumination with polarized light, was elaborated and described previously [16].

Results and Discussion

A typical set of polarized absorption spectra is shown in Fig. 1. Both polarized components of absorption, A, of unstretched sample coincide one other, but in case of stretched samples they are well resolved: the maximum of the parallel component, $A_{\parallel}$, is located at 580 nm, and that of the perpendicular, $A_{\perp}$, at 560 nm. It is known that in solution [17] the maximum at about 545 nm is contributed predominantly by B-phycoerythrin, whereas in long-wavelength region *b*-phycoerythrin is stronger contributing; therefore, it seems that in poly(vinyl alcohol) *b*-phycoerytrin has a tendency to be parallel to the direction of poly(vinyl alcohol) films stretching, whereas B-phycoerythrin is oriented rather perpendicularly to this direction. Hoarau et al. [18] have measured electric linear dichroism of phycobilisome and their fragments. They also have found different orientations of transition moments absorbing in 545 nm and 570 nm regions. From polarized absorption of native (Fig. 1) and of partially dissociated (not shown) phycobilisomes in poly(vinyl alcohol) it follows that in shortwavelength side of band linear dichroism (LD) has a negative value, whereas in longer-wavelengths region LD exhibits positive values. The resolution of polarized components is improved with the increase in the degree of

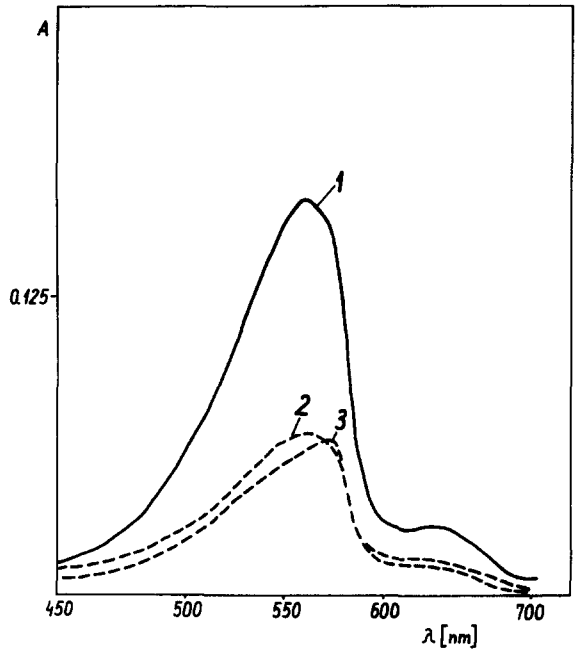

Fig. 1. Polarized absorption spectra of native phycobilisomes in deuterated polyvinyl alcohol films. (1) Unstretched film (0%), parallel (||) and perpendicular ($\perp$) component; film thickness, $d = 2.09 \cdot 10^{-2}$ cm; (2) and (3) Stretched film ($\Delta L/L) \times 100 = 300\%$) (2) $\perp$; (3) ||; $d = 0.71 \cdot 10^{-2}$ cm; $c \approx 4 \cdot 10^{-5}$ M.

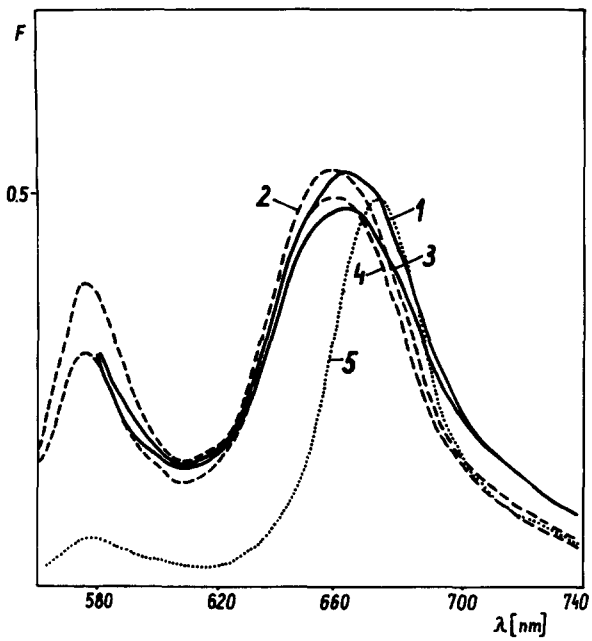

Fig. 2. Polarized fluorescence spectra of native phycobilisomes in deuterated poly(vinyl alcohol) films (in arbitrary units). (1) 300%, HHH; (2) 300%, HVV; (3) 300%, HHV; (4) 300%, HVH; (5) 0%, natural (n) light; H, horizontal; V, vertical; first and last letter: polarization of excitation and emission beams, between direction of sample axes. $\lambda_{exc} = 550$ nm.

