

Time-Resolved Polarized Fluorescence of C-Phycocyanin and Its Subunits from *Mastigocladus laminosus**

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The influence of aggregation and temperature on the excited state kinetics of C-phycocyanin from *Mastigocladus laminosus* has been studied. Polarized fluorescence decay curves have been recorded using a synchronously pumped dye laser in conjunction with a synchroscan streak camera. The experimental data for all samples can be fit satisfactorily assuming a biexponential decay law. Fluorescence depolarization times have been interpreted in terms of energy transfer among the different chromophores. The influence of temperature is only moderate on the intramolecular relaxation, but pronounced on the rates of energy transfer. Both are dependent on the size of the aggregate. The biexponential decay of the α -subunit containing only one chromophore, indicates the presence of different subsets of chromophores in these samples. The results are discussed in terms of variations of the chromophore arrangements upon temperature induced changes in the protein conformation.

Introduction

Phycobiliproteins are light harvesting pigments in certain algae. They contain 2–3 polypeptide subunits each bearing up to 4 covalently bound linear tetrapyrrolic chromophores [1–3]. Unlike most other photosynthetic pigments, the phycobiliproteins are not integral membrane proteins and readily water soluble. The phycobiliproteins from blue-green and red algae are aggregated *in vivo* into microscopic particles, the phycobilisomes, which act as light harvesting and energy transferring units mainly to photosystem II. They also have a tendency for aggregation *in vitro*. Here, much smaller and less complex structures are formed, which are believed to represent the phycobilisome building blocs [4, 5]. Earlier static fluorescence studies (see [6–8]) of the readily accessible biliproteins have prompted a series of investigations by picosecond time-resolved spectroscopy using different excitation and detection conditions [9–19]. The complex aggregation has, on the other hand, led to considerable technical and interpretational problems [11, 14, 16]. In order

to elucidate the influence of aggregation and other environmental factors (e.g. temperature) on the primary photophysical processes, we have begun systematic studies using polarized picosecond time-resolved emission spectroscopy [15, 16]. As a continuation of earlier work on higher aggregates including integral phycobilisomes from *Mastigocladus (M.) laminosus* [15], we here wish to report the results obtained with a series of increasingly complex aggregates of one of its biliproteins, C-phycocyanin (PC). This alga was chosen, because the primary structure of its biliproteins [20–22] as well as preliminary X-ray results of its PC [23] are known. The comparison with similar studies [16] on PC from a different alga, *Spirulina (S.) platensis* may also shed some light on the different properties of the two functionally similar pigments derived from a thermophilic and mesophilic organism, respectively.

Materials and Methods

Biochemistry

Cells of *M. laminosus* were grown photoautotrophically in Castenholz' medium [24] at 40–45° in 10 l cultures. They were either used fresh for the measurement with whole cells, or stored frozen for

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the isolation of PC. The latter was isolated as described for *S. platensis* [16]. The trimeric aggregates ($S_{20,w} = 5.6$) were dissociated into the monomers ($S_{20,w} = 2.8$) with NaSCN (1 M). Subunits were obtained by preparative isoelectric focusing on Sephadex G75 gels (Pharmacia, Uppsala) and renatured without delay on Biogel P2 desalting columns (Biorad, München). The isoelectric points were at 6.3 (α -) and 5.1 (β -subunit). The subunits were concentrated with aquacide (Calbiochem) to an optical density of $\leq 0.5 \text{ cm}^{-1}$ at the red maximum. Both subunits were $\geq 95\%$ pure from the respective other subunit and from colorless peptides if judged from SDS-PAGE. The absorption and fluorescence spectra of the subunits are shown in Fig. 2. Analytical SDS polyacrylamide gel electrophoresis was performed according to Laemmli [25]. Analytical ultracentrifugation was done as described earlier [16] on a model E (Beckman, München) ultracentrifuge. The S -values were corrected for density of the solutions. Standard correction factors for viscosity and partial volume of the protein were taken from the literature [26]. The viscosity correction for NaCl was used for KSCN. The $S_{20,w}$ coefficients were then extrapolated to $t = 0$. Stationary fluorescence was measured with a model DMR 22 (Zeiss, Oberkochen) photometer equipped

with a single monochromator on the excitation side and a double monochromator on the emission side. Standard bandwidths were 33 and 15 nm, respectively, at 600 nm. Sedimentation and fluorescence measurements were done with aliquots of the same preparations. All buffer compounds and other chemicals used were reagent grade. Stationary fluorescence measurements were performed as described in the previous paper [16].

Time-resolved fluorescence measurements and data analysis

The experimental setup is similar to the one used in the previous communications [15, 16]. The excitation pulses are derived from a dye laser (rhodamine 6G, tuned to 600 nm) synchronously pumped by an acousto-optically mode-locked Argon ion laser. The pulse intensity at the sample is $10^{13} \text{ photons} \cdot \text{pulse}^{-1} \cdot \text{cm}^{-2}$. The fluorescence is monitored at 90° and passes through a filter (Kodak Wratten No. 23, 620 nm cutoff) and a polarizing film directly into the entrance slit of the streak camera. The streaked image is monitored and digitized (PAR model 1025 optical multi-channel analyzer (OMA)), and the data are transferred to a minicomputer.

