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Fluorescence Anisotropy of Cyanobacterial Phycobilisomes Oriented in Polyvinyl Alcohol (PVA) Films

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Polarized absorption (at 296 and 85 K), fluorescence, and photoacoustic (at 296 and 85 K) spectra of antenna complexes—phycobilisomes isolated from cyanobacteria *Tolypothrix tenuis* and *Oscillatoria* and embedded in isotropic and anisotropic polyvinyl alcohol films—were measured. From the sets of polarized components of emission, the anisotropy of fluorescence for the pools of differently oriented molecules was calculated. On the basis of polarized photoacoustic and emission spectra, the competition between the process of thermal deactivation of excitation and excitation energy transfer in a chain of excitation donor and acceptor chromophores of phycobilisomes is discussed.

KEY WORDS: Cyanobacteria; excitation energy transfer; photoacoustic spectra; phycobilisomes; polarized fluorescence; polyvinyl alcohol (PVA) films.

INTRODUCTION

Phycobilisomes (PBS),[4] supermolecular structures of biliproteins occurring in cyanobacteria and red algae, are efficient transducers of excitation energy [1–3]. PBS are the light-harvesting pigment–protein complexes that tnansfer excitation energy to chlorophyll–protein complexes in thylakoid membranes. In many species of cyanobacteria, PBS are hemidiscoidally shaped and consist of rods of phycoerythrin (PE) and/or phycocyanin (PC) and an allophycocyanin (APC) core complex. The rods may be composed of two to four hexamer disks, while the core complex is usually composed of three dodecamers [4]. The PBS structure is rather rigid and, to some extent, known; therefore this complex can be used as a model system in investigations of the interactions between chromophores and of the process of excitation energy transfer (ET) in photosynthetic antenna complexes. The light energy absorbed by the pigments of photosynthetic organisms can be transferred to other molecules, exchanged into heat, emitted as fluorescence, or used for photochemical reaction. There is competition among all these possibilities, but in the case of antenna pigments, the process of excitation ET to the molecule being the next participant in the donor–acceptor chain is very efficient. As a result, in separated PBS the fluorescence of the final acceptor of excitation—the long-wavelength form of APC (APC-680)—is predominantly observed.

The mutual orientation of chromophores in PBS strongly influences the process of excitation ET. The structures of some biliproteins have been established by diffraction methods [5] but additional information about the mutual orientations and interactions of bilin chrom-

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[4] Abbreviations used: APC, allophycocyanin; LD, linear dichroism; PAS, photoacoustic spectra; PBS, phycobilisomes; PC, phycocyanin; PE, phycoerythrin; PVA, polyvinyl alcohol; TD, thermal deactivation; TM, transition moment; ET, energy transfer; H, horizontal; V, vertical; PBS-O, PBS from *Oscillatoria*; PBS-T, PBS from *Tolypothrix*.

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ophores can be obtained by means of polarized light spectroscopy of oriented PBS or their fragments [6–14]. It was suggested that there are at least two independent pathways of excitation ET [4] and that light absorbed by differently oriented acceptor molecules reaches, in isolated PBS, different groups of final emitters of fluorescence [9].

To prove these suppositions, in the present study polarized absorption and photacoustic spectra (PAS) of oriented PBS separated from two types of cyanobacteria were measured at a low temperature (85 K). The resolution between contributions to absorption spectra from various types of chromophores was improved. We were able to correlate the maxima of absorption and PAS with those of fluorescence excitation spectra, and as a result, we draw a conclusion of the process of ET between fractions of differently oriented chromophores.

The parameters describing anisotropy of emission of differently oriented groups of molecules are calculated. This provides additional information about the spatial distribution of biliprotein chromophores and their role in excitation ET.

The competition among the process of excitation ET, thermal deactivation (TD), and emission of fluorescence occurring in differently oriented pools of chromophores is also discussed.

MATERIALS AND METHODS

Phycobilisomes were isolated from cyanobacteria *Tolypothrix tenuis* and *Oscillatoria* sp. (from the collection of the Institute of Soil Sciences and Photosynthesis, Pushchino, USSR), according to Gantt *et al.* [15] as modified by Erokhina *et al.* [16], and introduced into polyvinyl alcohol (PVA) films as described previously [6]. The anisotropic films were obtained by stretching to four times their original length $\{[(1-1_o)/1_o]*100 = 300\%\}$.

The polarized absorption spectra at 296 K were recorded on a Perkin–Elmer 552UV/VIS spectrophotometer with a Data Station for computation. Absorption spectra at 85 K were measured using a Cary17D spectrophotometer equipped with polarizers. $A_{\parallel}$ and $A_{\perp}$ refer to the absorption light polarized parallel and perpendicular to the orientational axis of the sample, respectively.

