Photosynthetic Light-Harvesting Systems

Organization and Function

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PICOSECOND TIME-RESOLVED ENERGY TRANSFER KINETICS WITHIN C-PHYCOCYANIN AND ALLOPHYCOCYANIN AGGREGATES

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Introduction

The light-harvesting complexes of cyanobacteria and red algae, are supramolecular aggregates, so-called phycobilisomes (PBS), situated at the outer surface of the thylakoid membranes (1,2,3). They are composed of a central core of 2-3 cylinders to which usually six rods are connected. The core is mainly composed of allophycocyanin (APC) trimers, while the building blocks of the rods are hexameric units of phycocyanin (PC), phycoerythrocyanin (PEC) or phycoerythrin (PE) (1).

Due to the complex structure of phycobilisomes and the presence of several hundred chromophores that interact with each other in a complicated way it is difficult to determine experimentally the rate of each individual transfer step. One way to obtain a more detailed understanding of the excitation energy transfer between neighbouring chromophores would be to study the energy transfer in smaller biliprotein aggregates. Of special interest are C-phycocyanin (C-PC) aggregates, since the structures of C-PC trimers of Mastigocladus (M.) laminosus (5,6) and C-PC hexamers of Agmenellum quadruplicatum (4) have recently been determined at high (2.1-2.5 Å) resoultion by X-ray crystallography. From the crystallographic and spectroscopic (7,8) data it should in principle be possible to calculate the energy transfer rates in these systems assuming that the Förster merchanism (9) for energy transfer is in

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operation. Efforts in this direction have already been made by Sauer et al (10). The aim of this work was to study the energy transfer kinetics of C-PC and APC monomers and trimers of M. laminosus on the picosecond and femtosecond scale and to study the relaxation of the light-induced anisotropy. One interesting aspect would be to compare the energy transfer in C-PC and APC monomers and trimers. For structural and spectral reasons one might expect that the transfer rate between the α -84 and β -84 chromophores should be similar in C-PC and APC monomers.

Experimental

The C-PC and APC trimers of M. laminosus were prepared according to the method given in ref 11 and 12, respectively. Monomers were obtained by adding NaSCN to 1.2 M. No further check of the aggregation state for the monomers was made. The absorption maximum was at 615 and 611 nm for the C-PC trimer and the monomer, respectively. For APC trimers it was 652 nm and for monomers 615 nm. The picosecond measurements were made in a rotating cell of 1mm optical pathlength and the absorbance for both the trimers and the monomers were in the range 0.8-02 (in 1mm cells).

In order to follow the kinetics of energy transfer we employed the picosecond absorption recovery method with continuously tunable excitation and probing ligth. The laser system used to generate the picosecond pulses as well as the measuring technique have previously been described in detail (13). In short, the picosecond pulses were generated in a mode-locked and cavity-dumped dye laser, which was syncronously pumped by a mode-locked argon ion laser. The cavity dumper was operated in the 80-800 kHz range and typically gave ca. 10 ps long pulses (FWHM) of 1-2 nJ energy in the 580-670 nm wavelength range. The polarization of the pump and probe beams were controlled by a Soleil-Babinet compensator and prism polarizers, so that the absorption recovery kinetics could be measured with any relative orientation of the pump and probe polari-

zations. Measurements with parallel (I_{11}) and perpendicular ($I_{\perp}$) polarization were used to monotor the decay of induced anisotropy, $r(t) = (I_{11} - I_{\perp}) / (I_{11} + 2I_{\perp})$ and measurements at the magic angle (54.7°) were used to obtain the isotropic decay, free of depolarization effects. In some pump-probe experiments we used ca. 400 fs pulses from a fiber-grating pulse compressor.

