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# Femtosecond spectral and anisotropy study of excitation energy transfer between neighbouring $\alpha$-80 and $\beta$-81 chromophores of allophycocyanin trimers

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**Abstract**

Polarization pump-probe femtosecond spectroscopy was used to investigate photoinduced optical density changes in allophycocyanin (APC) trimers at 635–690 nm after excitation with 230-fs pulses at 618 nm. The initial bleaching observed at $\lambda < 645$ nm is followed by subpicosecond absorption recovery corresponding to $430 \pm 40$ fs recovery kinetics measured at 615 nm with 70-fs pulses. Only the red part of the APC absorption band remains strongly bleached at 3 ps after excitation. The spectral and kinetic results can be described in terms of two different models of interaction between neighbouring $\alpha$-80 and $\beta$-81 chromophores of APC trimers. According to the first one, the observed subpicosecond kinetics corresponds to relaxation between the levels of excitonically coupled, spectrally identical $\alpha$-80 and $\beta$-81 chromophores. Excited state absorption to doubly excited excitonic state should in this case contribute to the measured difference spectra. According to the second one, the femtosecond excitation energy transfer in APC trimers takes place between a donor chromophore absorbing predominantly at 620 nm and an acceptor chromophore absorbing at 650 nm. The high anisotropy value observed at 615 nm during the first 1.2 ps is in good agreement with the donor-acceptor model. Anisotropy values calculated in the 635–675 nm spectral region at 3 ps after excitation are in the 0.1–0.25 range corresponding to an angle of 30°–45° between donor and acceptor transition dipole orientations. The high anisotropy obtained at 658 nm during the excitation is probably due to stimulated emission of the donor chromophore.

*Keywords:* Allophycocyanin; Excitation energy transfer; Femtosecond spectroscopy; Anisotropy; Photosynthesis

## 1. Introduction

Allophycocyanin is a photosynthetic antenna pigment of cyanobacteria and red algae. It is located in extra-membraneous light-harvesting complexes, so-called phycobilisomes, which distinguish cyanobacteria and red algae from other algae and green plants. Allophycocyanin (APC) and other pigments of phycobilisomes, namely C-phycocyanin (C-PC), phycoerythrin (PE) and phycoerythrocyaninn (PEC), harvest solar energy in regions of the visible spectrum where chlorophyll absorption is low and subsequently transfer the excitation energy to chlorophylls in the photosynthetic membrane. These pigments are chromoproteins (called phycobiliproteins) containing linear tetrapyrrole chromophores covalently linked to the apoprotein via a cysteine linkage [1–3].

The basic building blocks of the phycobilisomes are trimeric units of the different biliproteins. Each monomer unit of APC consists of two subunits ($\alpha$ and $\beta$) each containing one phycocyanobilin chromophore ($\alpha$-80 and $\beta$-81, respectively). In comparison, the $\beta$-subunits of C-PC and PEC contain two phycocyanobilin chromophores ($\beta$-84 and $\beta$-155) and the

Abbreviations: APC, allophycocyanin; c-Pc, C-phycocyanin; PE, phycoerythrin; PEC, phycoerythrocyanin; CPM, colliding pulse mode locked.

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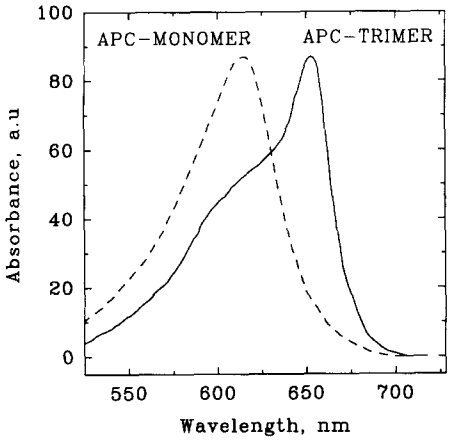


Fig. 1. Mutually normalized absorption spectra of APC trimers in 100 mM potassium phosphate buffer, pH 7 (solid line) and APC monomers prepared by adding 1.2 M of NaSCN (dashed line), both measured at room temperature. The trimer absorption maximum is located at 653±2 nm. The ratio of absorption at 653 nm (15314 $cm^{-1}$) to 620 nm (16129 cm) is 1.6. The monomer absorption maximum is located at 615 nm (16260 $cm^{-1}$). The excitation with femtosecond pulses was achieved at 618 nm (16181 $cm^{-1}$), and the spectral pulse width was 4 nm (100 $cm^{-1}$).