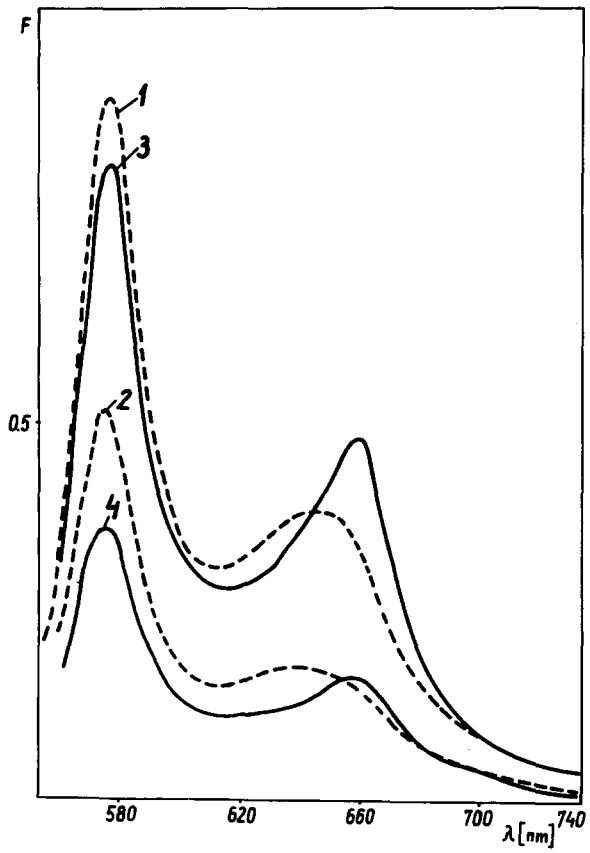

Fig. 3. Polarized fluorescence spectra of dissociated phycobilisomes in undeuterated poly(vinyl alcohol) films. (1) 300%, VVH; (2) 300%, VVV; (3) 0%, VVH; (4) 0%, VVV. $\lambda_{exc} = 550$ nm.

phycobilisome dissociation, and is a little higher in a case of films prepared from H_2O solution of poly(vinyl alcohol) than for ^2H_2O samples. Fig. 2 shows an example of fluorescence spectra of phycobilisomes in deuterated poly(vinyl alcohol). The long-wavelength maximum of emission of unstretched sample is located 675 nm. This is the emission of long-wavelength allophycocyanin-type emitter. The emission at 575 nm from phycoerythrin in this sample is very low. Thus excitation-energy transfer from phycoerythrin to the long-wavelength form of allophycocyanin is very efficient. Stretching of the film causes the broadening of the long-wave band related with the decrease in the yield of energy transfer between various allophycocyanin ($F_{660-680\,nm}$) forms and probably also between R-phycocyanin ($F_{640\,nm}$). Emission of phycoerythrin (575 nm) increases as a result of stretching (Fig. 3), but a considerable amount of energy transfer between phycoerythrin and phycocyanin still exists. In the allphycocyanin region two polarized components, located at 665 nm (perpendicular to the film stretching: $F_{\perp}$), and at 655 nm (parallel: $F_{\parallel}$) appear on film stretching. Allophycocyanin occurs in several forms [8,9] having different emission maxima and lifetimes of fluorescence [19,20]. Similarly, as in the case of polarized absorption, the polarized fluorescence spectra depend on sample dissociation and poly(vinyl alcohol) deuteration. As a result of film stretching, phycoerythrin and phycocyanin emission increases and allphycocyanin decreases. Upon greater dissociation (Fig. 4) emission of phycoerythrin increases and a new long-wavelength maximum with the strong parallel component appears, whereas the 580 nm maximum is polarized perpendicular to film axes. The short-wavelength maximum may be related to B-phycoerythrin and the longwavelength maximum with *b*-phycoery-