Results and discussion

C-Phycocyanin monomers and trimers

In Fig. 1 we show the absorption recovery kinetics of C-PC monomers at 580 nm with different polarization of the excitation and the probe pulses. The data were analyzed by fitting them to a sum of two or three

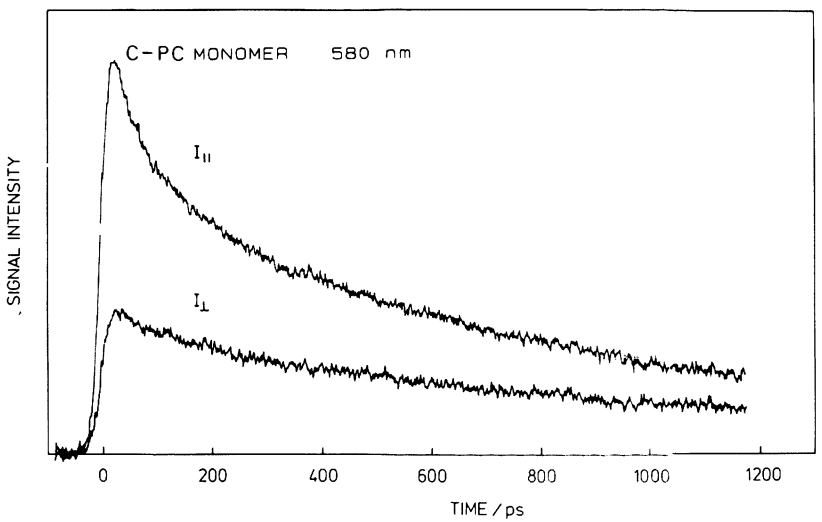

Fig.1

exponentials. The mean values of lifetimes and amplitudes obtained at different wavelength intervals are shown in table 1. In table 2 the corresponding anisotropy relaxation times and amplitudes are shown. From these data it is clear that besides a long lifetime in the ns range.

Table 1

Lifetimes (τ_1) and relative amplitudes (R_1) obtained from isotropic signals of C-PC monomers at different wavelength, intervals.

nm	τ_1 (ps)	R_1 (%)	τ_2 (ps)	R_2 (%)	τ_3 (ps)	R_3 (%)
580-600	57±4	27±2	665±101	62±1	4000	14±9
635-640	178±64	25±5	893±151	75±5		

Table 2

Anisotropy relaxation times (τ_{r_1}) and amplitudes (r_1) of C-PC monomers at different wavelength intervals.

nm	τ_{r_1} (ps)	r_1	τ_{r_2} (ns)	r_2	r(0)
580-590	52±19	0.13±0.03	2.6±1.5	0.28±0.03	0.41±0.01
635-640	36±8	0.08±0.04	4.4±0.1	0.29±0.04	0.37±0.02

we observe a lifetime of ca. 57 ps at shorter wavelength (∿ 590 nm), where the β-155 chromophore absorbes strongly (7,10). At longer wavelength, i.e. 635-540 nm, the fastest lifetime increases to 178 ± 64 ps. The most direct interpretation of these data is that the 57 ps lifetime is due to energy transfer between β-155 and β-84 within a C-PC monomer unit. As can bee seen in table 2 this transfer step is accompanied by an equally fast relaxation of the anisotropy from 0.41 + 0.01 to 0.28 + 0.03. This last anistropy is similar to that found in steady state fluorescence measurements (7).The centrum distance (R) between β-155 and β-84 in C-PC of M. laminosus 34.3 Å (5) and the

orientation factor $\kappa = 0.84$. With the radiative lifetime $\tau_o = 2$ ns we obtain a Förster radius (R) of 61 Å, using the equation;

$$\frac{1}{\tau} = \frac{3}{2\tau_o}\kappa^2\left(\frac{R_o}{R}\right)^6$$

where τ is the measured lifetime of energy transfer and under the assumption that the rate of back transfer is comparatively small. Because the observed transfer rate (k) between two chromophores is the sum of the ratios in the forward (k_1) and back directions (k_{-1}) and inclusion of 25 % back transfer would just reduce the calculated value of R_o with 3.5 %. The calculated Förster radius is in fair agreement with litterature data and (10) thus one might conclude that the Förster mechanism for energy transfer is in operation in the C-PC monomers. A similar analysis of the long wavelength lifetime of 178 ps indicates that this is due mainly to the transfer step α-84 → β-84. Assuming similar rates for the forward (k_2) and back (k_{-2}) energy transfer, we calculate (with R = 50.2 Å and κ = 1.73) that R_o = 52 Å.This value is reasonable and as expected, due to the smaller overlap between donor emission and acceptor absorption, lower than for the β-155 → β-84 transfer.