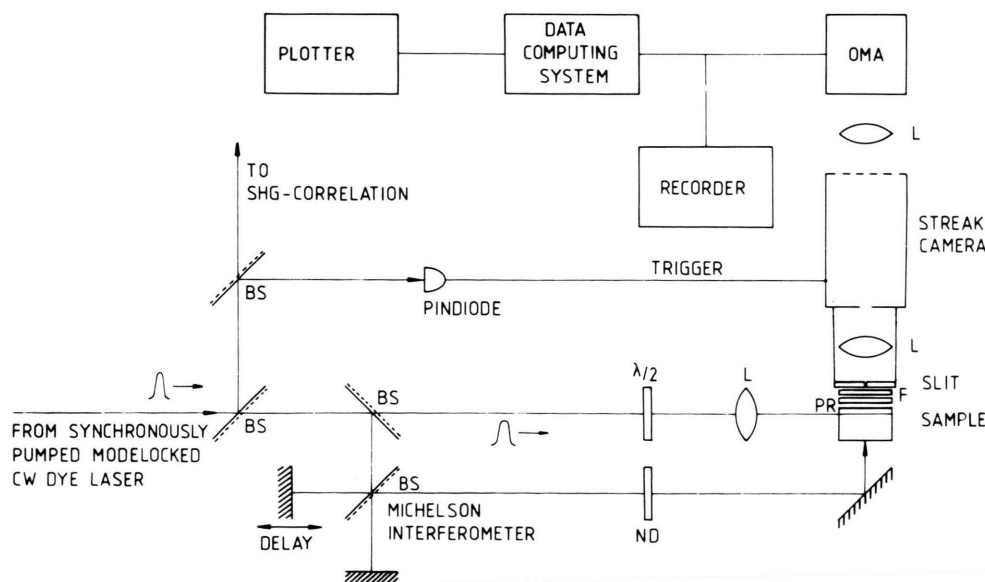


Fig. 1. Schematic of the experimental setup for the fluorescence measurements. L = lenses; PR = fixed polarizer, f = emission filter, BS = beam splitters, $\lambda/2$ = adjustable polarizer, ND = neutral density filter. The Michelson interferometer is used for calibration of time scale.

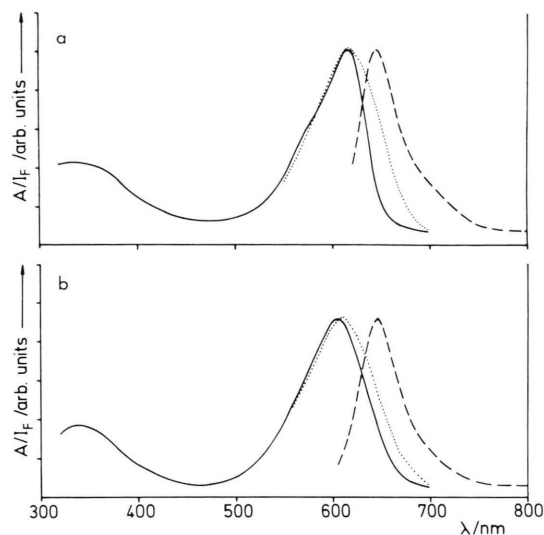


Fig. 2. Absorption (—), fluorescence excitation (----) and emission spectra (· · · · ·) of the α (a) and β -subunit (b) of PC from *M. laminosus*. All spectra are normalized with respect to the red absorption maximum. Experimental conditions for the α -(β)-subunit: $A = 0.52$ (0.48) at 617 (602) nm, emission at 650 nm for the excitation spectra, excitation at 600 (590) nm for the emission spectra. The skewing of the excitation as compared to the absorption bands is due to the experimental conditions.

The expressions $I(t) = I_{\parallel}(t) + 2I_{\perp}(t)$ and $D(t) = I_{\parallel}(t) - I_{\perp}(t)$ were calculated from the decay curves with the analyzer being parallel ($I_{\parallel}(t)$) and perpendicular ($I_{\perp}(t)$) to the adjustable polarization of the exciting laser beam. $I(t)$ corresponds to the decay of the excited state population, the “difference function” $D(t)$ to the product of the former with the correlation function of the absorption and emission dipoles [27, 28]. $D(t)$ can be evaluated by means of a convolution (in contrast to the fluorescence anisotropy $R(t)$) both if the individual functions are convoluted by the slower instrument response function (see e.g. [28]) and if the recorded fluorescence is a superposition of fluorescence from two (or more) emitting species (see e.g. the appendix in [16]). The latter condition is prevalent in most biological samples.

In the case of fluorescence depolarization by orientational relaxation of the photoselected excited molecules, the correlation function is (multi-)exponential and the analytically simple function $D(t)$ can be evaluated to give the orientational relaxation time(s) τ_{or} [29]. In the case of depolarization by energy transfer, the functional dependence of the

correlation function on energy transfer parameters is not yet solved. We have here assumed that the correlation function can be approximated by a (multi-)exponential and thereby derived a formal set of parameters τ_{dep} which give a rough measure of the energy transfer kinetics.