The PAS of isotropic and anisotropic samples were measured with a single-beam spectrometer [17,18] using illumination with polarized light [19]. A single-beam photoacoustic spectrometer equipped with polarizers was proposed by us [19,20] and used for investigation of oriented anisotropic biological samples. The aim of this method is to photoselect molecules having a similar orientation and to measure separately the TD of differently oriented fractions of the excited molecules. The frequency of light modulation was 12 Hz. The light beam intensity at 680 nm was 7.89 W/m^2. The photoacoustic signal was not saturated for all investigated samples. TD was determined in arbitrary units (au) as a ratio between the amplitude of PAS and the percentage of absorption.

The fluorescence emission and excitation spectra were obtained using a Spex Fluorolog 2 spectrophotometer Model 1870 equipped with a Datamate DM1 data acquisition system and a thermoelectrically cooled Hamamatsu Model R928 photomultiplier.

Four polarized components of fluorescence (F) were measured for stretched samples: (VVV), (VHV), (VVH), and (VHH) (H, horizontal; V, vertical). The first and last letters in the parenthese refer to the direction of the electric vector of exciting and fluorescence light beams, respectively, and the middle letter refers to the position of the orientational axis of the polymer matrix.

For comparison and proper normalization of polarized emission spectra, the following components of fluorescence of unstretched samples were measured: (000), (V0V) and (V0H) (0 means unpolarized light or unstretched sample).

This notation, used previously [21,22] for uniaxially oriented samples, is unequivocal and can easily be converted to the two-letter notation traditionally used in nematic liquid crystal investigations.

For elongated bilin chromophores the angle between absorption and fluorescence transition moments (TMs) is low, therefore the large angle between TMs of chromophores excited directly by polarized light and emission TMs of final acceptors in a donor–acceptor chain can be related only to the stepwise ET between several molecules having different orientations. Therefore to describe the chain of donors and acceptors, it is convenient to introduce the four coefficients of emission anisotropy described below.

From two pairs of components—(VVV),(VVH) and (VHV),(VHH)—after normalization [22], the anisotropy of fluorescence emitted by two pools of chromophores, one with small and the other with large angles between the emission TMs and the electric vector of the exciting light, can be obtained from the formula:

$$r_a = \frac{(VVV)/(VOV) - (VVH)/(VOH)}{(VVV)/(VOV) + 2(VVH)/(VOH)} \quad (1)$$

$$r_b = \frac{(VHV)/(VOV) - (VHH)/(VOH)}{(VHV)/(VOV) + 2(VHH)/(VOH)} \quad (2)$$

Formulas (1) and (2) are valid under the supposition that unstretched samples are isotropic, which is a reasonable hypothesis due to the fact that the linear dichroism (LD) of unstretched samples vanished.

The components with the same polarization of excitation and fluorescence beams can be compared without additional corrections. The two pairs of such components, (VVV),(VHV) and (VVH),(VHH), are not equivalent. In the first case, we measure emission of molecules which have their emission TMs oriented in the same direction as the TMs of molecules directly excited by the light absorption. In the second case, we measure the emission depolarized by several acts of ET in a chain of differently oriented molecules. Two types of anisotropy, related to the fluorescence emitted by molecules of the two groups, are defined:

$$r_c = [(VVV) - (VHV)]/[(VVV) + 2(VHV)] \quad (3)$$
$$r_d = [(VVH) - (VHH)]/[(VVH) + 2(VHH)] \quad (4)$$

The value of r_a describes anisotropy of fluorescence of "well-oriented molecules" with a low inclination of their absorption TMs of the direction of the film stretching. This coefficient is high when TMs of this group of molecules form a narrow angular distribution function.

r_b describes the anisotropy of emission of molecules with a wide distribution function. In a case of various values of r_a and r_b, the final acceptors of excitation transferred by these two groups of molecules have to be different.

r_c gives the size of a fraction of molecules excited by light molecules with absorption TM parallel to the film axis transferring excitation energy to final acceptors emitting fluorescence polarized parallel to this direction. Therefore the r_c and r_d values describe the difference between the contributions of the molecules oriented parallel and perpendicular to the film axis to parallel (||) and perpendicular ($\perp$) polarized components of fluorescence, respectively. r_c is large when energy is depolarized by several acts of ET in a chain of differently oriented molecules.

Bilin chromophores in PBS have a linear shape in most cases [23], so the angles between absorption and emission TMs are low. Therefore r_d for PBS can have high values only in the case of ET in a chain of chromophores that is not parallel oriented.

The calculation of all r_a, r_b, r_c, and r_d emission anisotropy values provides information about the spatial distribution of chromophores in the donor–acceptor chain in PBS.

RESULTS AND DISCUSSION

Absorption and Photoacoustic Spectra

Absorption and PAS of PBS from *Oscillatoria* and *Tolypothrix* in PVA films are shown in Fig. 1. The shape of absorption and PAS at room temperature of unstretched samples are similar. For stretched samples, the anisotropy of absorption is much lower than that of PAS. The orientation factor $s = A_{\parallel} - A_{\perp}/(A_{\parallel} + 2A_{\perp})$ is positive in a case of PBS from *Oscillatoria* (PBS-O) and negative for PBS from *Tolypothrix* (PBS-T) as shown in Fig. 2. Absolute values of s in both decrease with the increase in wavelength of absorption (Fig. 2), which is in agreement with the literature data [9,10,14].

