Photosynthetic Light-Harvesting Systems

Organization and Function

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ENERGY TRANSFER IN "NATIVE" AND CHEMICALLY MODIFIED C-PHYCOCYANIN TRIMERS AND THE CONSTITUENT SUBUNITS

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Introduction

The light-harvesting complexes of blue-green and red algae, called phycobilisomes, are highly organized assemblies of the biliproteins allophycocyanin (APC) and phycocyanin (PC), often containing also phycoerythrin (PE) or phycoerythrocyanin (PEC) [1,2]. These biliproteins carry one or several tetrapyrrol chromophores bound covalently to polypeptides. In case of PC, which is discussed here, the monomeric unit consists of the α-polypeptide chain with one chromophore (A84) and the β-chain with two phycoyanobilin chromophores attached to it (B84 and B155) [3,4].

From X-ray work [5,6] on PC from two different organisms, the crystal structure of the chromoprotein, the conformations, distances and the approximate relative orientations of the chromophores have become known recently. Very similar results have been obtained for a trimer $(\alpha\beta)_3$ from Mastigocladus laminosus and a hexamer $(\alpha\beta)_6$ from Agmenellum quadruplicatum, which is composed of two trimeric units aggregated face-to-face. For the understanding of the energy transfer process within these units and in the whole phyco-

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bilisome, three features deduced from this analysis are important.

(i) The chromophore arrangement exhibits a 3-fold symmetry around the central axis orthogonal to the plane of the trimeric unit.

(ii) The shortest distance is found between the 1A84 chromophore of one monomeric unit and the 2B84 chromophore of the neighbouring monomer; it is only about 21 Å center to center.

(iii) All three chromophores adopt an extended geometry; they exhibit hydrogen bonds to a conserved aspartate residue (A87, B87, B39, resp.) and are subject to strong coulombic interaction with the net charges localized on the surrounding amino acids.

If the threefold symmetry established in the crystal structure is also present in the native trimeric unit (i.e., there are only three types of non-equivalent chromophores), then theory predicts that any measurement, which monitors the time course of the excited state population, should be fittable by a sum of three exponentials provided that the evolution of the system can be described by a set of linear differential equations (radiative and intramolecular radiationless decay, energy transfer by dipole-dipole interaction (Förster transfer)).
Because of the short distance between pairs of chromophores, like 1A84 and 2B84, it was concluded that the energy transfer rate between these chromophores should be very high and/or the coupling so strong that Förster theory may not be applicable anymore [7]. Alternatively the model of localized excitonic states should then be used for the description of the energy transfer kinetics (see fig. 1). Besides of the very fast

(and eventually unresolvable) internal conversion process, only two decay times should then be observed in a picosecond time-resolved experiment: one related to the transfer between the s-chromophore and the excitonically coupled state of chromophores A84/B84 (devoted C^+ or C^- in fig.1) and intramolecular decay of the corresponding state (C^+). Since the important factors in excitonic coupling are the energy difference of the unperturbed states and their interaction energy, a modification of one of the chromophores by a distinct perturbation could result in an intermediate situation between the two above described limiting cases and therefore help to elucidate the situation in PC-trimers.

In a previous publication [8] it was shown that titration of solutions of biliproteins in various states of aggregation with the mercury salt p-chloromercurybenzenesulfonate (PCMS)