Almost all decay curves can be fitted on the basis of a biexponential:

$$F(t) = A_0 + \int E(t - t') \cdot [A_1 \exp(-t'/\tau_1) + A_2 \exp(-t'/\tau_2)] dt'$$

where $E(t)$ represents the excitation profile as recorded by the streak camera and A_0 a constant background. The five parameters, A_0 , A_1 , A_2 , τ_1 and τ_2 are determined using a non-linear least squares routine based on the algorithm of Marquardt [30] and Berington [31]. The precision of the fit parameters depends not only on the signal-to-noise ratio (S/N), but also on their relative magnitudes [32]. The noise distribution is not well defined in a combined streak camera-optical multichannel detector system, in contrast to e.g. single photon timing methods [13]. We have, therefore, performed simulations in order to establish some criteria for the reliability limits of the computed parameters. Two examples with S/N ratios of 2 and 5% are shown in Fig. 3 (see figure legend for details), which correspond roughly to the situation encountered with the β -subunit (Figs. 4a and 4b). The deviation of the amplitude ratios (A_1/A_2) is comparably small in the cases studied. The time constants can, however, deviate by up to 20% from the “true” value ($\delta = 1$) for an S/N = 5%. This is in particular true for the long-lived component due to the limited time window (2–3 ns) of the streak camera system. If judged from these reliability tests, the absolute values of the calculated parameters are only approximate, but their variations with temperature and aggregation state should reliably reflect the trends.

Results and Discussion

Temperature dependence of the fluorescence

The fluorescence intensity of all samples decreased markedly with increasing temperature. This decrease is demonstrated in Fig. 3 for the α - and β -subunits (normalized to equal excitation and detection conditions). The integrated fluo-

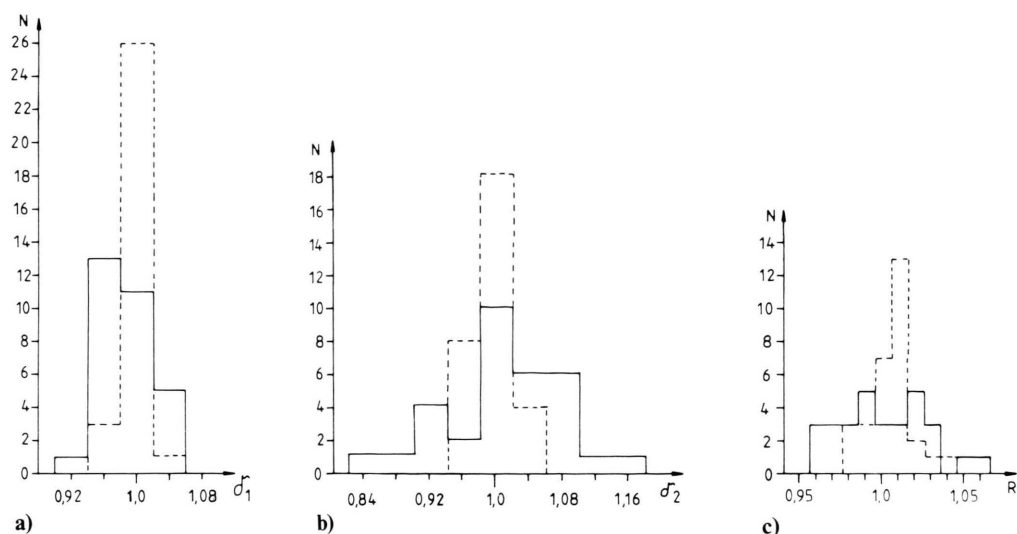


Fig. 3. Error analysis for the derived decay times. Noise from a random number generator corresponding to 2% (dashed lines) and 5% intensity (solid lines) with respect to the maximum amplitude has been superimposed on a theoretical decay curve (convolution of an excitation function with a biexponential, $\tau_1^0 = 100$ ps, $\tau_2^0 = 1500$ ps, $R^0 = 5$). The procedure has been repeated 30 times, and the fit parameters were then determined by means of a program based on a Marquardt algorithm. The distribution of deviations (in relative units with respect to the "true" values, e.g. $\delta_1 = \tau_1/\tau_1^0$, $\delta_2 = \tau_2/\tau_2^0$, $R = (A_1/A_2)/R^0$) are given for the short decay time τ_1 (a), the long decay time τ_2 (b), and the amplitude ratio R of the two components (c).

rescence intensity (expressed as $A_1 \tau_1 + A_2 \tau_2$) is then proportional to the fluorescence yield, provided that the spectral distribution of the fluorescence is temperature independent. The yields decrease at increasing temperature (see Table I). The changes are reversible to a large extent, if the samples are kept at elevated temperatures only for the relatively brief time necessary for thermal equilibration and data acquisition (see Ref. 16).

Increased temperature leads generally to a (partial) unfolding of the peptide chain. The bili-

proteins are particularly suited to study this process, because they contain with the covalently bound chromophores very sensitive probes for the state of the peptide chain. The unfolding of the protein both by increased temperatures or chemical denaturants like urea is reversible and accompanied by drastic changes in their absorption [1, 33] and circular dichroism spectra [34] and fluorescence yields [8, 34]. The oscillator strength of the visible absorption band is decreased by a factor of five, which is probably due to a conformational change of the chromophore (see 1 for leading references). The steady-state fluorescence is, however, decreased by four orders of magnitude [8, 34]. This much stronger effect must therefore be connected to other changes of the chromophore state.