Lowering the temperature or stretching the sample improves the resolution of bands, therefore several maxima related to various types of chromophores can be distinguished (Figs. 1A and B). Exact positions of these additional maxima depend on the source of PBS, polarization of light, and type of perturbation (temperature or stretching), but there are still some common features for

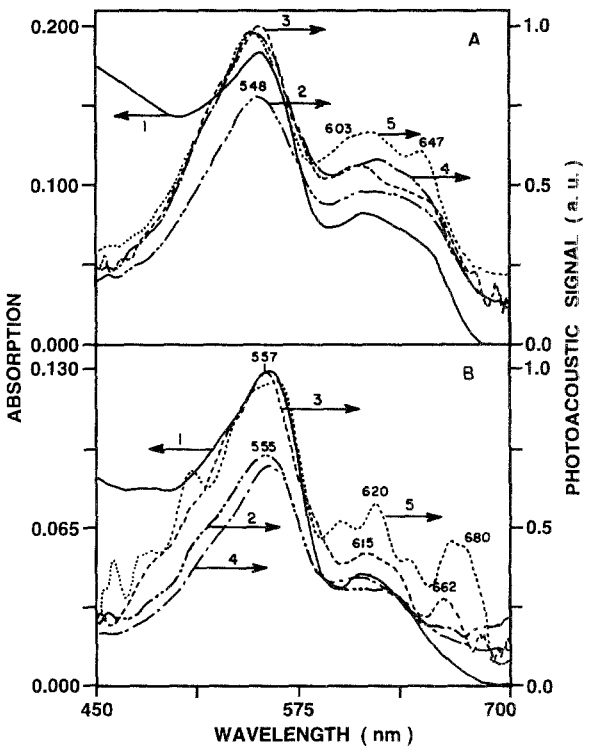

Fig. 1. Absorption and photoacoustic spectra of PBS in PVA. (A) From *Oscillatoria*; (B) from *Tolypothrix*. (1) Absorption, unstretched sample (0%), 296 K. (2) PAS, 0%, 296 K. (3,4) PAS, 300%, 296 K, || and $\perp$ components. (5) PAS, 0%, 85 K.

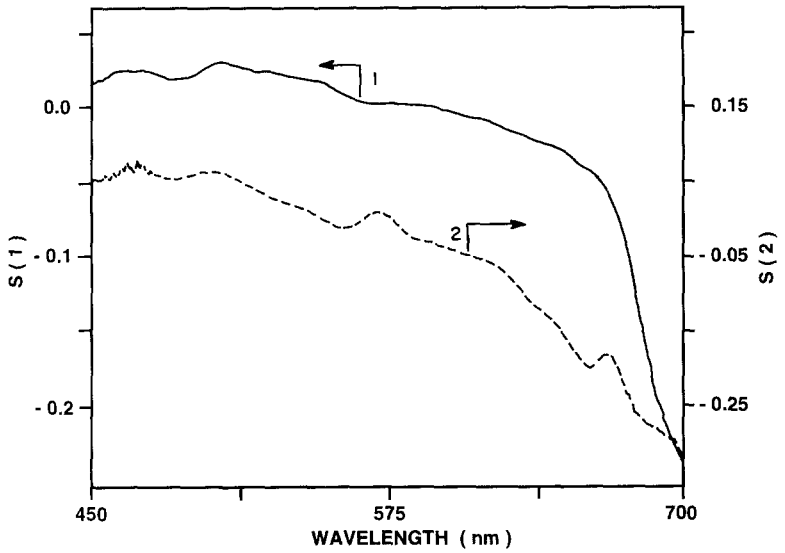

Fig. 2. Degree of chromophore orientation $s = A_{\parallel} - A_{\perp}/A_{\parallel} + 2A_{\perp}$. (1) PBS from *Oscillatoria; (2) from Tolypothrix.*

the absorption, photoacoustic, and fluorescence excitation spectra which enable the band classification in Table I.

On the basis of literature data [1–3,13,24–27], for PBS from various organisms and for isolated biliprotein complexes, some tentative assignment of observed bands is proposed (Table I). The stretching or lowering temperature perturbs the interactions between macromolecules in PBS, therefore some maxima, characteristic of smaller subunits of these giant macromolecules, can be visible even in absorption spectra, not just in PAS and fluorescence excitation spectra. Polarized light photoselects pools of molecules of a given orientation of absorption TMs, shown in Table I as a splitting of one band observed in natural light into two components in polarized spectra. The intensities of bands observed in absorption, PAS, and fluorescence excitation spectra are different. It shows that the yield of TD and the yield of ET of various groups of chromophores are different. Fluorescence excitation maxima are related to chromophores efficiently transferring excitation to the long-wavelength form of APC, which emits fluorescence in the 720-nm region. But the presence of the same band in all three types of spectra confirms the existence of definite types of chromophores.