$\alpha$-subunits contain only one ($\alpha$-84) phycocyanobilin (or phycoviolobilin in the case of PEC) chromophore. Therefore, the trimeric unit of APC contains six chromophores, whereas those of C-PC and PEC contain nine chromophores.

The conformation of the linear tetrapyrroles is modified by the apoprotein. This is probably the most important factor by which the spectral characteristics of phycobiliproteins are optimized for high absorption and for efficient excitation transfer processes. APC is situated in the core of the phycobilisome and is closest to the Photosystem II reaction center which finally accepts the excitation energy. This position close to the final acceptor is reflected by the red-shifted absorption spectrum of APC trimers in comparison with other biliproteins of cyanobacteria, even those containing the same phycocyanobilin chromophores (C-PC, PEC). The absorption spectrum of monomeric subunits of APC has a maximum near 615 nm which shifts to 653 nm upon trimer formation, indicating changes in the chromophores and their surroundings [2] (Fig. 1).

It is known from X-ray crystallography that $\alpha$-84 and $\beta$-84 chromophores of different monomeric subunits in C-PC trimers are separated by only 20.8 Å [4–6]. Similar structures have been found for PEC [7] and PE [8] trimers. The X-ray structure for APC trimers is not yet available, but one might assume that conformational changes and/or excitonic interaction between the $\alpha$-80 and $\beta$-81 chromophores are responsible for dramatic changes in the absorption spectra upon trimer formation.

In intact phycobilisomes several hundred chromophores interact and participate in excitation energy transfer processes. Among them excitation transfer between the closest $\alpha$-80 and $\beta$-81 chromophores is expected to be fastest and to occur on the femtosecond time-scale. We have recently measured with 70-femtosecond resolution the absorption polarization kinetics in APC trimers at 615 nm [9–11]. The measurements were made using a one-colour scheme (i.e., excitation and probing were at the same wavelength). A 440 ± 30 fs decay of the initial bleaching was observed in different APC preparations. These kinetics were attributed to Förster excitation energy transfer between a donor chromophore absorbing at about 620 nm and an acceptor absorbing at 650 nm. No excitonic interaction was invoked to explain this femtosecond process. The complete recovery of the initial absorption at 615 nm was observed in intact preparations with femtosecond kinetics. A model of energy transfer in APC trimers taking into account both femtosecond and picosecond (> 10 ps processes was developed based on distorted or heterogeneous APC trimers [10,11].

In this publication we report results on APC trimers in the 635–690 nm spectral region obtained by two-colour polarization pump-probe femtosecond spectroscopy. In this spectral region we assume the absorption of 650-nm acceptor (or absorption to the lowest excitonic state), while excitation at 618 nm is assumed in the region of 620-nm donor absorption (or absorption to higher excitonic state). The situation where the absorptions of the two chromophores overlap strongly and the 620-nm shoulder of APC absorption band is vibronic band for both $\alpha$-80 and $\beta$-81 chromophores is also discussed. The high anisotropy value observed at 615 nm during the first 1.2 ps is in agreement with the donor-acceptor model assuming spectrally different $\alpha$-80 and $\beta$-81 chromophores.