The denaturation of proteins can often be described by a two state model, where the equilibrium is shifted by the denaturant from the native to the fully denatured state [35, 36]. The absorption and circular dichroism data of PC have indicated earlier, that the unfolding of PC from *S. platensis* could not be described satisfactorily by the two-state model, but that an intermediate state exists in equilibrium with the native and the denatured state [33, 34]. These data were, however, derived from

Table I. Temperature dependence of integrated fluorescence intensity normalized to emission at 18 °C. Values in parenthesis are derived from steady state experiments.

$I_F(T)/I_F[18^\circ\text{C}]$ [%]	$T[^\circ\text{C}]$		
	18	36	51
α -subunit	100	73	17
β -subunit	100	67	33
monomer	100 (100)	42 (71)	27 (41)
trimer	100 (100)	67 (72)	42 (44)
algae	100	95	70

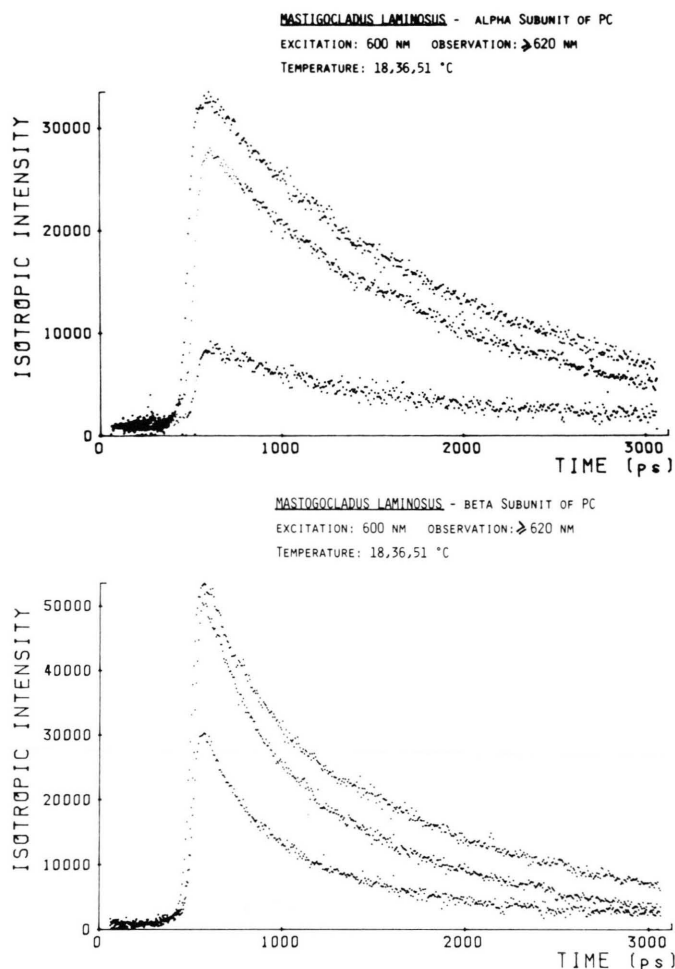


Fig. 4. Isotropic decay $I(t)$ of the chromophore fluorescence in the α -(top) and β -subunits (bottom) of PC from *M. laminosus*. Curves are normalized to equal excitation at 600 nm, maximum intensity decreases with increasing temperature. All fluorescence with $\lambda \geq 620$ nm has been recorded.

integral PC consisting of two subunits, and the dissociation of the former could be the process leading to this intermediate. The results obtained here for the α -subunit indicate, that another type of intermediate must be invoked, *e.g.* one in which the peptide chain has changed its conformation. The two-state model would predict a monotonous decrease of the fluorescence yield with temperature, and temperature independent decay constants. Since both the yield *and* the rates change, the data presented in this paper must be taken as further evidence for an intermediate state being present during the unfolding. As shown in Fig. 4, this is also true for the more complex aggregates.

Aggregation state and fluorescence

A similar set of data as shown in Fig. 4 has been collected for the monomeric ($\alpha\beta$) and trimeric PC

($\alpha\beta$)₃ and for the whole algae. The data are summarized in Fig. 5 for both the isotropic ($I(t)$) and the anisotropic decay ($D(t)$, see experimental part for the definition of these functions). To emphasize the different kinetics with increasing temperatures, the curves have here been normalized to the maximum amplitude except in those cases, where an identification of the individual curves would be impossible due to an extensive overlap of data points. The results are in the following discussed in the order of increasing size of the aggregates.

α -subunit

The α -subunit of PC contains only a single chromophore, and the decay should, therefore, be monoexponential, if all chromophores were kept in the same conformation by noncovalent interactions

with the apoprotein. The decay curves can nonetheless only be fitted by biexponentials (Fig. 5). A similar observation has been made earlier for the isotropic fluorescence of the α -subunit of PC from *S. platensis* [16] and *Anabaena variabilis* [19]. An aggregation of the α -subunit is unlikely from (i) the low concentration, (ii) from the fact that NaSCN is present [16] and (iii) the ultracentrifuge measurements ($S_{20,w} = 2.35$). One must then assume, that there are two species present with different chromophore-protein arrangements and hence fluorescence lifetimes. The “long-lived” species has a lifetime in the range of the integral biliproteins, whereas that of the “short-lived” species is unusual in the sense that lifetimes of this intermediate range have only occasionally been reported for integral phycobiliproteins [16, 19].