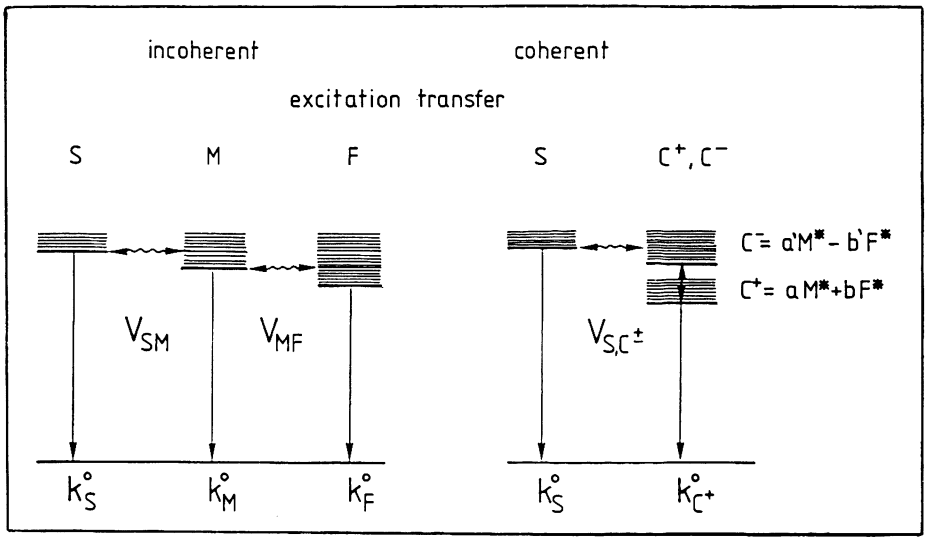


Fig. 1: Left : Förster type energy transfer
Right: Excitonic interaction between M and F chromophores

leads to a binding of this compound to the free cystein B111, and concomitantly to a selective change in the absorption and circular dicroism spectra of the close-by chromophore B84. Resonance-enhanced CARS spectra (Coherent Anti-Stokes Raman Scattering) of such modified PC-trimers confirm the evidence for a change in geometry of B84, which was deduced from the X-ray data [9]. In this contribution the effects of PCMS-binding on CARS spectra (structural information) and excited state kinetics are compared. It is concluded that in smaller aggregates (up to trimers) a microheterogeneity of the chromophore-protein arrangement exists, which produces a rather complex fluorescence decay pattern.

Materials and Methods

PC-trimers without linker peptides were prepared according to published procedures [10]. β-subunits were isolated by a modified flat-bed gel electrofocusing technique [11]. In particular, care was taken to avoid oxidative bleaching of the chromophores by gassing with Argon and work under subdued light. The pigment is present as a dimer (β_2).
Both types of samples were titrated with PCMS in approximately 1.1 fold excess with respect to the number of free cysteins to assure complete modification.

The fluorescence decay curves were recorded by means of a Hamamatsu synchroscan streak camera using interference filters for spectral narrowing of the monitored emission. Excitation was performed under magic angle conditions at low average intensity (≈10 mW or 10^{13} photons $pulse^{-1} cm^{-2}$) by the output of a synchronously pumped mode-locked dye laser (pulse repetition rate 84 MHz). The decay curves were analysed by applying a least-squares fit routine and assuming a 3-exponential decay law (More details are given in references 12-14).

Resonance-enhanced Coherent Anti-Stokes Raman Scattering (CARS) was measured with an apparatus described in detail in references 15 and 16. Two eximer laser-pumped dye lasers generate the so-called pump- and Stokes-beams, with wavelengths λ_p (fixed) and λ_s (variable), which are focused in the sample under a small angle. Recording the intensity of the generated anti-Stokes radiation as a function of the wavenumber difference $\tilde{\nu}_p - \tilde{\nu}_s$ yields a vibrational spectrum. Since λ_p coincides with an electronic transition (absorption band) of the chromophore, only the chromophore's vibrations are resonance-enhanced and, therefore, dominate the spectrum.

