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STUDY OF ENERGY TRANSFER IN THE ANTENNA SYSTEM ISOLATED FROM *MASTIGOCLADUS LAMINOSUS* COHN

D. Frąckowiak[1*], A. Skibiński[1], B. Zelent[2], and R.M. Leblanc[2]

[1]Institute of Physics, Poznań Technical University, Piotrowo 3, 60-965 Poznań, Poland

[2]Centre de Recherche en Photobiophysique, Universite du Quebec a Trois-Rivieres, G9A 5H7 Quebec, Canada

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SUMMARY: Phycobilisomes oriented in stretched polymer films exhibit anisotropy of absorption and fluorescence. Up to now it was not clear in which way, by the deformation of their shape or by orientation of undeformed phycobilisome disk, this anisotropy of chromophore absorption and emission is reached. The photographs of two different kind of phycobilisomes obtained from cyanobacteria *Tolypothrix tenuis* and *Mastigocladus laminosus* were taken using a fluorescence microscope. From these photographs follows that phycobilisomes form in polymer big clusters and that these clusters are deformed as a result of stretching. It shows that predominantly the interaction between phycobilisomes is responsible for their orientation. Probably in clusters most phycobilisomes are oriented with disk diameter along the direction of film stretching. The fluorescence decay curves were recorded for *Mastigocladus laminosus* phycobilisomes embedded in PVA films. At least biexponential decay law has to be assumed. Calculated lifetimes are discussed in terms of energy transfer from primarily excited to fluorescent chromophores.

The excitation energy transfer (ET) in immobilized supramolecular antenna complexes phycobilisomes (PBS) was investigated in a case of isotropic and anisotropic samples [1,2]. Anisotropic samples were obtained by mechanical stretching of poly(vinyl alcohol) (PVA) film in which PBS were embedded. In data interpretation it was supposed that the film stretching has no influence on PBS shape [1,2]. Such, a supposition was in agreement with the time resolved emission spectra in presented the ps range. But up to now, it was not clear in which way, by the deformation of their shape or by orientation of undeformed PBS disk, this anisotropy of chromophore absorption and emission is reached.

Now, we present the photographs of PBS in isotropic and stretched PVA films taken under fluorescence microscope as well as the fluorescence lifetimes (τ) taken for the same samples.

*To whom correspondence should be addressed.

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Materials and methods

The culturing methods of *Tolypothrix tenuis* (Kutz)(M-29) and *Mastigocladus laminosus* Cohn (Genus Fischerella) cyanobacteria were previously described [3,4]. *Tolypothrix tenuis* and *Mastigocladus laminosus* PBS were isolated according to [5] and [6], respectively. Samples were embedded in PVA film. The PVA films after drying were uniaxially stretched three (200%) and four (300%) times their initial lengths. Unstretched and stretched samples were photographed using a Nikon Measurescope UM 2 (Japan) provided with Nikon-Super High Pressure Mercury Lamp with Power Supply Model HB-10101 AF Sony Video Graphic Printer UP-850. Samples were illuminated with λ_{exc}= 420-490 nm, photographed in light with $\lambda >$ 590 nm. The fluorescence lifetimes were measured using a Photochemical Research Associates, Inc. International Model 3000 fluorescence lifetime, single photon, counting apparatus. The excitation light was generated by a nitrogen flash lamp (4 ns pulse width, 10^6 photons per flash, 30 kHz repetition rate). Fluorescence was collected throught a 10 nm band-pass interference filter (Ditric Optics, Inc.), by an ellipsoidal mirror focused on a thermoelectrically cooled photomultiplier (Hamamatsu R928). Data were analysed using PRA statistical deconvolution program.