PBS from two cyanobacteria exhibit different anisotropy in PAS. In PBS-O bands IIIa and b have low positive values of linear dichroism of PAS [LD(PAS) = $PAS_{\parallel} - PAS_{\perp}$], whereas bands IV and V exhibit rather high negative values of LD(PAS). PBS-T always exhibit positive LD(PAS), but with different values in various band regions. The value is especially high in a region of the IIIb band.

The APC-680 band is observed clearly in PAS, showing that in isolated PBS, when ET to chlorophyll is cut, this form of APC exchanges a large amount of excitation into heat. Similar effects have been found previously for PBS from other organisms [7,8]. It was established previously [28] for the same strains of cyanobacteria cultured under the same conditions that the content of C-PE, C-PC, and APC is equal to 10.2, 8.4, and 4.1 10^{-3} mg cm^{-3}, respectively, in PBS-O, whereas for PBS-T this content is 16.4, 8.7, and 4.7 10^{-3} mg cm^{-3}, respectively. This means that the C-PC/APC ratio of concentrations is similar in both types of PBS, but from polarized spectra, the mutual orientations of these biliproteins are different.

Fluorescence Spectra

Figure 3A and b show some of the polarized fluorescence spectra of PBS-O and PBS-T in PVA films. In both samples the film stretching perturbs the ET from C-PE to C-PC, and as a result the intensity ratio of C-PE emission (in the 570-nm region) to that of the final acceptor of energy (APC-680 at 670 nm) increases. IN the case of PBS-O, the shape of (VVV) and (VHV) polarized components is similar, and the (VVV) intensity is higher than the (VHV) intensity. This shows that a large pool of molecules with well-ordered absorption

Table I. Positions of Absorption Maxima from Absorption (A), Photoacoustic (PAS), and Fluorescence Excitation (F_{ex}) Spectra

Spectrum and PBS type	Sample deform. (%)	Light polar.	Temp. (K)	Band position (mm) and notation[a]								
				I	II_a	II_b	III_a	III_b	IV	V_a	V_b	VI
A, PBS-0	0	n	296		548			610		645		
	0	n	85		548			610		647		
PAS, PBS-0	0	n	296		548			607		648		
	300	∥	296		549			606		648		680
	300	⊥	296		545				621	648		
	0	n	85		546		603		617	647		
F_{ex}, PBS-0	0	n	296	512	554			606	629	642	656	681
	300	∥ (VVV)	296	512	542	560	596	611	629	644	658	
	300	⊥ (VHV)	296	512	535	550		605	629	644	658	
A, PBS-T	0	n	296		558			612	630			
	0	n	85		557			612	630			
PAS, PBS-T	0	n	296		557			615	630			
	300	∥	296		557			615	631		662	
	300	⊥	296		555			615	630			
	0	n	85	509	548	560	600	620		641	669	680
F_{ex}, PBS-T	0	n	296			565	608	629		641		
	300	∥ (VVV)	296			561	608	614	629	642		
	300	⊥ (VHV)	296			560	601	615	630	641		

[a] Tentative assignments of bands on the basis of literature data: I, bilin complexes [24]; II_a, C-PE hexamer [12]; II_b, C-PE trimer [12]; III_a, β subunit of C-PC dimer [26], β-155 of C-PC trimer [25]; III_b, α subunit of C-PC dimer or monomer of C-PC [26] and/or β-84 of C-PC trimer [25]; IV, C-PC with linkers [26], APC-sensitizing chromophore [12]; $V_{a,b}$ various APC aggregates [25,27], APC fluorescent chromophores [12]; VI, APC 680 [1–3].

TMs contributes to parallel components of the emission. In PBS-T, both pairs of components, (VVV),(VHV) and (VHH),(VVH), differ in shape. Comparison of emission spectra of unstretched and stretched samples shows that, in PBS-T, stretching causes an increase not only in C-PE (570 nm) emission, but also in that in the C-PC region (630 nm), suggesting that, in this case, the interaction between C-PC and APC is also perturbed.