In contrast to earlier work [16], it has now been possible to obtain also information of the depolarization with an acceptable S/N (Fig. 5). The difference function $D(t)$ of the α -subunit can again be fit only by a biexponential, with shorter decay times τ than the respective τ' of the isotropic decay. Under the assumptions discussed above, the depolarization times τ_{dep} can be obtained separately for both components from the relation

$$1/\tau_{\text{dep}} = 1/\tau - 1/\tau'.$$

The slow depolarization components are subject to a large possible error (values in parenthesis in Table II) and will, therefore, not be discussed. The depolarization time of ≈ 1500 ps for the short-lived component at lower temperatures (18 and 36 °C) can be reconciled with the torsional motion of a loosely bound chromophore. The chromophores of PC from *M. lamosus* are covalently attached to the peptide chain by a single thioether bond [38], but they are believed to be rigidly bound to the latter by additional strong non-covalent interactions (see Ref. [1]). The comparably rapid depolarization of the fast decaying fluorescence component would then indicate, that these interactions are weakened in the

Table II. Fluorescence depolarization times τ_{dep} (in ps) in dependence on temperature and state of aggregation (for error analysis see text; values in parenthesis correspond to the slow decaying component).

τ_{dep} [ps]	T [°C]		
	18	36	51
α -subunit	1575 (10778)	1355 (3306)	573 —
β -subunit	403 (2838)	162 (2701)	145 (773)
monomer	580 —	561 (7165)	649 (4400)
trimer	70 —	242 —	160 (3520)
algae	0	0	0

“short-lived” species. This “loosened bolt” model would be supported by the decreased depolarization time of 570 ps at higher temperature (51 °C), where an even higher mobility of the less tightly coupled chromophore is expected. This is also in accordance with the decreased isotropic lifetime of the “short-lived” species, while that of the “long-lived” one is fairly insensitive to temperature.

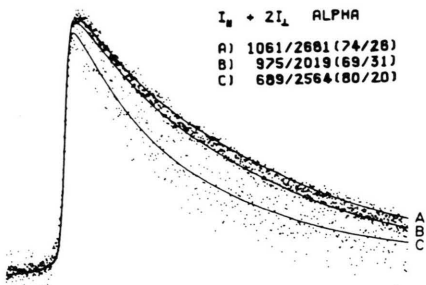
There are two explanations for these results: The first is, that the fast-decaying species is an experimental artefact due to irreversible denaturation. The isolation of the α -subunit involves a complete unfolding of the peptide chain over several hours, which may cause such problems. The second explanation suggested by Sauer (personal communication, 1983) is based on the finding (contrary to our data) of a similar decay constant in the β -subunit and in integral PC from *Anabaena variabilis* [19]. It suggests, that the native biliproteins might be heterogeneous *per se*. The two possibilities are presently difficult to distinguish, but the microheterogeneity of a biopolymer is an intriguing and potentially far-reaching idea. It may be supported by the fact, that biexponential decays have been observed for α -subunits of PC's from different

Fig. 5. Influence of temperature and aggregation on the isotropic ($I(t)$, left side) and anisotropic decay ($D(t)$, right side) of the chromophore fluorescence in PC from *M. lamosus*. Top: α -subunit, second row: β -subunit, third row: monomeric PC, fourth row: trimeric PC and bottom: whole algae. No anisotropic fluorescence was detectable in the latter. Excitation wavelength was generally 600 nm, and all fluorescence with $\lambda \geq 620$ nm has been recorded. All curves are normalized with respect to peak intensity, except for few to allow a better identification of the individual curves. The original data are given by the points, and the fit parameters for the solid curve are given in the insets in the order τ_1 , τ_2 (in ps), amplitude ratios A_1/A_2 (in %). The data were collected at 18 °C (A), 36 °C (B), and 52 °C (C), in the figures the amplitudes at longer decay times decrease in that order (see labels Fig. 5a) except for the anisotropic decay of the trimer (notice labels).

Isotropic

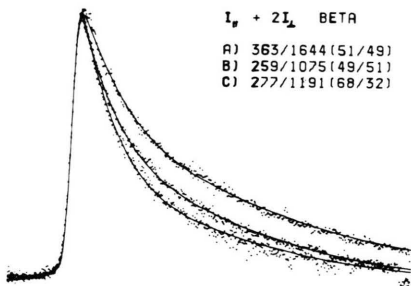
$I_{\parallel} + 2I_{\perp}$ ALPHA

A) 1061/2681 (74/28)
B) 975/2019 (69/31)
C) 689/2564 (80/20)



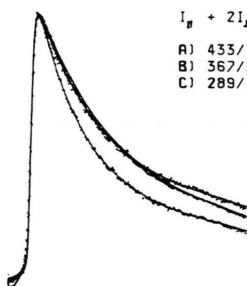
$I_{\parallel} + 2I_{\perp}$ BETA

A) 363/1644 (51/49)
B) 259/1075 (49/51)
C) 277/1191 (68/32)