Results and discussion

Fig. 1 shows the photographs of PVA film with PBS isolated from *Tolypothrix tenuis* (A,B) and *Mastigocladus laminosus* (C,D). From these photographs done under fluorescence microscope, it follows that both types of PBS investigated form large clusters seen as strongly fluorescent regions. Comparing the pictures of clusters one can see that the degree of aggregation of PBS obtained from *Tolypothrix tenius* is lower than that for *Mastigocladus laminosus*. The number of PBS in cluster has been calculated using PBS dimensions taken from literature [1,7] and the averaged (from several photographs) dimensions of clusters. The accuracy of such calculations is estimated using the standard deviation method. The results of calculations are gathered in Table 1.

In unstretched PVA film, the number of *Mastigocladus laminosus* PBS in cluster is about three times that in a cluster of *Tolypothrix tenuis* PBS. As a result of film stretching, the aggregates are dissociated into the smaller particles. In both cases, they are three times smaller than in the case of isotropic film. Stretching causes the deformation of clusters, which become in approximation ellipsoidal. The degree of deformation of *Mastigocladus laminosus* is larger than that of *Tolypothrix tenuis*. It is reasonable because it has been shown previously [8] that larger particles are more easily deformed than smaller. The average angle of inclination of the long axis of ellipsoids from the direction of film stretching is, for both types of PBS the same, about 5°. Absorption and fluorescence spectra of PBS in PVA were published previously (for *Mastigocladus* [9], for *Tolypothrix* [1,2]). From the polarized absorption and fluorescence spectra follows the orientation of biliprotein chromophores with respect to the stretching axis. From the photographs follows that PBS form in polymer big clusters and that these clusters are deformed as a result of stretching. It shows that predominantly the interaction between PBS is responsible for their orientation. Probably in clusters most of PBS is oriented with disk diameter