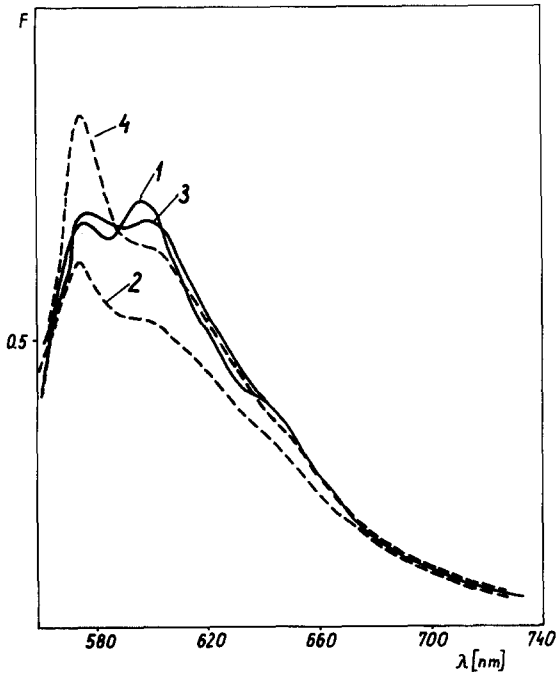

Fig. 4. Polarized fluorescence spectra of strongly dissociated phycobilisomes in undeuterated poly(vinyl alcohol) films. (1) 300%, HHH; (2) 300%, HVH; (3) 300%, HHV; (4) 300%, HVV. $\lambda_{exc} = 550$ nm.

thrin, but the new maximum could also be related with some phycoerythrin-phycocyanin complexes oriented parallel to the stretching direction [18].

The shape of fluorescence spectra of deuterated stretched samples (Fig. 2) shows that upon stretching energy transfer between phycoerythrin and phycocyanin as well as between phycocyanin and allophycocyanin is not interrupted, but only diminished. This fact, as well as the stepwise increase of LD with phycobilisome dissociation, as the influence of deuteration on LD suggests rather mutual reorientation of parts of phycobilisomes than phycobilisomes fragmentation. Under such a supposition long cylinders build from phycoerythrin and phycocyanin hexamers have to be reoriented parallel to the direction of the film stretching, therefore transition moments located parallel to the cylinder axes probably contribute parallel to components of absorption and fluorescence.

The lifetimes of fluorescence of investigated samples are gathered in Table I. All emission was excited at 550 nm in a region of phycoerythrin absorption. Two emission regions were investigated: at 626 nm and 658 nm. It is predominantly R-phycocyanin that can contribute to the 626 nm emission, whereas in long-wavelength region predominantly different forms of allophycocyanin contribute [19]. For an unstretched sample of phycobilisome in deuterated poly(vinyl alcohol) lifetime in 626 nm emission region is shorter than at 658 nm. In both regions the decays are strictly uniexponential (Table I).

Unstretched sample in deuterated poly(vinyl alcohol), as it follows from the emission spectrum (Fig. 2), contains phycobilisome undissociated, because the ratio of allophycocyanin to phycoerythrin emission is very high and emission in R-phycocyanin region is low. Therefore in both regions (626 nm and 658 nm) predominantly only some types of allophycocyanin are emitting. Both of them are emitting independently of one other, without strong energy transfer between short- and long-wavelength forms, because in the case of the efficient energy trasfer the decay has to exhibit strong declination from uniexponential character, what is not observed (Table I). Probably these two forms are oriented mutually almost perpendicularly, because in another case excitation energy transfer has to be very efficient as usually between forms having strongly overlapped spectra.