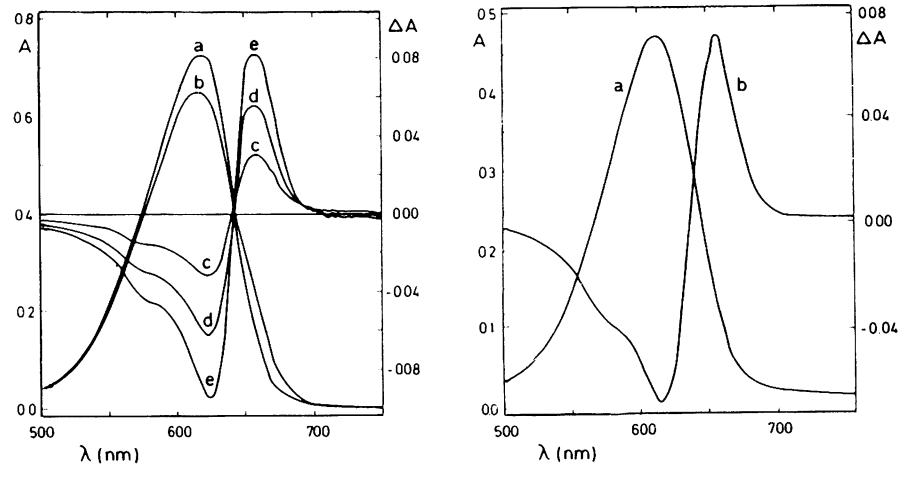


Fig. 2: Left: Absorption spectra of PC trimers before (a) and after titration with PCMS (b). Difference spectra recorded 30 (c), 60 (d) and 100 min. (e, final absorption) after PCMS addition.
Right: Absorption spectrum of β-subunit (a) and final difference spectrum after PCMS titration (b).

Results and Discussion

In figure 2, the effect of PCMS titration (binding) on the absorption spectra of β-subunits (present as dimers β_2) and trimeric PC are shown . In both cases the absorbance at the long-wavelength side of the absorption maximum (λ_{max} = 604 nm and 616 nm, resp.) is reduced by about 10 % and there is a concomitant increase in the long wavelength slope of this band (wavelength of maximum absorbance increase is ≈655 nm).

Figure 3 displays the CARS spectra recorded for the four samples in the fingerprint region. At first glance it is already apparent that the band centered at 1247 cm^{-1} in native PC-trimers disappears upon addition of PCMS, whereas the other bands remain essentially unchanged. The spectrum of β_2, on the other hand, exhibits no change which is beyond experimental uncertainty. Furthermore it is interesting to

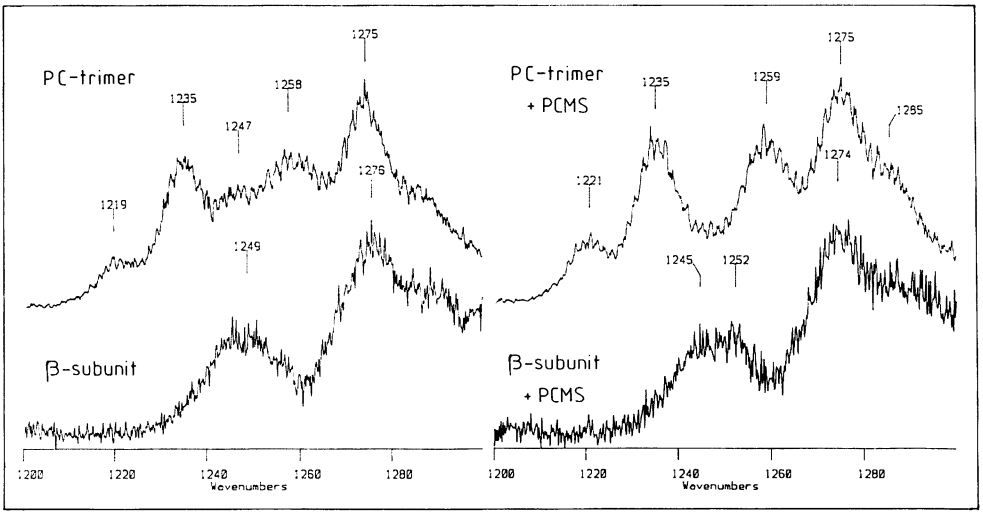


Fig. 3: Resonance-enhanced CARS spectra of PC trimers (left, top), PC β-subunits (left, bottom), PC trimers titrated with PCMS (right, top), PC β-subunits titrated with PCMS (right, bottom). Pumpwavelength 640 nm.

note that PC trimers, which have only one additional type of non-equivalent chromophore, show at least 3 more bands, namely at 1221, 1235 and 1259 cm^{-1}. The band which appears in both spectra (1245 - 1252 cm^{-1}) disappears upon PCMS binding in the trimer spectrum. This fact can be taken as additional evidence that it is the chromophore B84, whose geometry is changed predominantly upon PCMS binding. In the double bond stretching region (1550 - 1700 cm^{-1}) no changes upon PCMS titration have been observed for β_2 and only small, but significant ones are observed for PC trimers [16].