## 2. Materials and methods

Pulses from a colliding pulse mode-locked (CPM) dye laser at 618 nm central wavelength with a pulse duration of less than 100 fs were amplified at 40 Hz repetition rate in a multi pass jet amplifier to an energy of 1 $\mu$J. The pump source for the amplifier was an excimer laser (Lumonix EX520) operating at 308 nm. An additional 15-mm length dye cell with transverse excitation was used to increase the pulse energy to 15 $\mu$J. The 6-aminophenalenone dye was used in both amplifier stages [12]. The amplified pulses were equally split into two channels (pump and probe). The pump beam was passed through a saturable absorber jet (malachite green in ethylene glycol) for suppression of the amplified spontaneous emission from the amplifier and the nonamplified background from the CPM laser. The pump beam then passed through a variable optical delay line and was focused on the sample cell to a spot

diameter of 150 μm. The final pump energy after additional attenuation was 180 nJ and the corresponding energy flux was $2.5 \cdot 10^{15}$ photons/cm$^2$ pulse.

The probe pulses passed through a $\lambda/4$ plate to produce circular polarization and were then focused into a 1-cm water cell for generation of femtosecond continuum. A RG12 colour filter placed after the cell cuts off the 620-nm light and allows us to use probe wavelengths longer than 635 nm. The difference spectra were measured over the spectral region 635–690 nm. After passing a dichroic thin film polarizer, the probe beam acquired polarization parallel or perpendicular to the polarization of the pump beam. A part of the probe beam was directed with a 50% reflection mirror into the reference channel. The energy of the references pulses at a given wavelength (after momochromator) and the probe pulses after sample cell and monochromator were measured with photodiodes. The data acquisition system has been described elsewhere [13]. The photoinduced changes of optical density $\Delta A(t)$ and anisotropy $r(t)$ were calculated according to standard formulae. in the case of kinetic measurements at 658 nm and 635 nm an interference filter was placed after the continuum generator instead of a monochromator. The pulse duration was estimated from an intensity correlation function measured for probe (at selected wavelengths) and pump pulses with a KDP nonlinear crystal. Assuming equal pulse duration for the pump and probe pulses, the pulse width was determined to be 230 fs due to group velocity dispersion in some optical elements.

The APC trimers of *Mastigocladus laminosus* were prepared as reported [14]. The ratio of the maximum absorption at 653 nm to that of the shoulder at 620 nm was 1.6. In our previous work [10] this preparation was identified as free of the 8.9 kDa linker peptide. The absorption spectrum is shown in Fig. 1. The preparations were kept frozen until used. Monomers were prepared by adding 1.2 M of NaSCN to the sample immediately before the measurements. All measurements were made at 20°C in a rotating cell of 2 mm optical path length. The optical density of the sample at the excitation wavelength was 0.5 for both monomers and trimers.

## 3. Results

Fig. 2 shows the trimer kinetics of the photoinduced optical density changes at 635 nm (thick dashed line) and at 658 nm (solid line) for parallel polarization of the pump and probe pulses. The initial bleaching observed at 635 nm is followed by absorption recovery. At the same time, only a rise term was observed at 658 nm. The kinetics obtained at 635 nm and 658 nm are essentially described by convolution of the molecular response function with the intensity correlation function of the pump and probe pulses. The integral of the intensity correlation function for 230-fs pump and probe pulses is shown in Fig. 2 by the thin dashed line. One can see some delay in the bleaching kinetics recorded at 658 nm. For comparison to results obtained at 658 nm, the bleaching kinetics obtained with APC monomers at 635 nm is shown by the dashed line in Fig. 3. According to the results obtained at 615 nm [9–11], no femtosecond processes except for the initial bleaching (excited state formation) were observed for APC monomers. Photoinduced optical density changes at 635 nm are in good agreement with the integral of the correlation function, additionally indicating the delay in bleaching kinetics measured with trimers at 658 nm. Decay kinetics measured at 635 nm very probably corresponds to the $440 \pm 30$ fs decay observed earlier at 615 nm [9–11]. Fig. 4 shows the kinetics obtained at