After dissociation of such a sample the difference between lifetimes is similar, but both τ values became shorter. Stretching of a dissociated sample does not change too much, but stretching of a 'native' sample causes declination from uniexponential decay, especially for short-wavelength form. This effect as well as the shortening of τ upon stretching is suggesting that reorientation of chromophores causes the change in excitation energy transfer between biliproteins constituting phycobilisomes. In all native and dissociated samples the parallel component of fluorescence has a longer lifetime than the perpendicular one. This is in agreement with previously measured photoacoustic spectra of biliproteins in poly(vinyl alcohol) [21], which show that the thermal deactivation of parallel ordered chromophores is lower than that of less ordered pigments. Lower thermal deactivation is accompanied by higher yield and a longer lifetime of fluorescence.

TABLE I

FLUORESCENCE LIFETIME OF PHYCOBILISOME IN POLYVINYL ALCOHOL FILMS (EXCITATION AT 550 nm)

Sample	Polarization of light and sample orientation	Wavelength of emission (nm)	τ (ns)	$m/\cos\phi$ (± 0.01)
1	2	3	4	5
Native	n	626	1.36 ± 0.05	1.00
0% ^2H_2O		658	1.76 ± 0.05	1.00
Native	VVV	626	1.20 ± 0.1	0.95
300% ^2H_2O	VHV	626	1.00 ± 0.2	0.95
(fluorescence as	VVV	658	1.60 ± 0.1	0.98
for Fig. 2)	VHV	658	1.40 ± 0.1	0.98
Dissociated	n	626	1.09 ± 0.02	1.00
0% ^2H_2O		658	1.43 ± 0.02	1.00
Dissociated	n	626	1.17 ± 0.04	1.00
300% ^2H_2O		658	1.42 ± 0.05	0.99
Dissociated				
0% H_2O	n	626	0.87 ± 0.1	0.96
(fluorescence as		658	0.70 ± 0.1	1.00
for Fig. 3)				
Dissociated	VV [a]	626	1.26 ± 0.1	–
300% H_2O	HV [a]	626	0.91 ± 0.1	–
(fluorescence as	VV [a]	658	1.28 ± 0.1	–
for Fig. 3)	VH [a]	658	0.87 ± 0.1	–
Strongly dissociated				
0% H_2O	n	626	1.65 ± 0.1	0.89
(fluorescence as		658	0.70 ± 0.1	1.00
for Fig. 4)				

[a] Polarized only in exciting beam.

Unstretched samples of dissociated in poly(vinyl alcohol) dissolved in H_2O exhibit only a short lifetime of fluorescence: below 1 ns. After stretching of such a sample it is possible to distinguish two components: in parallel component longer lifetime equal about 1.3 ns and in perpendicular short similar to that in unstretched sample. In a ^2H_2O-poly(vinyl alcohol) sample such short component of τ is not observed even after the sample partial dissociation. This component has to be related with some perpendicularly ordered species which are not separated from other parts of phycobilisomes in samples prepared in deuterated poly(vinyl alcohol). Hefferle et al. [20] have shown that besides the long-living component of phycobilisome emission some shorter components occur, emitting about 30% of absorbed light. On this stage of our work we cannot decide which is the character of our short lifetime emission. It occurs in dissociated samples in which excitation-energy transfer between phycobilisome pigments is less efficient than in native complexes, therefore it is not due to the competition between light emission and energy transfer processes. The 'partially dissociated' phycobilisomes are up to now not biochemically characterized. It will be a subject of forthcoming study.

Lifetimes of intact phycobilisome are reported differently ranging from 1.45 ns to 2.01 ns [20,21]. Also the reported lifetimes of separated biliproteins varies [19,22–25].

Yamazaki [24] for allophycocyanin isolated from *P. cruentum* reports allophycocyanin lifetime equal to 1.9 ns at excitation at 540 nm. Our

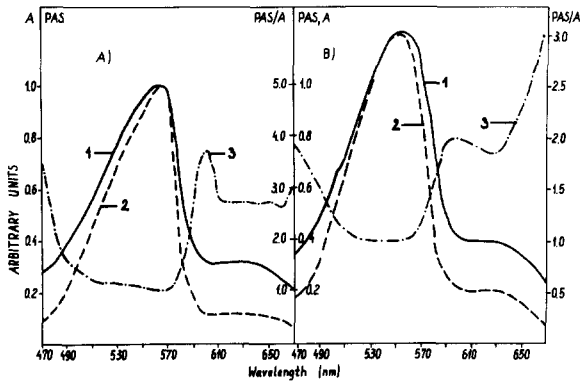

Fig. 5. Photoacoustic (1), absorption (2) and thermal dissipation (3) spectra of native (A) and dissociated (B) phycobilisomes in unstretched deuterated poly(vinyl alcohol) films.