In order to demonstrate the large change induced in the fluorescence decay of PC trimers upon PCMS binding, the decay curves recorded with 600 nm excitation, but different observation wavelengths are shown in fig.4. Even without analysis it is obvious that the wavelength, above which a delayed onset of the fluorescence is observed, is increased from about 620 nm to 670 nm. Furthermore, it is apparent that the contribution of the long-lived component is much larger in the native system and this effect is increasingly pronounced at shorter wavelengths. In contrast to the pronounced effect found in PC-trimers, however, only a minor effect is observed for β_2 (small reduction in the lifetime of the terminal emmitter). In figures 5 to 8, the results (lifetimes deduced from a 3-exp. fit) are summarized for easier comparison. The following conclusions can be drawn:

In case of dimers of the β-subunit the lifetime of the long-lived component shows a slight reduction upon PCMS binding. Its apparent value varies with observation wavelength, but appears to be fairly independent of the excitation wavelength. A similar statement holds for the intermediate component. For the short-lived component, the situation is quite different. With increasing observation wavelength, the apparent decay time of the fast fluorescence increases by more than a factor of 2 up to 60 ps. In the red part of the emission,

the derived rise times are generally speaking below 20 ps. In the measurements close to the "turning" wavelength, an excitation wavelength dependence is apparent.

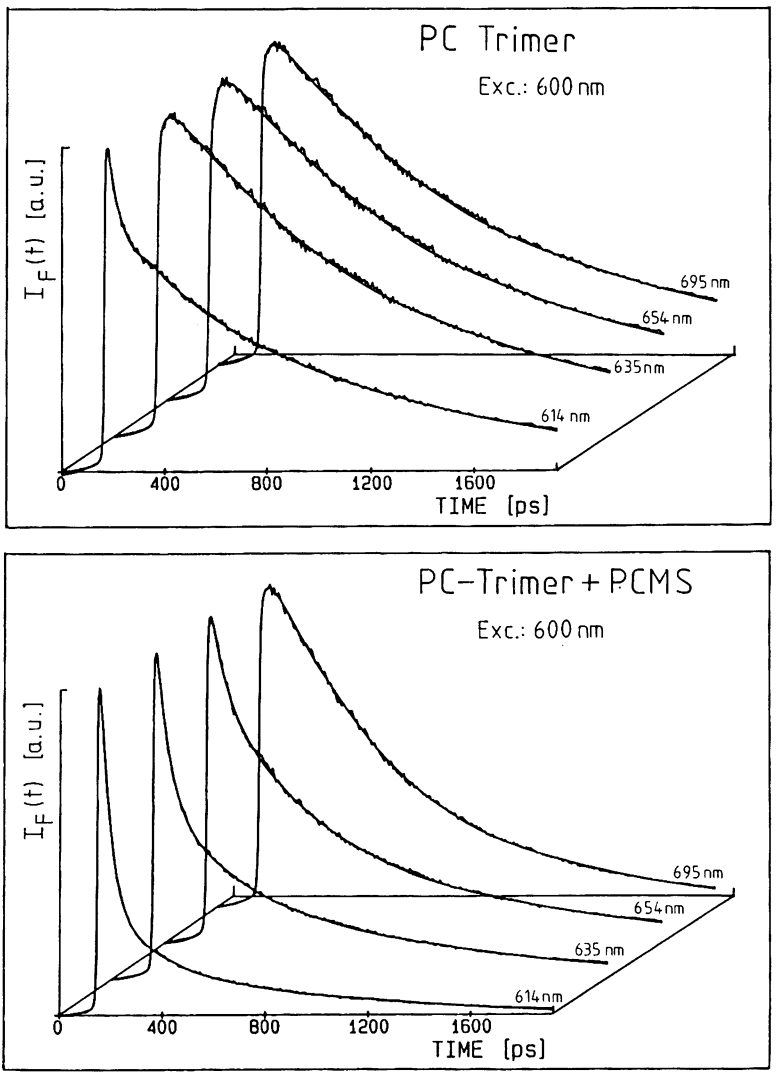
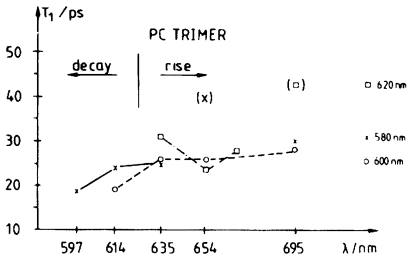
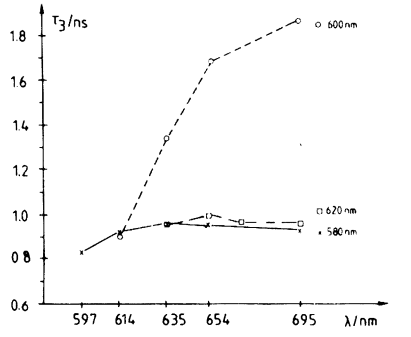
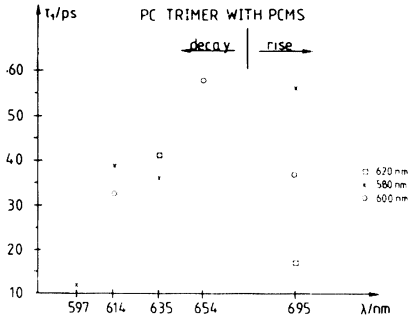