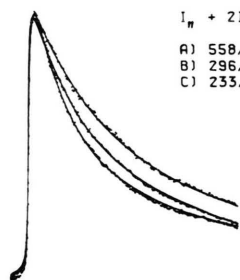
$I_{\parallel} + 2I_{\perp}$ MONOMER

A) 433/1870 (57/43)
B) 367/2478 (59/41)
C) 289/1785 (65/35)



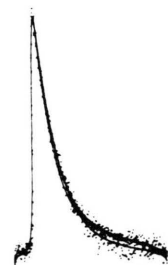
$I_{\parallel} + 2I_{\perp}$ TRIMER

A) 558/1392 (86/14)
B) 296/1507 (51/49)
C) 233/1633 (61/39)



$I_{\parallel} + 2I_{\perp}$ ALGE

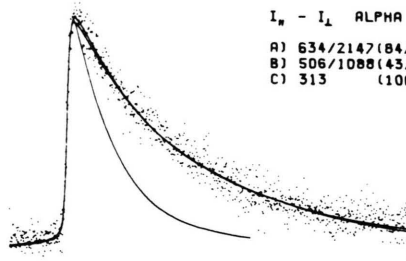
A) 112/ 705 (86/14)
B) 127/ 561 (83/17)
C) 155/



Anisotropic

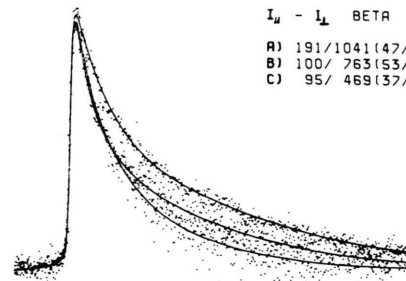
$I_{\parallel} - I_{\perp}$ ALPHA

A) 634/2147 (84/16)
B) 506/1088 (43/57)
C) 313 (100)



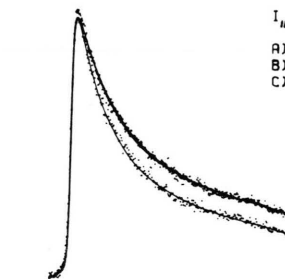
$I_{\parallel} - I_{\perp}$ BETA

A) 191/1041 (47/53)
B) 100/ 763 (53/47)
C) 95/ 469 (37/63)



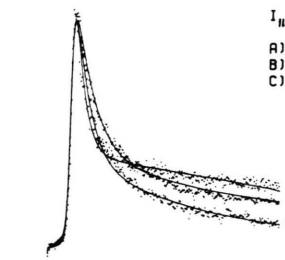
$I_{\parallel} - I_{\perp}$ MONOMER

A) 248/1811 (55/45)
B) 222/1763 (54/46)
C) 200/1378 (61/39)



$I_{\parallel} - I_{\perp}$ TRIMER

A) 62/ 260 (77/23)
B) 64/ 836 (72/28)
C) 95/1138 (75/25)



500 1000 1500 2000 t (ps)

500 1000 1500 2000 t (ps)

species and prepared by somewhat different procedures. It may also relate to the photochromic properties of phycobiliproteins under mildly denaturing conditions including low pH [39], moderate concentrations of urea [40] or monomer formation by chaotropic salts including NaSCN [41], or of isolated subunits of biliproteins [42].

β -Subunit

This subunit contains two chromophores, whose absorption maxima are about 20 nm or 550 cm^{-1} apart. Both chromophores are about equally well excited with the chosen wavelength, *viz.* 600 nm. The stationary emission spectrum of the β -subunit is at ambient temperature similar to that of the monomer, which indicates an efficient energy transfer from the high-energy (sensitizing = “s” in the nomenclature of Teale and Dale [6]) to the low energy (fluorescing = “f”) chromophore.

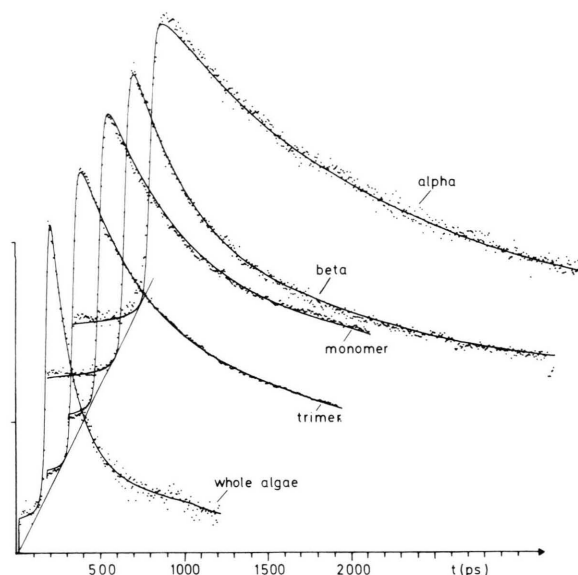
The postulated efficient energy transfer is supported by the kinetic data. A satisfactory fit of all decay curves is again obtained with a biexponential fit (Fig. 5c, d), although additional long-lived, low-amplitude components cannot be excluded in all cases. At increased temperatures, the decay times of both components are reduced, but less pronounced than in the α -subunit. The depolarization times τ_{dep} (see above) of the fast decaying component are much faster than those of the α -subunit and decrease with increasing temperature from $\tau_{\text{dep}} \approx 400$ to 150 ps (Table II). They are too fast for an orientational depolarization, but are rather assigned to a depolarization by energy transfer. A physical interpretation of τ_{dep} as the energy transfer time is, however, ambiguous. There is no dissipative continuum, and back transfer can, therefore, not be excluded, in which case τ_{dep} would be only an effective energy transfer time. Processes of this type have been discussed in chlorophyll antennas, which have similar energy differences as isolated PC [43].