All fluorescence excitation spectra were measured for 720-nm emission related predominantly to APC-680. Figures 4A and B show the influence of film stretching on the ET from various chromophores to APC-680. Stretching changes the shapes of fluorescence excitation spectra. For PBS-O (Fig. 4A) the maximum at 681 nm practically disappears. Because all complexes present in the unstretched sample are also present in the stretched one, the absorption TM of this complex is oriented perpendicularly to the film plane. Some changes are also observed in the C-PC region in both types of PBS. It is unexpected for PBS-T that the ET from C-PE to APC is still so efficient, when the intensity of C-PE fluorescence increases (Fig. 3). This shows, again [9], heterogeneity of the C-PE pool of chromophores. Parts of these chromophores are still able to transfer excitation to APC, when the second fraction having strongly perturbed ET to APC emits strong fluorescence. The stretching does not change the ratio of the photoacoustic signal in C-PE to that in the C-PC region (Fig. 1). This means that the ratio of the rate of TD processes in these biliproteins is similar in stretched and unstretched samples. Figure 5 shows the polarized components of fluorescence excitation spectra of stretched samples. These spectra have been used for gathering the data in Table 1. The very pronounced maximum at 512 nm in the VVV component is probably related to some bilin chromophore loosely attached to apoprotein. Such chromophores usually are efficiently ordered in stretched films [29]. The maximum at 509 nm observed in low-temperature PAS of PBS-T probably has a similar character.

The emission bands of various biliproteins in the PBS spectra were strongly overlapped, therefore a Gaussian analysis of fluorescence spectra was performed (see Figs. 6A and B). Prior to the Gaussian analysis, all spectra were expressed in terms of wavenumbers (cm^{-1}). Using this scale, the emission related to one electronic transition can be presented approximately as a Gaussian

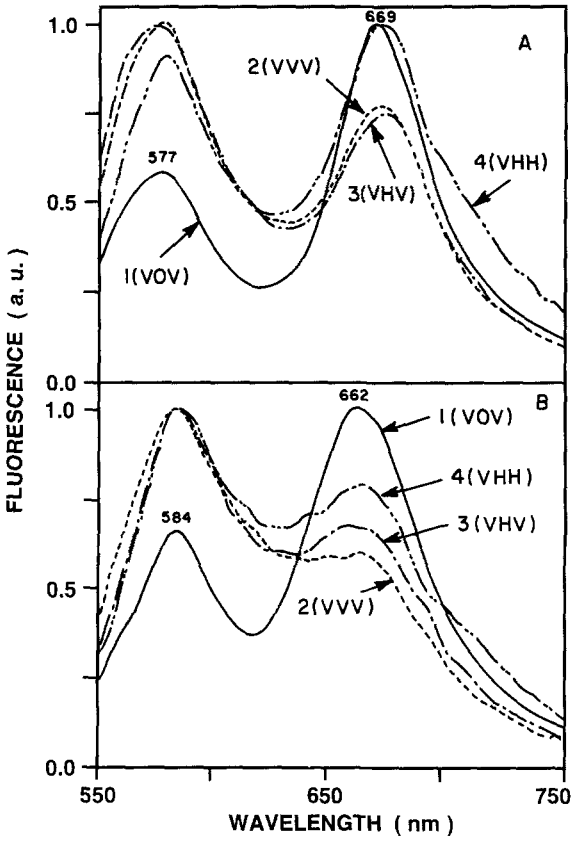

Fig. 3. Fluorescence spectra of PBS. (A) PBS from *Oscillatoria*; (B) from *Tolypothrix*. (1) Unstretched sample; (2–4) 300% stretching. Polarization of light and sample position marked (explanation in text). λ_{ex} = 500 nm.

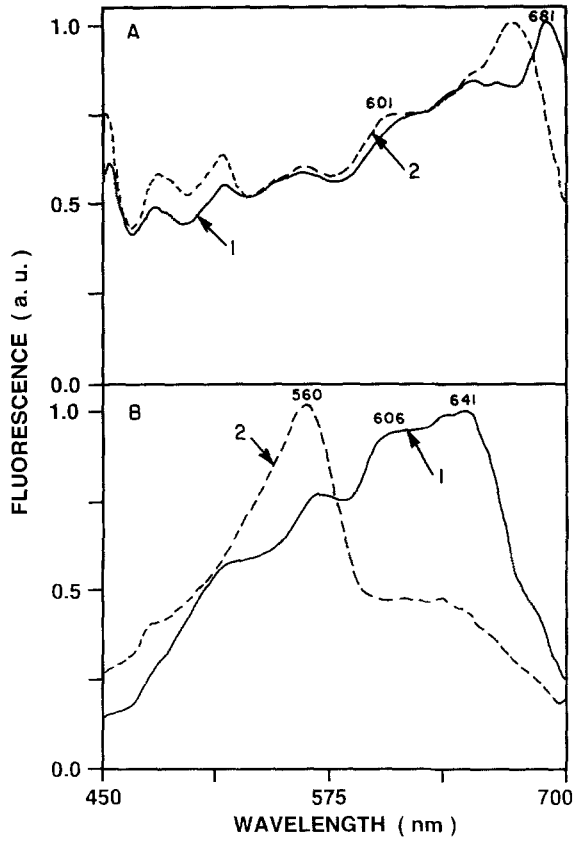

Fig. 4. Fluorescence excitation spectra of unstretched (1) and stretched (2) samples (natural light). (A) PBS from *Oscillatoria*; (B) from *Tolypothrix*. λ_F = 720 nm.