Fig. 4: Fluorescence decay of PC trimers and PC trimers titrated with PCMS.







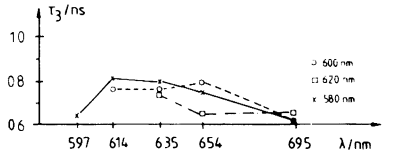
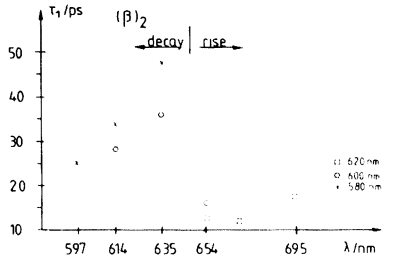
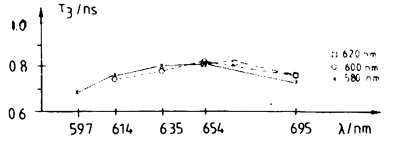
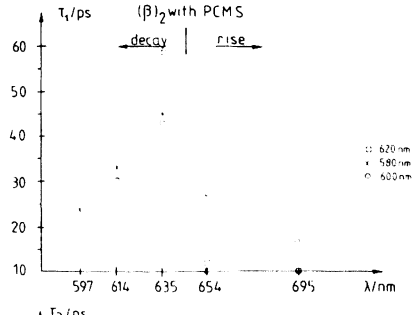


Fig. 5 ↑ Fig. 6 ↑

Fig. 7 ↓ Fig. 8 ↓







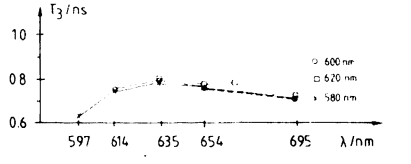


Fig. 5-8: Observed fluorescence lifetimes τ_1 and τ_3 of PC trimers in dependence of excitation (x=580nm, o=600nm, =620nm) and detection wavelength (abscissa).

Fig. 5: PC trimers. Fig. 6: PC trimers titrated with PCMS.
Fig. 7: Dimers of β-subunits from PC.
Fig. 8: Dimers of β-subunits from PC titrated with PCMS.