The longer lived component in the fluorescence decay is considerably shorter than that of the α -subunit and that of all other isolated PC's studied here (Fig. 5). This could indicate a partial uncoupling of the chromophore as compared to the integral PC, because the free chromophores have lifetimes ≤ 100 ps, whereas those of native chromophores are ≈ 1500 ps. There are two explanations to account for such a change: The first is again an

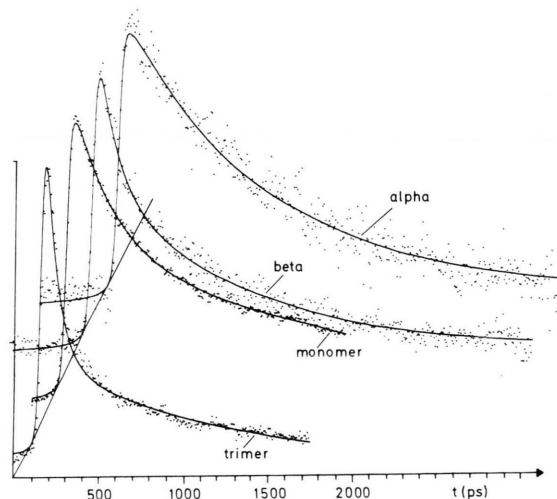
artefact due to the preparation, which involves the same denaturation-renaturation sequence as described above for the α -subunit. The second is a rearrangement of the peptide chain in the absence of the α -subunit. The absorption spectra of the two subunits add up to that of the monomer (as observed earlier for other biliproteins, see *e.g.* [8]), but it is also known that the absorption spectra are far less sensitive than the fluorescence towards changes in the state of the protein (see [1] for a discussion). A partial uncoupling of the chromophore is indicated by a small but distinct heterogeneity of the cw fluorescence (Fig. 2b). It is also supported by the pronounced temperature sensitivity of the lifetime. The reduction in the effective depolarization time would then indicate that the energy back transfer is more strongly reduced than the forward process.

Monomer and trimer

The isotropic fluorescence decay curves ($I(t)$) of the monomer ($\alpha\beta$) and the trimer ($\alpha\beta$)₃ are similar to each other and to those of the β -subunit. They can again be fit with biexponentials, with τ_1 in the range of 200–500 ps and τ_2 in the range of 1600–2500 ps (Fig. 4e–h). The slow component is assigned to the decay of the “f” chromophore(s) in their native state, and the fast one is probably associated – as in the β -subunit – with energy transfer. This interpretation is supported by the depolarization times. They are much shorter in the trimer bearing 9 chromophores than in the monomer bearing only three chromophores (Table II), *i.e.* they decrease with the number of possible acceptors. A similar dependence on the aggregation has been observed earlier for the static fluorescence depolarisation [6–8]. It increases with an increasing number of chromophores, which can all act as acceptors. It should be pointed out in this context that the fluorescence anisotropy in the kinetic experiments never extrapolates to 0.4, *e.g.* the theoretical maximum in a randomly oriented system. It has been estimated to ≈ 0.2 from the deconvoluted limits of $I(t)$ and $D(t)$ extrapolated to $t = 0$, a value which is similar to results from other laboratories [51]. One explanation is the non-statistical orientation of the chromophores in biliproteins, an assumption which is also supported by the non-vanishing anisotropy in steady-state experiments or



a)



b)

Fig. 6. Isotropic (a) and anisotropic decay (b) of the chromophore fluorescence of PC at the same temperature (36 °C), but in different aggregation states. The decay of whole algae is also shown in (a). All spectra are normalized with respect to peak intensity.

at long times after excitation in kinetic experiments [6–8, 50]. An alternative explanation is a third component in the fluorescence decay which is faster than the time-resolution of our equipment. Gilbro *et al.* [50] have recently found two short-lived components in phycobilisomes from *Synechococcus* 6301 with $\tau \approx 10$ and 90 ps, respectively. The latter is in the range observed by us for the fast

component of isolated PC. The presence of an additional ≈ 10 ps component (which is not contained in our biexponential fit) would also lead to a decreased limiting value of the anisotropy for $t = 0$.

It should be pointed out that the aggregation of biliproteins is at present not yet fully understood. Ultracentrifugal measurements of our PC preparations from *M. lamosus* (this work) and *S. platensis* [16] gave the trimer ($\alpha\beta$), as the predominant aggregate, with little to no hexamers ($\alpha\beta$)₆ detectable. This is at variance with a large body of earlier work showing the hexamer as the predominant species [4], but similar findings have occasionally been reported by others, too [see *e.g.* 44, 45]. There is growing interest in the function of the generally colorless linker peptides present in phycobilisomes and – in varying amounts – in preparations of isolated phycobiliproteins as well [46–49]. In particular have two of them been invoked in the aggregation of PC from *Synechococcus* 6301 [49].