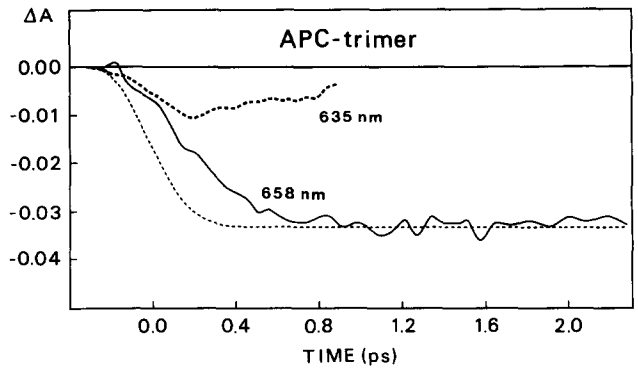


Fig. 2. Photoinduced optical density changes ($\Delta A$) measured at room temperature in APC trimers at 635 nm (thick dashed line) and at 658 nm (solid line) after the excitation with 230-fs pulses at 618 nm. The kinetics were measured at parallel polarization of the probe light relative to the polarization of the excitation light. The thin dashed line is a convolution of the step response function with the correlation function between the 230-fs pump and probe pulses.

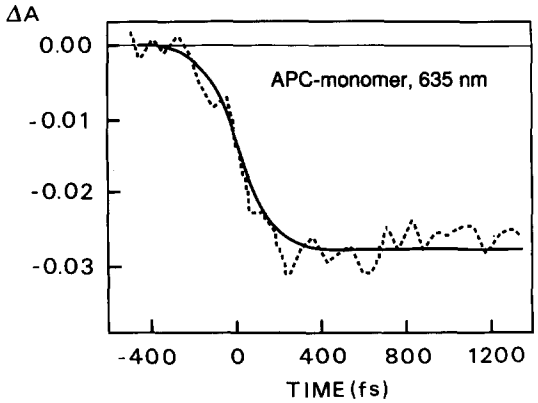


Fig. 3. Photoinduced optical density changes ($\Delta A$) measured at 635 nm in APC monomers at room temperature with parallel polarizations of pump and probe pulses (dashed line). The solid line is a convolution of the step response function with the correlation function between the 200-fs pump and probe pulses.

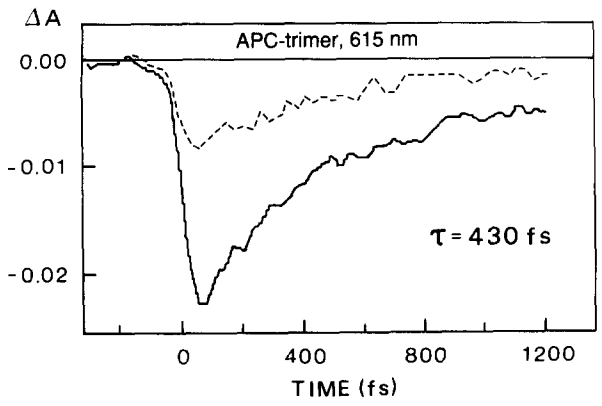


Fig. 4. Photoinduced optical density changes ($\Delta A$) observed at 615 nm on excitation of APC trimers at room temperature with 70-fs pulses at 615 nm. The polarization of the probe light is parallel (solid line) or perpendicular (dashed line) to the polarization of the pump light.

615 nm when the same experimental technique as described in Refs. [9–11,13] (70-fs pump and probe pulses at 615 nm) was used. The bleaching occurs immediately during the excitation as a result of the ground state depletion. This bleaching is followed by $430 \pm 40$ fs recovery kinetics to the level of the initial bleaching for both parallel and perpendicular polarization of the probe pulse. Fig. 5 shows the corresponding anisotropy, $r(t)$, calculated in accordance to the formula:

$$r(t) = (\Delta A_{\parallel} - \Delta A_{\perp})/(\Delta A_{\parallel} + 2\Delta A_{\perp}) \quad (1)$$

where $\Delta A_{\perp}$ and $\Delta A_{\parallel}$ are the optical density changes for perpendicular and parallel polarization, respectively.