As one might already expect from the behaviour of the CARS spectra, the effects of PCMS binding are substantially different in case of trimers. In the "native" system, excitation around 600 nm seems to preferentially select a chromophore-protein moiety, which is characterised by a very long fluorescence decay time (1.8 ns). By PCMS-binding the formation of this species must be prevented, because no such long time constant is observed for any of the excitation wavelengths (600, 580 or 620 nm).
The apparent lifetime of the predominant terminal emitter is reduced upon PCMS binding from about 0.9 to about 0.7 ns. Similarly, the decay time of the intermediate component is reduced from approx. 200 ps to approx. 100 ps.
The lifetime of the shortest-lived component experiences an interesting PCMS-effect. In the "native" system the decay times derived from the emission on the short-wavelength side of the fluorescence spectrum (20-30 ps) agree fairly well with the rise times found in the emission on the long-wavelength side. However, a situation similar to that described for β_2 is found in the PCMS treated trimers. There is an increase of the decay time with observation wavelength and a dependence of the rise time on excitation wavelength.

Because of the change in sign of its amplitude with increasing observation wavelength and the appearance of similar values in the decay of the β-subunit (in monomeric β-subunits prepared by application of detergent, a very similar decay pattern as in β_2 is observed) the 20 - 30 ps lifetime is assigned to the decay of the excited state population of B155 (in agreement with other authors [18, 19]).

The observed decrease in the lifetime of the terminal emitters upon PCMS binding could also be due to changes in radiationless decay rates, caused by the reduced energy gap ΔE ($S_1 \rightarrow S_0$). It can not be taken as unambigous indication for strong coupling between 1A84 and 2B84 chromophores.

The intermediate lifetime has been related in the past [20, 21, 22] to the energy transfer between 1A84 and 2B84 chromophores. Our present results do not support this interpretation for several reasons:

(i) A component exhibiting a lifetime of around 200 ps was found not only in the trimers, but also in monomers and both α-and β-subunits.

(ii) This component was always detected as a decaying component. Representing a transfer time, its amplitude should change sign for long wavelength detection similar to the one of the fast component or at least decrease in amplitude at long wavelength detection.

(iii) The amplitude was always above 10%. If it were related to a transfer between two chromophores with similar absorption and emission characteristics, the amplitude should be very small (it is expected to be proportional to the difference of the spectral properties, i.e. emission or absorption in case of pump-probe experiments).

We therefore believe that the intermediate component is connected with a modified chromophore-protein arrangement. E.g., we have found a lifetime in the 200 - 300 ps time domain in chromopeptides, where it is suspected that the chromophore adopts a ZZE configuration.

Before entering the discussion about the coupling of the 1A84 - 2B84 pair, it should be mentioned that, when pump-probe experiments were performed on PC trimers [14], all absorption recovery curves could be fit very well by assuming a biexponential decay law. The derived long lifetime varied around 950 ps, the shorter one between 20 and 100 ps, depending on wavelength of pump and probe beam.

Combining the results of transient absorption and fluorescence experiments we conclude that the energy transfer time between the 1A84 - 2B84 pair is on the order of or faster than the measured fast component. Then, the fast component is actually a superposition describing two processes (i.e. a s-to-f transfer from B155 and a f-to-f transfer A84/B84). Since the coupling strength between the A84 - B84 pair is sensitive to variations in the electronic properties of the chromophores, the pronounced wavelength dependence could be understood. That is to say we relate the observed dependence of the short lifetime on excitation and detection wavelength to the existence of a microheterogeneity in chromophore-protein arrangement which effects mainly the coupling between A84 and B84 of neighbouring monomers. Model calculations [23] have shown that small changes in the protein environment (e.g. formation of tautomeric forms of amino acid residues) are sufficient to shift the excitation energy of one chromophore by 10 nm.

Preliminary experiments using the CARS technique suggest that the microheterogeneity postulated here for the smaller aggregates is reduced when linker peptides are present as in phycobilisomes [24]. Deviations from exact threefold symmetry seem nevertheless to occur even in the larger systems. Because of the overall arrangement of the chromophores in the light-harvesting complexes a few "defects" should not reduce significantly the efficiency of energy transfer to the reaction center.

Acknowledgement

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