Table 3

Lifetimes (τ_i) and relative amplitude (R_i) obtained from the isotropic signals of C-PC trimers at different wavelengths.

nm	τ_1(ps)	R_1 (%)	τ_2 (ps)	R_2 (%)	τ_3 (ps)	R_3 (%)
580-590	27±3	40±7	106±27	14±5	1162±67	46±5
616-625	27±6	40±7	173±85	27±8	1228±242	33±8
635-645	48±12	35±9	429±143	24±9	1190±287	41±17

Turning now to the C-PC trimers, we observe (see table 3) that the fastest process has a lifetime of 27 ps at 625 nm and increases to 48 ps at about 640 nm. There is also a long (fluorescence) lifetime of about 1.2 ns at all wavelengths. In addition, there is an intermediate lifetime that varies from about 200 to 400 ps when going to longer excitation wavelengths. Similar lifetimes were observed in the anisotropy measurements (table 4).

Table 4

Anisotropy relaxation lifetimes (τr_1) and amplitudes (r_1) of C-PC trimers at different wavelengths.

nm	τr_1(ps)	r_1	τ_2(ps)	r_2	r(0)	r(∞)
580-600	24±5	0.18±0.02	108±36	0.10±0.02	0.42±0.02	0.14±0.03
616-635	21±8	0.17±0.03	222±77	0.06±0.03	0.38±0.02	0.15±0.03

The final anisotropy at longer times of 0.05 is just about half the anisotropy found for the monomers. This of course is a reflexion of the final distribution of the excited state is over more chromophores in the trimers.

The interpretation of the observed lifetimes is of course more complex in the trimers than in the monomers, since the number of possible interactions is larger. From the crystallographic data, however, the by far closest pair of chromophores is α -84 and β -84 of adjacent monomers. With R = 20.8 Å and κ= -1.34 and R_o= 52 Å (see above) one would expect lifetime of about 1.5 ps (assuming that the backward and forward rates

are similar). No such fast process was found, however, in our picosecond study. We therefore also performed some experiments on C-PC trimers at 618 nm with 400 fs pulses (Fig. 2)., but we were unfortunately

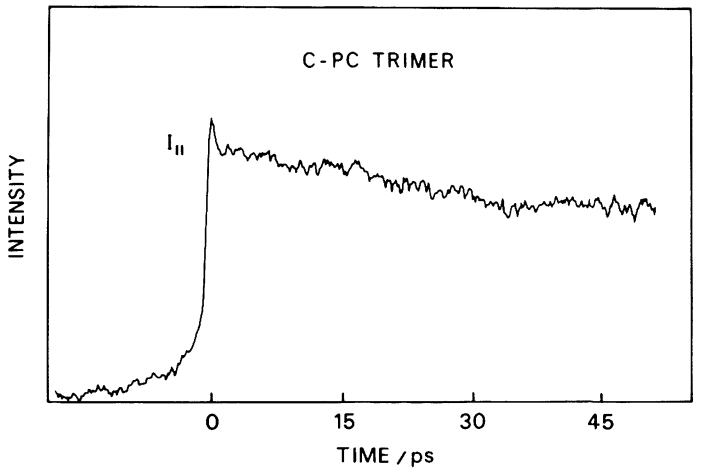

Fig.2

not able to resolve any lifetime (isotropic or anisotropic) in the interval 0.5-25 ps. The 27 ps component thus seems to be to slow for an α-84 → β-84 transfer. It has been attributed to transfer from β-155 to α-84 and/or β-84 from time-resolved fluorescence stdues (16) and our data at 580-590 nm would support this interpretation, however, going toward longer wavelengths, i.e. 616-625 nm, the relative amplitude of this component should decrease and it should only be about 10% at 640 nm. Where the absorption of β-155 is small (7,10). Since this is contrary to our data (see table 3) we must conclude that transfer among α-84 and β-84 chromophores or other processes also contribute to this component.