During the initial 1.2 ps after excitation the anisotropy value is within $0.36 \pm 0.03$. The excitation energy flux was varied in this experiment from the value described in Section 2 ($2.5 \cdot 10^{15}$ photons/cm$^2$ pulse) to 20-times less. No difference in the kinetics recorded

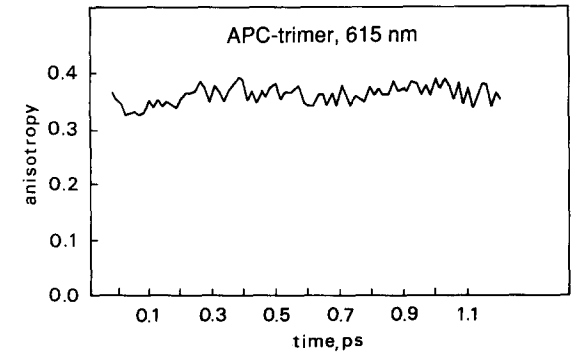


Fig. 5. Absorption anisotropy calculated from the decay kinetics measured for parallel and perpendicular polarization of the probe pulse at 615 nm relative to polarization of the pump pulse at the same wavelength.

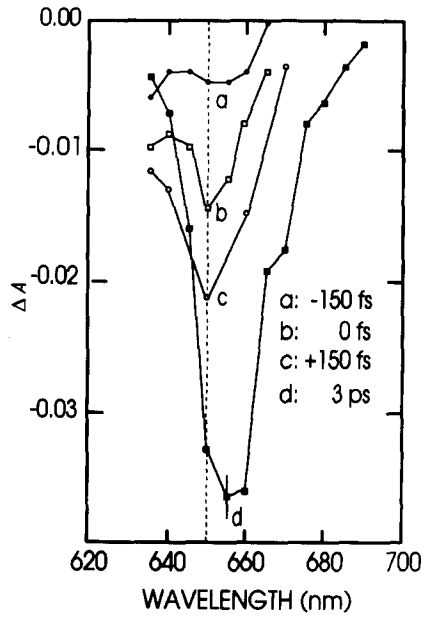


Fig. 6. Photoinduced optical density changes measured in the 635–690 nm spectral range at −150 fs, 0 fs, 150 fs and 3 ps after the excitation of APC trimers with a 200-fs pulse at 618 nm. The measurements were made in a 2-mm cell at room temperature. A typical standard deviation in the measurement is shown in the minimum of the 3-ps spectrum. Polarization of the probe light is parallel to polarization of excitation light. The dotted vertical line corresponds to the absorption maximum of the sample. A difference spectrum corresponding the bleaching of the APC absorption band as a whole and normalized at 650 nm with the 3-ps spectrum is shown by the dashed line.

at different excitation energies was observed, indicating that an excitonic annihilation process does not occur. The measured anisotropy value which is close to the theoretical maximum value of 0.4 indicates that no saturation effects take place at used excitation energies. It means that only a small part of the ground state molecules is excited by each pulse.

Fig. 6 shows difference spectra measured at −150 fs (a), 0 fs (b), + 150 fs (c) and 3 ps (d) delays between 230-fs pump and probe pulses in the 635–690 nm spectral region. The polarization of pump and probe beams was parallel. Comparing spectra a and b we conclude that bleaching of the 650-nm band probably occurs with a delay relative to the bleaching around 635 nm. The optical density changes at −150 fs delay near the maximum of the APC absorption spectrum are smaller than optical density changes at 635 nm. The measurements were repeated several times to assure reproducibility in the determination of the small absorption changes in the initial phase of the bleaching process. Nevertheless, the conclusion that the bleaching of the 620-nm band starts before the bleaching of the 650-nm band is not completely evident. We cannot exclude that different delays of different spectral parts of femtosecond continuum relative to the pump pulse are not completely compensated.