curve. In most investigations of pigment–protein complexes at room and low temperatures (e.g., Refs. 4 and 30–32), the absorption and emission spectra were deconvoluted, assuming a Gaussian band shape of contributions related to each type of chromophore. Such treatment should be regarded as an approximation. The band shape depends on the shape of ground- and excited-state Franck–Condon potential curves. In a case of pigment–protein complexes the shape of the band is not exactly Gaussian or exactly Lorentzian. It depends also on the temperature [33]. In biological samples chemically identical chromophores located in slightly different surroundings do not have identical spectral properties. Therefore the division of all chromophores on different types is not "sharp" and is, to some extent, arbitrary. In this situation we used Gaussian analysis of spectra, which enabled us to compare our results with literature data.

In the case of biliproteins in PBS, the approximation is even more crude because every biliprotein possesses at least three pools of chromophores of various spectral properties [1–3]. Therefore, the component related to one biliprotein is the result of superimposition of contributions from various types of chromophores. Because these chromophores are oriented differently [8,9], these contributions are different in various polarized components of fluorescence. All fluorescence spectra of PBS-O can be easily analyzed for the four following components: C-PE (570 nm), C-PC (654 nm), APC (665 nm), and APC-680 (686 nm). Exact positions of components for various polarized spectra are given in Table II. It was found previously [9,27] that in the case of PBS-T, the C-PE absorbing in shorter (560-nm) and longer (580-nm) regions differ strongly in orientation. Therefore emission spectra of PBS-T were analyzed for five components located in the following spectral ranges: C-PE, 571–581 and 587–594 nm; C-PC, 630–636 nm; APC, 660–664 nm; and APC-680, 679–685 nm. Examples of the Gaussian analysis are shown in Fig. 6, and the results are presented in Table II.

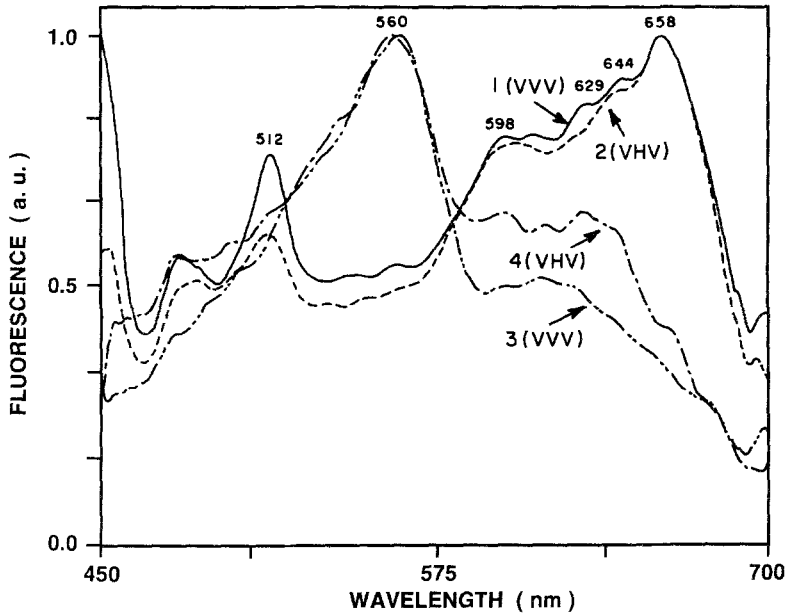

Fig. 5. Polarized fluorescence excitation spectra. (1, 2) PBS from *Oscillatoria*; (3, 4) from *Tolypothrix*. λ_F = 720 nm.

The shift of maxima of various polarized components shows that contributions from various chromophores are different. Anisotropy of emission parameters was calculated twice: first from whole emission spectra (Fig. 7 and Table III) and then from separated Gaussian components (Table III). In the first case, the contributions from various chromophores are superimposed and some averaged values are obtained; even such values for two types of PBS are strongly different. The value of r_a of PBS-O is negative, showing that the angle of emitting TMs and absorption TMs by light-excited chromophores is large. Absolute values of this coefficient are higher in the short-wavelength region than in a region of longer wavelengths. The difference is related to differently oriented C-PE chromophores contributing to short-wavelength emission [9]. On the basis of r_a values we can conclude that in PBS-O the major fraction of molecules emitting perpendicularly polarized emission obtains excitation energy directly from C-PE molecules absorbing light with absorption TMs oriented parallel to the PVA stretching axis. The r_a(PBS-T) > 0 shows that, in this case, most C-PE molecules with parallel orientation of absorption TMs contribute to the parallel component of observed emission. The r_b values are positive for PBS-O and negative for PBS-T, showing that, in the first case, the fraction of molecules which can be excited by an electric vector of light directed under a large angle with respect to PVA film axis is bigger than in the second case. It means that the distribution function of C-PE absorption TMs is wider for PBS-O than for PBS-T. The r_d(PBS-O) > r_d(PBS-T) shows once more that, in the first case a large fraction of parallel excited molecules contributes to the perpendicular component of emission. The higher values of r_c for PBS-O than for PBS-T show that biliproteins are oriented to a greater degree in the first than in the second type of PBS. The values of r_a of PBS-O are similar to that of PBS-T r_b, and vice versa, suggesting that the major fraction of chromophores of one type of PBS is the minor fraction of the second.