longest long-wavelength emission is close to this value (1.76 ns $\pm$ 0.05 nm). In this region the final acceptor of excitation in phycobilisome is emitting. But after dissociation in the same region some species with short τ are also appearing because other forms of allophycocyanin start to fluoresce upon diminishing in energy transfer; and long-wavelength allophycocyanin form emission could be hidden in the emission of other complexes.

Figs. 5–7 show absorption and photoacoustic spectra of native and dissociated phycobilisomes in deuterated unstretch and stretch poly(vinyl alcohol). The ratio of photoacoustic spectra to absorption can be taken as a measure of thermal dissipation. Calculated on such a way thermal dissipation is also shown in Figs. 5–7. The accuracy of these spectra is low in a region of low absorption. Both photoacoustic and absorption spectra are normalized at main maximum, therefore of the maximum the photoacoustic-to-absorption ratio equals 1. For unstretched films (Fig. 5), this ratio in a region of R-phycocyanin absorption (553 nm, 615 nm) increases, showing that thermal dissipation in phycocyanin is higher than in phycoerythrin. The native phycobilisomes (Fig. 5a) dissipation decreases in a region of allophycocyanin absorption much more abruptly than for dissociated sample (Fig. 5b). It shows that, as a result of dissociation, energy transfer between various allophycocyanin forms is less efficient; consequently, some allophycocyanins of lower yield of fluorescence are loosing their excitation energy instead of transfering it to other forms. The shape of the dissipation spectrum in stretched films is different (Figs. 6 and 7). In the perpendicular component (Figs. 6a and 7a), the maximum in photoacoustic spectra is shifted to a longer

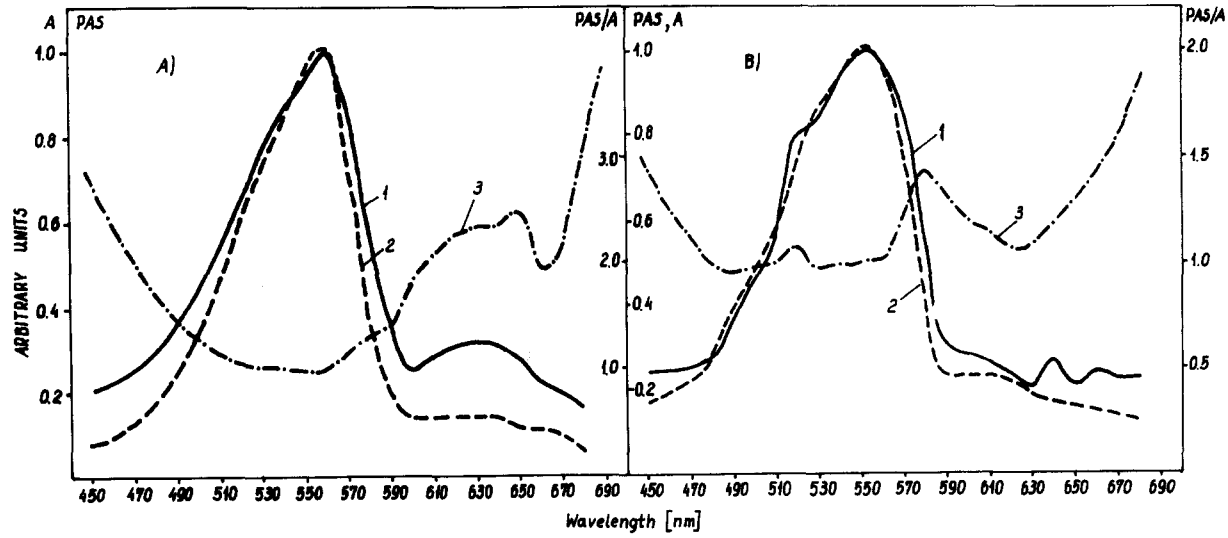

Fig. 6. Photoacoustic (1), absorption (2) and thermal dissipation (3) spectra of native (A) and dissociated (B) phycobilisomes in stretch deuterated poly(vinyl alcohol) films for parallel component.