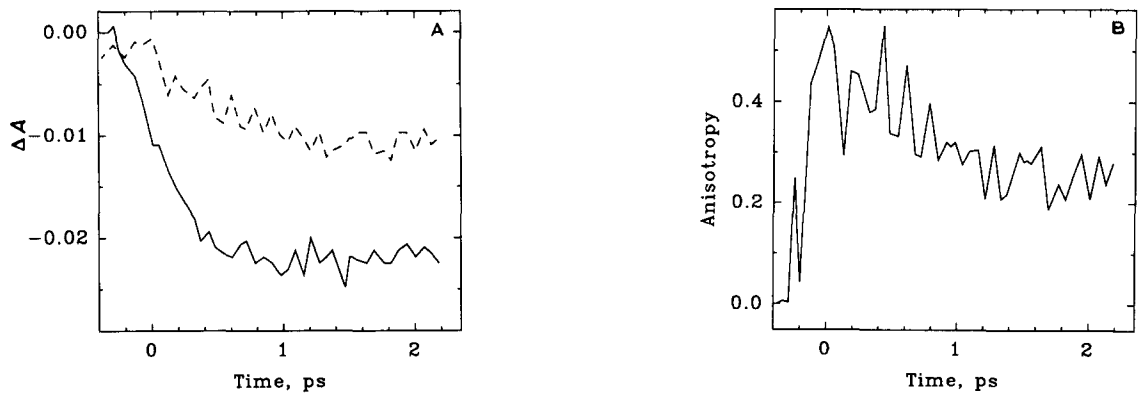


Fig. 7. Optical density changes ($\Delta A$) measured in APC trimers at 658 nm. Probe pulse polarization is parallel (solid line) or perpendicular (dashed line) to the polarization of the excitation light (top), and the anisotropy calculated from these results according to formula: $r(t) = (\Delta A_{\parallel} - \Delta A \perp)/(\Delta A_{\parallel} + 2\Delta A \perp)$ (bottom). The measurements were made at room temperature. High noise at $t < 0$ is due to the small optical density changes.

The spectra obtained at 0 fs and + 150 fs delays (spectra b and c in Fig. 6) show an evolution of the APC absorption band bleaching. The shape of these difference spectra is similar to the shape of APC absorption spectrum in the 635–670 nm region (Fig. 1), clearly indicating that both bands (650-nm peak and 620-nm shoulder) are bleached at those times.

Spectrum d in Fig. 6 represents the difference spectrum obtained at a 3 ps delay. Absorption changes measured at 635 nm are very small for this delay in accordance with the bleaching recovery kinetics represented in Fig. 2 by a dashed line. We conclude that bleaching in the 620-nm shoulder has almost completely disappeared. On the other hand, further pronounced bleaching takes place at 650 nm and at longer wavelengths.

The 3-ps difference spectrum is red-shifted relative to the APC absorption peak at 653 nm. Optical density changes are rather noticeable in the 680–690 nm (14700–14500 $cm^{-1}$) spectral region where the APC absorption is very small (Fig. 1). The most evident explanation of the 3-ps spectrum is that stimulated emission contributes in the red part of the difference spectrum in addition to the bleaching of the 650-nm absorption band.

All measurements described above were performed with parallel polarization of pump and probe pulses. Additional anisotropy measurements should be performed for further understanding of the femtosecond processes in APC trimers.

Trimer optical density changes measured at 658 nm for parallel (solid line) and perpendicular (dashed line) mutual polarization of the pump and probe beams are shown in Fig. 7A. The corresponding calculated anisotropy is shown in Fig. 7B. Although the data are very noisy, they indicate that the anisotropy decays during the excitation to a value of about 0.25. Fig. 8 (bottom) presents trimer difference spectra measured at 3 ps delay with parallel and perpendicular polarization of the pump and probe pulses (filled and open squares, respectively). The top figure (filled circles shows anisotropy values calculated in the 635–675 nm spectral region from these spectra and from the kinetic measurements. One can see rather large variations in the $r(t)$ values from point to point although all values are in the 0.10–0.25 range. For comparison the result obtained with picosecond pulses by Gillbro et al. [15] at 648 nm ($r = 0.2$) are indicated by the open circle in Fig. 8. Another situation occurs at 615–620 nm [9–11,16].