The values of emission anisotropy calculated from Gaussian components are in qualitative agreement with the results obtained from whole spectra, but the following additional conclusion can be drawn.

1. In the region of C-PC emission for both types of PBS, the r_c and r_d values are similar, showing agreement with previous data [9], i.e., a lower degree of orientation of C-PC than of other biliproteins.
2. In the APC region, r_c is positive for PBS-O and negative for PBS-T.
3. The r_d values for all biliproteins of PBS-O are higher than that for PBS-T.
4. After spectrum deconvolution, the r_a values for both types of PBS are negative in C-PE and C-

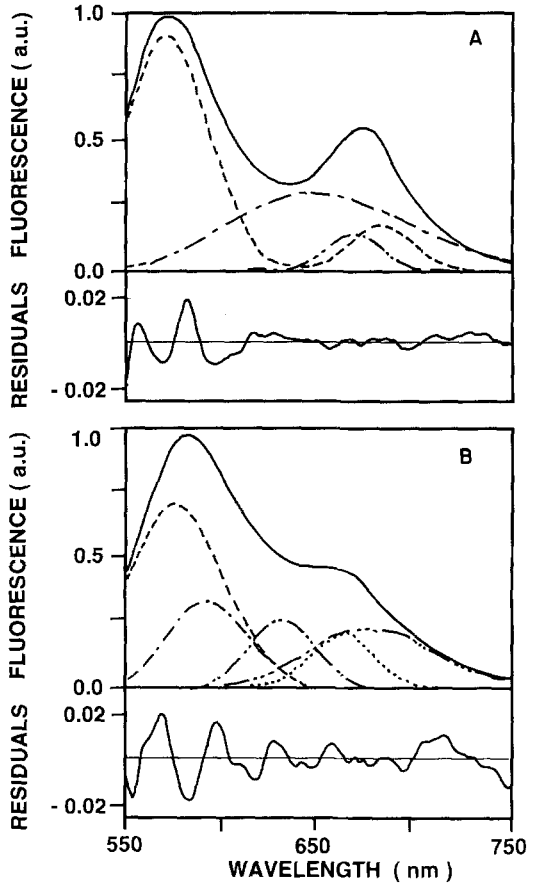

Fig. 6. Examples of Gaussian analysis of fluorescence spectra. (VVV) components for PBS from *Oscillatoria* (A) and from *Totypothrix* (B).

Table II. Gaussian Analysis of Fluorescence Spectra[a]

Polarized component	PBS from *Oscillatoria* Max	*y*	FWHM	% surface	PBS from *Tolypothrix* *Max*	*y*	FWHM	% surface
VOH	—	—	—	—	571	4.0	1.4	22
	573	5.1	1.4	31	587	2.7	0.8	8
	654	2.6	3.0	35	636	2.2	2.4	30
	665	5.1	0.7	16	660	4.9	0.8	17
	686	2.9	1.4	18	685	3.0	2.0	23
VOV	—	—	—	—	571	5.4	1.5	30
	570	7.3	1.51	41	588	2.8	0.7	7
	654	3.2	3.0	37	636	3.6	21	28
	665	4.7	0.7	12	662	5.2	0.9	18
	680	2.7	0.9	10	684	2.5	1.8	17
VVV	—	—	—	—	576	7.2	1.7	50
	570	9.1	1.7	59	594	3.3	1.4	18
	648	3.4	2.7	32	632	2.6	1.0	10
	665	1.4	0.8	4	663	2.1	0.9	7
	678	1.7	0.8	5	679	2.2	1.7	15
VHV	—	—	—	—	570	6.7	1.6	43
	570	9.5	1.7	59	591	3.5	1.2	18
	648	3.1	2.7	32	631	3.1	1.1	14
	665	1.3	0.8	4	664	2.5	0.9	9
	678	1.6	0.8	5	682	2.3	1.7	16
VHH	—	—	—	—	582	6.3	1.7	46
	574	8.7	1.5	43	588	2.9	1.1	12
	655	4.5	3.0	46	630	3.1	0.9	11
	665	1.9	0.6	4	663	2.9	0.8	9
	678	2.3	0.9	7	684	3.1	1.8	22
VVH	—	—	—	—	581	6.8	1.7	45
	573	5.1	1.6	50	589	3.1	1.1	13
	655	4.2	2.8	41	630	3.0	0.9	11
	665	1.8	0.6	4	663	2.5	0.8	8
	679	1.7	0.8	5	679	2.9	2.0	23

[a] Max, position of maximum; *y*, intensity at maximum; % surface, percentage of surface of a given component in the surface under whole emission spectrum; FWHM, fluorescence component width at half-maximum intensity.