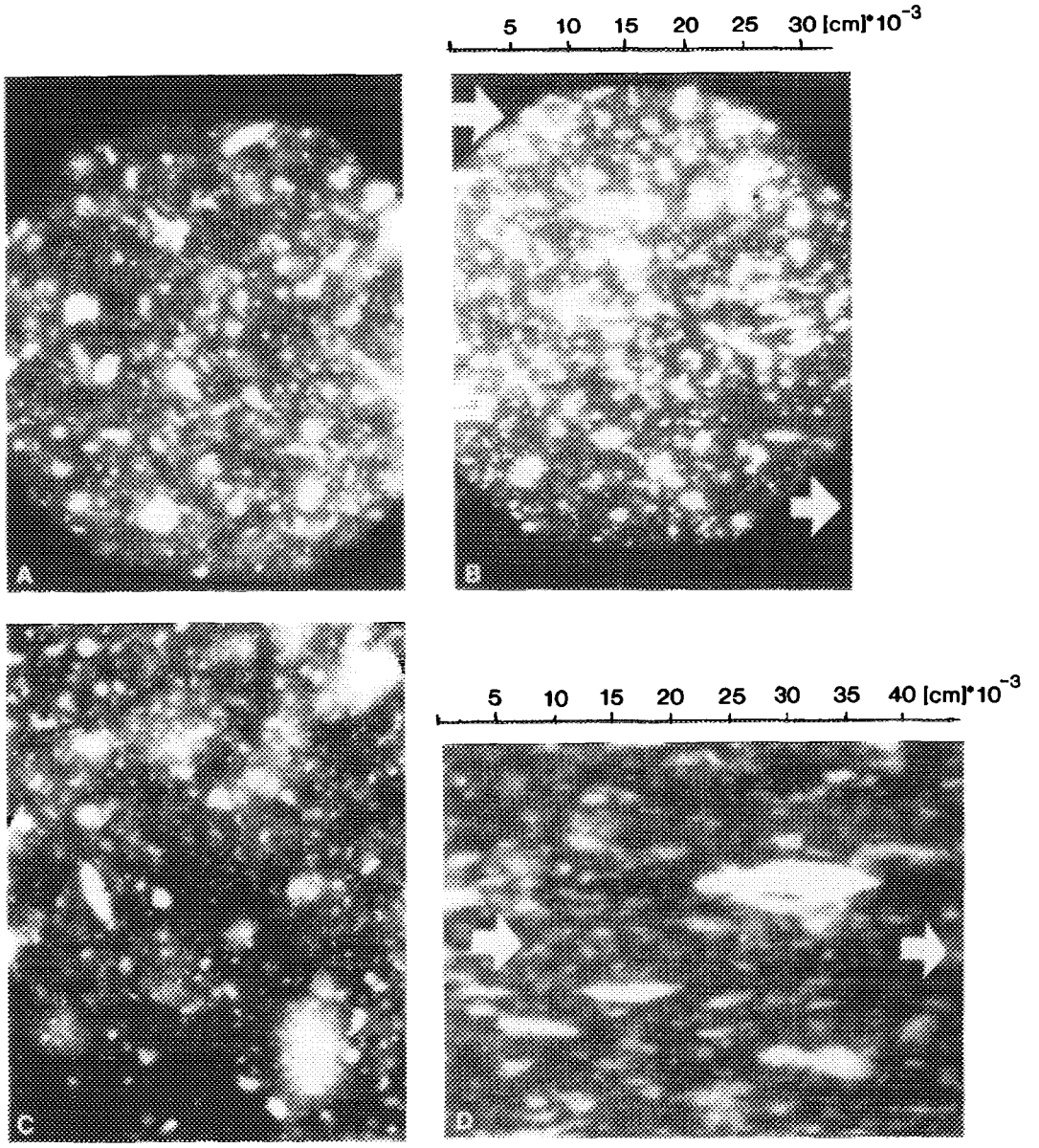

Fig.1. Photographs of *Tolypothrix tenuis* PBS (A,B) and *Mastigocladus lamniosus* PBS (C,D) in unstretched (A,C) and stretched (B,D) PVA films. The stretching directions are marked on the photographs. Magnification X 160. Exposition time for all samples was the same. Pictures are taken as polaroid technics in very short time. The thickness of unstretched sample was 0.1 mm and stretched sample 0.01 mm, respectively.

along the direction of flim stretching. The picture shown in Fig. 1 could suggest that in cluster migration of excitation between biliproteins belonging to various PBS can occur. Fig. 1 presents the deformation of a cluster, but does not give information about a single PBS shape. The deformation of PBS shape or/and possibility of ET between biliprotein belonging to various PBS

Table 1

Calculated numbers of PBS in unstretched and stretched PVA films

	(Number of PBS) * 10^3		
Phycobilisomes	0 % PVA	200 % PVA	300% PVA
Tolypothrix tenuis	390±20		125±15
Mastigocladus laminosus	1187±77	330±25	

has to have some influence on the ET between biliproteins in stretched films and in a result on the lifetime of fluorescence of PBS. In native PBS, because of efficient ET between biliproteins, APC emission is predominantly observed. In PVA, some PE and PC fluorescence, in the case of *Tolypothrix tenuis*, is observed whereas in the case of *Mastigocladus laminosus* only PC and APC are fluorescent. As follows from Table 2 and Fig. 2, in the case of *Mastigocladus*

Table 2

Fluorescence lifetimes (τ_i) of *Mastigocladus laminosus* PBS

PBS	Sample	λ_{exc}	λ_{em}	τ_1(ns)	A_1	τ_2(ns)	A_2	τ_3(ns)	A_3	χ^2
Mastigocladus laminosus	U	440	680	5.98±0.08	0.054	0.74±0.05	0.557			1.39
				6.73±0.34	0.041	2.70±0.53	0.052	0.30±0.05	1.69	1.16
	S	440	680	6.15±0.07	0.042	0.71±0.06	0.245			1.02
				6.53±0.31	0.054	2.75±1.10	0.042	0.36±0.08	0.681	0.97
Tolypothrix tenuis**	U	546	650	0.583	0.007	0.131	-0.008			0.61
				1.717	0.010	0.136	-0.012			0.94
	S	546	650	2.32	0.012	0.098	-0.013			0.99

U - unstretched PVA film.
S - stretched PVA film.
A_i - amplitude of τ_i component (in arbitrary units).
** - data from the paper D.Frąckowiak, A.Kowalczyk, A.Skibiński,Biophys.Chem. 42(1992)153-161.