Allophycocyanin monomers and trimers

Again we will start with the monomer units. In Fig. 3 and absorption recovery measured at 610 nm is displayed cleary shows a biphasic decay. The fast small amplitude component has a lifetime of 144 ps, while the dominating decay has a lifetime of 1.3 ns. Since APC monomers only have two chromophores (α-80 and β-81) the short lifetime should be due to energy transfer between these chromophores. The fact that we can observe this signal means that the absorption spectra are not identical, however, the small amplitude of the signal indicates that the spectra are strongly overlapping. This might be expected when the spectral similarity to C-PC monomers is considered. We also note that within the experimental error the 144 ps component is the same as the corresponding energy transfer component found in C-PC monomers. Thus one may can conclude that the chromophores are situated on similar positions in the C-PC and APC monomers. This also what one would expect from the homology between the two proteins (12).

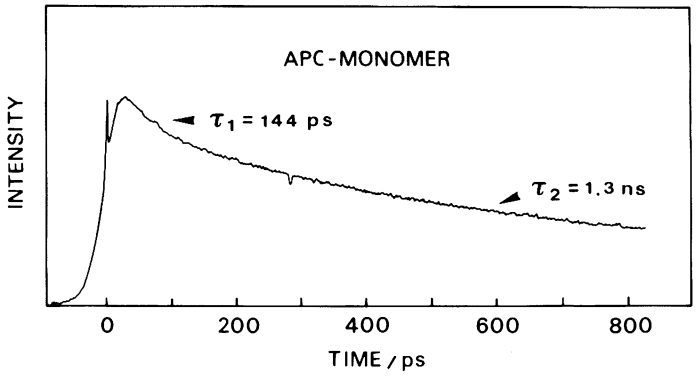

Fig. 3

APC trimers are the smallest aggregates of APC occuring in the core of the phycobilisomers. So far there has been no report on subnanosecond energy transfer processes in APC trimers. In this work we have observed a fast process with a lifetime of 45 $\pm$ 10 ps (see Fig. 4). Since the amplitude of this component is substantial (ca 60%) at 642 nm, the transfer has to take place between chromophores with different absorption spectra. The relative amplitude also increases in going from 670 to 630 nm as expected if this is a normal Förster (donor-acceptor) type of transfer. It is interesting to compare the APC with the C-PC trimer data about 640 nm, where a 48 $\pm$ 7 ps process was observed in CPC. This suggests that a transfer between α -80 and β -81 chromophores is responsible for this component. Since our preparation contained a small amount (< 10%) of chromophore with a red-shifted absorption spectrum (max $\sim$ 675 nm) it is however not possible to exclude that the process is partly due to a transfer of excitation energy to this chromophore in combination with a quenching process. We used 400 fs pulses at 648 nm in some experiments (Fig. 4) to investigate it there is any fast process in the range 0.5-10 ps. However, we could not observe such a process in the isotropic or anisotropic decay. One interesting finding was that the anisotropy at t=0 was only about 0.2 (Fig. 4) in stead of 0.4 as expected. This indicates that there is a fast (< 0.5 ps) unresolved anisotropy relaxation process. This might be a transfer of excitation between closely spaced states with differently directed transition dipole moments. Such states could for instance be formed in a strongly coupled dimer, (excitonic states), which has been suggested to give rize to the 652 nm absorption band in APC-trimers (15). The anisotropy at longer times is similar to the steady state value (r $\sim$ 0.05) (14).

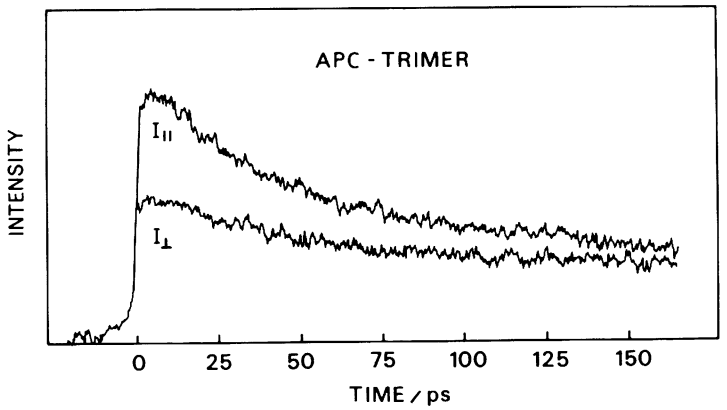

Fig. 4

It is clear from this study of APC trimers that the energy transfer and related processes are more complex than what one would expect considering the relative simplicity of the system. To understand the physical meaning of these processes further work is urgently needed.

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