The differences in aggregation could then be due to different amounts of the linker peptides in different preparations. The samples studied by us contained only traces of these peptides if judged from SDS-PAGE after staining with Coomassie blue, and the failure to observe aggregates higher than trimers may be linked to this fact. Earlier work involving two of us [15] on PC from the same organism, *M. lamosus*, but isolated by the controlled dissociation of phycobilisomes and subsequent ultracentrifugation had indeed produced both trimers (as well as hexamers) with significantly different fluorescence decay times. In particular was at ambient temperatures the shorter component of $I(t)$ more pronounced and its lifetime was about half of the values given here, and the difference function $D(t)$ could be fit satisfactorily with a single short-lived exponential. For the present preparation a second component is needed with a lifetime in the range of the isotropic decay ($\tau \approx 2600$ ps). Since the residual fluorescence polarization in biliproteins is increased with a decreasing number of coupled chromophores [6–8] and also order-dependent, these differences in decay pattern probably reflect differences in aggregation and/or non-covalent chromophore-protein interactions in the two preparations. This point adds yet another hitherto neglected parameter in the sample characterization (besides the measuring technique, data analysis and species related differences) which renders the

comparison of data in a generalized description rather difficult.

In spite of these problems, it is evident that the set of data obtained here for *M. laminosus* shows distinct differences as compared to the data obtained earlier [16] under rather similar isolation and measuring conditions for PC from *Spirulina platensis*. The depolarization times are rather different for PC derived from the two organisms. In both cases, the fast components of the monomer and trimer have similar isotropic, but different anisotropic decay times. The latter is ≈ 600 ps in monomeric PC from *M. laminosus*, and shorter and more sensitive to increased temperatures in the trimer. The situation is opposite to that in PC from *S. platensis*, where this decay constant is rather sensitive in the monomer (300–760 ps), but constant (≈ 90 ps) in the trimer. This difference in temperature dependence of the energy transfer characterized by the depolarization time, may be related to the fact that *M. laminosus* is thermophilic, although there seems to be no obvious ecological advantage in the observed behavior of PC from *M. laminosus*. It is furthermore not yet clear how the excited state kinetics of small biliprotein aggregates relate to those of integral phycobilisomes.

Whole Algae

The fluorescence of whole algae is completely depolarized at our time resolution; therefore, only the isotropic fluorescence decay has been analyzed. The recorded fluorescence is leakage fluorescence from different members of the energy transfer chain, which are to the most part indirectly excited. At ambient temperatures, the integral fluorescence is dominated by a short lived component ($\tau \approx 120$ ps). The less intense long-lived component is also comparably short ($\tau \approx 600$ ps), and disappears at 52°C . Since all fluorescence with $\lambda \geq 620$ nm is recorded, it includes leakage not only from PC but also from its acceptor pigments, *e.g.* allophycocyanin and chlorophyll. A distinction is possible by a spectral analysis of the emission [15, 18], which was beyond the scope of this project. A tentative assignment is, however, possible from comparison with data from the literature. The isotropic fluorescence of whole phycobilisomes from another alga, *Synechococcus* 6301 has recently been shown [50] to have two short-lived components (≈ 10 and 90 ps). The first com-

ponent is beyond our time resolution, but the second one could correspond to the 120 ps component in *M. laminosus* (differences are expected from the differences in the phycobilisome composition and organization in the two organisms). The short-lived component is thus assigned to indirectly excited PC, quenched by transfer to allophycocyanin. The longer lived component in *M. laminosus* would then arise from allophycocyanin and/or chlorophyll *a*, whose lifetime is determined by the energy transfer to the reaction centers. If this tentative assignment were correct, the decrease in amplitude of the longer-lived component at higher temperatures could indicate an increased rate of radiationless processes in the acceptor pigments (internal conversion, photochemistry) leading to similar short lifetimes of both PC and allophycocyanin. *M. laminosus* is generally grown at $\approx 50^\circ\text{C}$, so that photosynthesis and in particular the energy transfer between the two pigments is still efficient at this temperature. Otherwise, the long-lived allophycocyanin emission should be detectable as in the case of integral phycobilisomes [15].

Concluding Remarks

The data presented demonstrate a strong influence of chromophore-protein and chromophore-chromophore interactions on the photophysics of the chromophores in PC. This interaction is modified by changes in the quaternary structure, but also in the protein conformations, as shown for the α -subunit. Only two parameters, *e.g.* the state of aggregation and temperature have been investigated here, in addition to the species dependent differences. The major biochemical problem is the functional relationship of isolated pigments to the *in situ* antenna system, since the state of the former depends on the isolation procedures. The major problem in deriving parameters for the photophysical processes is the presence of more than one emitting species (in all but the α -subunit), and the large range of decay times. The main advantage of the repetitive streak-camera, *viz.* the intrinsic high sensitivity, is in part lost by the necessity to record simultaneously very long and very short decay times. This has in addition significant consequences on the accuracy of the derived parameters. Since chromophore-protein interactions are a key for the understanding of chromo-protein structure and

function, further studies using additional pico-second-time resolved techniques on the same system are in preparation.

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