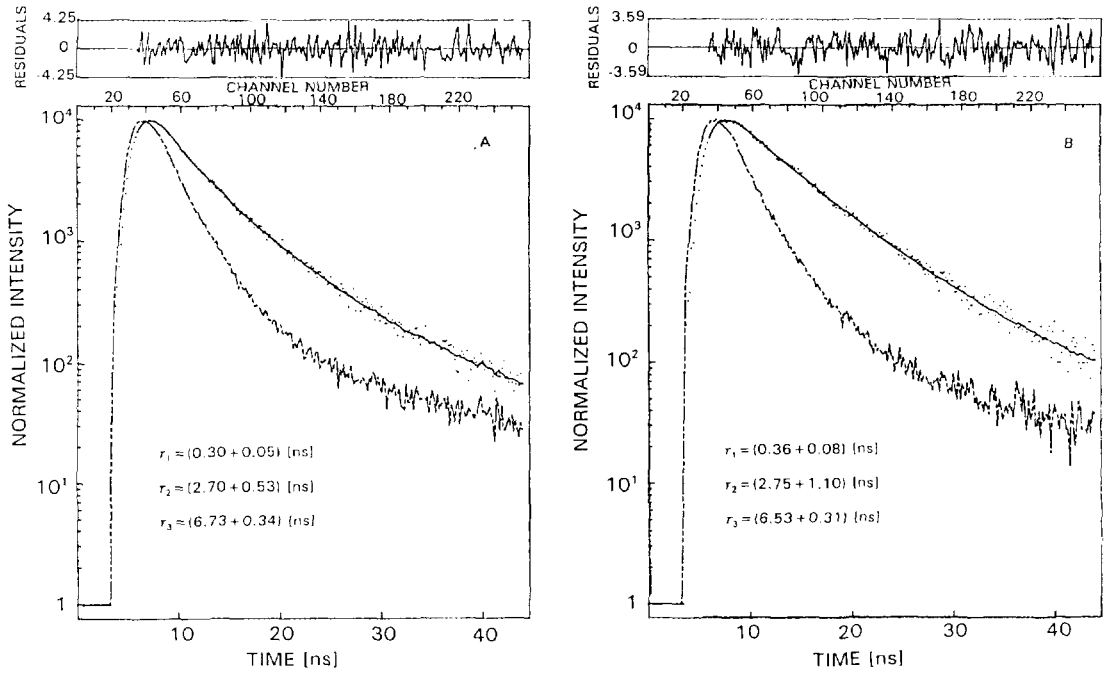

Fig.2. Fluorescence decay (λ_{exc}=440 nm, λ_{em}=680 nm) of *Mastigocladus laminosus* PBS in unstretched (A) and stretched (B) PVA films.

laminosus a good fitting to experimental decay is reached at analysis on three exponential components. At such analysis the τ values for stretched and unstretched samples are very close. The differences in amplitude of components is related to different orientation of various chromophores with respect to PVA plane. Before stretching the contributions from differently oriented PBS were averaged. After stretching, the projections of various chromophores' transition moments on the PVA plane are different. In the case of *Tolypothrix tenuis* the APC τ components in unstretched and stretched PVA are different (Table 2). This fact can be explained by different contributions from sensitizing, medium and fluorescent chromophores to measure fluorescence in isotropic and oriented samples. The discussion about types of chromophores taking part in the ET process and in emission is not possible on the basis of lifetime measurements done in ns range because the time of ET is much shorter (in ps range). But on the basis of *Mastigocladus laminosus* results, previous *Tolypothrix tenuis* spectra and similar pictures of aggregation in both cases it seems that ET between biliproteins belonging to various PBS can be excluded. Practically the same τ components of PBS in isotropic and anisotropic samples suggests strongly that the shape of the PBS giant molecule is not changed as a result of cluster deformation.

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