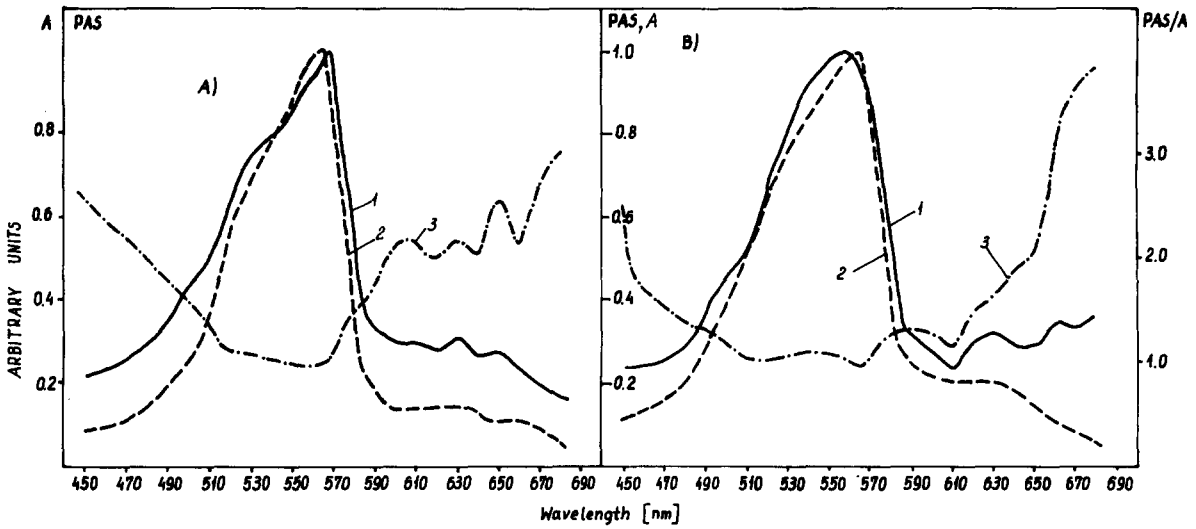

Fig. 7. Photoacoustic (1), absorption (2) and thermal dissipation (3) spectra of native (A) and dissociated (B) phycobilisomes in stretch deuterated poly(vinyl alcohol) films for perpendicular component.

wavelength: at about 568 nm. Stretching causes some dissociation of allophycocyanin, therefore Fig. 6a is similar to Fig. 5b. For a dissociated stretch sample (Figs. 6b and 7b) the two polarized components are very different from one another. In the perpendicular component, the maximum of the photoacoustic spectra is located at 558 nm, it look that B-phycoerythrin, is loosing a little more energy than b-phycoerythrin. The shift of absorption suggests that the transition moment of B-phycoerythrin now contributes less to the perpendicular than the parallel component. It means that after dissociation B-phycoerythrin, is differently reoriented than in native phycobilisome. In the region of R-phycocyanin thermal dissipation is higher in the parallel than in the perpendicular component, suggesting that after dissociation orientation of R-phycocyanin or phycocyanin-phycoerythrin complexes is parallel to the stretching direction. Contributions for allophycocyanin are lower than in native form; this menas that they have high yield of fluorescence.

From the presented results the following conclusions are drawn. (1) Several forms of allophycocyanin having various lifetimes of fluorescence and exhibiting different reorientations in stretched poly(vinyl alcohol) films are present in phycobilisome from *P. cruentum*. Different reorientations suggest that they exist in complexes of different shapes. (2) Two types of phycoerythrin B-phycoerythrin and b-phycoerythrin are differently reoriented in stretched samples, which strongly influences excitation-energy transfer. (3) The partial dissociation of phycobilisome favors the reorientation of biliprotein, whereas partial deuteration of phycobilisome diminishes the dissociation. (4) Mutual orientation of biliproteins strongly influences their lifetimes of fluorescence, thermal deactivation and excitation-energy transfer between them.

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