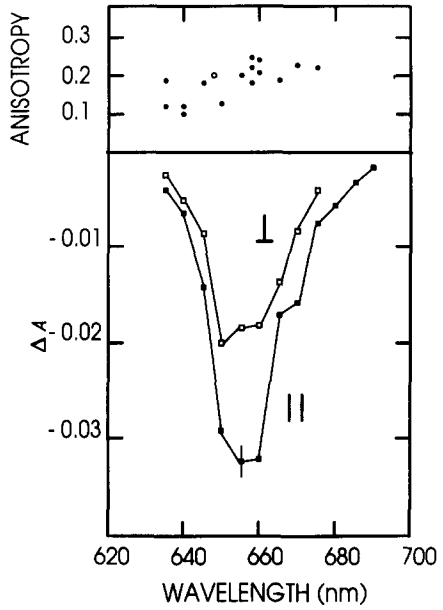


Fig. 8. Photoinduced optical density changes measured at 3 ps after the excitation of APC trimers with a 200-fs pulse at 618 nm. The polarization of the probe light is parallel (filled squares) or perpendicular (open squares) to the polarization of the excitation light (bottom). Anisotropy calculated in the 635–675 nm spectral range from the difference spectra presented in the figure and from additional polarization kinetic measurements (filled circles), and from the work of T. Gillbro et al. [14] (open circle) (bottom).

Anisotropy was equal to 0.4 for at least several picoseconds after the excitation and no anisotropy decay was observed during this period. This conclusion is also evident from Fig. 5.

## 4. Discussion

The absorption spectrum of APC monomers has its maximum at 615 nm. This maximum shifts to 653 nm and a 620-nm shoulder evolves upon trimer formation. Two different models have been proposed to rationalize these dramatic changes in the allophycocyanin spectrum. According to the first one (excitonic model), two excitonic states arise as a result of the splitting of the doubly degenerate $S_1$ electronic state of spectrally very similar $\alpha$-80 and $\beta$-81 chromophores. This idea was first proposed by MacColl and coworkers [17] and later further elaborated in fluorescence [18] and absorption [16] ultrafast measurements. In this model the trimer absorption spectrum as a whole belongs to both ($\alpha$-80 and $\beta$-81) spectrally nearly identical chromophores or, more correctly, to the excitonically coupled dimer formed upon aggregation.

The alternative explanation of the APC trimer absorption spectrum has been proposed recently on the basis of femtosecond absorption measurements [9–11]. In monomeric subunits both $\alpha$-80 and $\beta$-81 chromophores have absorption maxima near 615 nm. New chromophore surroundings in the trimer change the initially identical spectra of $\alpha$-80 and $\beta$-81 in different ways. It has been proposed that absorption spectrum of one of them ($\alpha$ or $\beta$) is very similar to the absorption spectrum in the monomer, indicating that this chromophore is almost unchanged in the process of trimer formation. Such chromophores form the 620-nm shoulder of the APC trimer absorption spectrum. The second chromophore is changed strongly upon trimer formation, forming the 650-nm absorption maximum. Efficient Förster energy transfer between neighbouring chromophores ($\alpha$-80 and $\beta$-81 chromophores of different monomeric subunits in trimer) due to better spectral overlap could be achieved in this way. It was proposed that one of the chromophores is an energy donor absorbing predominantly at 620 nm and the second chromophore is an acceptor absorbing at 650 nm (donor-acceptor model) [10]. This model is supported by the results obtained at 615 nm with 70-fs resolution. The recovery of the initial bleaching was observed as a result of 430-fs kinetics (Fig. 4). Such kinetics are typical for recovery of molecules to the ground state. The relaxation of donor molecules to the ground state is a result of excitation energy transfer and acceptor molecules in their excited state should be observed finally. However, it is rather unlikely that the residual bleaching observed at 615 nm is due to the bleaching of the 650-nm acceptor (see discussion of anisotropy results). According to the model of partial trimer distortion [10], this residual bleaching can be attributed to a fraction of the excited donor molecules which are still in their excited state having a picosecond lifetime.