PC regions, but with absolute values much higher for PBS-O than for PBS-T.

As follows from Table III, the values of emission anisotropy of PBS from two organisms obtained before and after Gaussian deconvolution of emission suggest similar distributions of absorption and emission TMs.

Both types of PBS contain the same biliproteins, and chromophore attachment in every biliprotein is established to be similar [1–3]. Therefore the differences in polarization of emission between the two types of PBS can be related to

(1) different fractions of absorbing C-PE chromophores ordered parallel and under a large angle with respect to the PVA axis and/or

(2) different spatial distributions of PBS "rods" [1–3].

No simple correlation can be established between the yield of TD of excitation and the yield of ET to the final acceptor of excitation. For example, PBS-T $TD_{\parallel} > TD_{\perp}$ in the region of IIIb and IV bands, but these bands are still efficient in the VVV component in the ET to APC. Of course, for a given type of chromophores, the sum of energy (i) emitted as fluorescence, (ii) converted into heat, and (iii) transferred to other chromophores is equal to the energy absorbed or obtained by ET, but because of chromophores and paths of ET heterogeneity, the relations are complex.

The polarized PAS and time-resolved fluorescence spectra [9,10,34] suggest that in some PBS there is more than one chain of excitation donors and acceptors. The efficiency of ET depends on the distance and mutual orientation of donor and acceptor molecules and their energy levels. Various chromophores are differently oriented and have different yields of TD of excitation, therefore polarized PAS and absorption and fluorescence

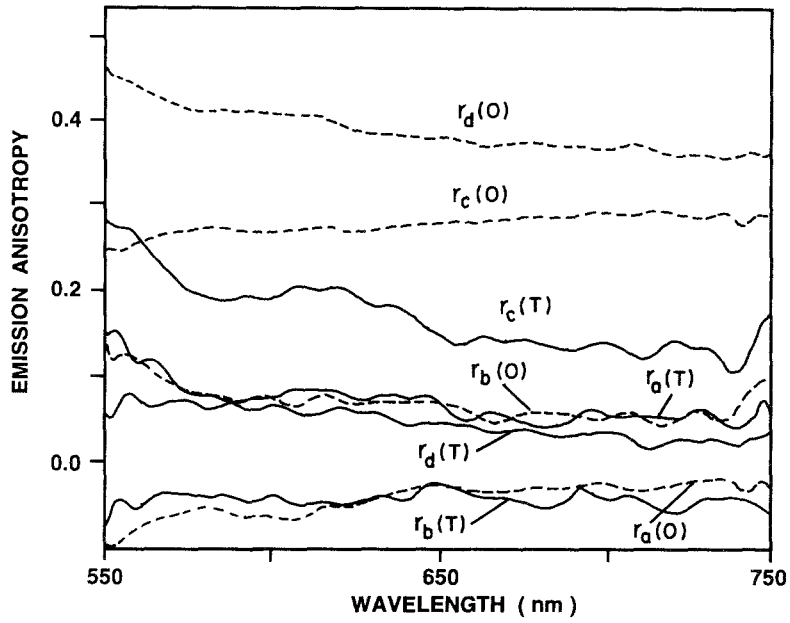

Fig. 7. Emission anisotropy calculated from whole emission spectra (explanation in text).

Table III. Anisotropy of Fluorescence

Source of PBS	Biliprotein emission	λ (nm)	r_a	r_b	r_c	r_d
		(1) From whole spectra				
Tolypothrix	C-PE	585	+0.07	−0.04	+0.19	+0.07
	APC	662	+0.06	−0.04	+0.15	+0.04
Oscillatoria	C-PE	578	±0.06	+0.07	+0.27	+0.42
	APC	670	−0.03	+0.05	+0.28	+0.37
		(B) From Gaussian components				
Tolypothrix	C-PE(1)	575	±0.16	±0.27	+0.26	+0.08
	C-PE(2)	590	0.00	−0.09	+0.21	+0.10
	C-PC	631	−0.11	−0.13	+0.08	+0.06
	APC	662	−0.16	−0.22	+0.10	+0.01
	APC 680	680	−0.14	−0.21	+0.18	+0.07
Oscillatoria	C-PE	572	−0.20	−0.06	+0.23	+0.42
	C-PC	655	−0.20	−0.19	+0.31	+0.39
	APC	665	−0.11	−0.03	+0.27	+0.38
	APC 680	680	+0.08	+0.01	+0.30	+0.22

excitation spectra enable us to distinguish between chromophores belonging to various paths of ET. The results presented suggest that in both type of PBS there is more than one path of ET and more than one type of final energy acceptor. On the basis of the data presented, one can also conclude that there are serious differences in biliprotein interaction and orientations in PBS from various organisms.

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