Assuming a donor-acceptor model the relaxation of the excited donor molecules to their ground state is also reflected in the kinetics measured at 635 nm with 230-fs pulses (Fig. 2). Although we cannot exactly evaluate the femtosecond lifetimes from the data presented in Fig. 2, the delayed kinetics observed at 658 nm most probably corresponds to the formation of the acceptor in its excited state as a result of excitation energy transfer. A corresponding bleaching of the acceptor absorption band was also observed. 3-ps spectra measured with parallel and perpendicular probe pulse polarization (Fig. 8) clearly indicate that only the red part of APC absorption spectrum is still bleached at that time. The 3-ps spectrum is far more intense than the spectra measured at earlier times (Fig. 6). In the Results section we assigned the optical density changes in the red wing of the 3-ps difference spectrum to stimulated emission. The steady-state fluorescence of APC trimers has a maximum at 664 nm [18]. In the donor-acceptor interpretation we attribute this fluorescence to the 650-nm acceptor emission. Assuming that the fluorescence band is a mirror image of the acceptor absorption band, one can suggest equal contributions of ground state depletion and stimulated emission to the 3-ps spectrum around 660 nm. Therefore, the 3-ps spectrum should be twice as intense around 660 nm as a spectrum originating only from ground state depletion. Very small absorption changes are recorded in the blue part of the 3-ps spectrum because the 620-nm donor molecules are in their ground state at that time.

In the alternative explanation the 3-ps spectrum is a result of a 430-fs relaxation between excitonic states or vibrational relaxation. Strong excited state absorption should contribute in the blue part of the 3-ps spectrum (635 nm–640 nm) superimposing the bleaching signal and giving small absorption changes in that spectral region. This conclusion is also valid for 615 nm. The observed recovery kinetics (Fig. 4) can be attributed to the formation of excitonically or vibrationally relaxed excited state having an excited state absorption at 615 nm. The suggested excited state absorption peak arising at 615–640 nm after 440-fs relaxation process is not surprising for excitonic interaction. In the case of the $\alpha$-80/$\beta$-81 dimer such an excited state absorption could originate from the excitation of the second molecule in the pair and as a result a doubly excited dimer state will be formed [16].

Difference spectra measured at −150 fs, 0 fs and +150 fs delays indicate bleaching at 635 nm which corresponds to population of the donor excited state (or to

population of the unrelaxed excitonic state). At −150 fs delay the absorption changes at 635 nm are higher than around 650 nm. This fact is in good agreement with a donor-acceptor model because at earliest times only the donor is excited and the acceptor band should be bleached with a 440-fs delay. Nevertheless the 0-fs and + 150-fs spectra already indicate bleaching at 650 nm. Very probably a part of this bleaching is due to stimulated emission of the donor molecules. This transient stimulated emission should be blue-shifted relative to the stimulated emission of the acceptor. This fact is reflected in the red shift of the 3-ps bleaching maximum to 655–660 nm in comparison with 650 nm bleaching maximum at 0-fs and + 150-fs spectra.

Assuming the excitonic model, less bleaching at 650 nm in comparison with the bleaching at 635 nm observed at the −150-fs spectrum can be interpreted in the same manner as the 3-ps spectrum if the higher excitonic state has a strong absorption at 650 nm and a weak absorption at 615–635 nm. This means that excited state absorption peak is shifted from 650 nm to 620 nm as a result of relaxation between the excitonic states.

Summarizing our spectral and kinetic results we conclude that they are in agreement with the donor-acceptor model assuming that ground state depletion of 620-nm donor and 650-nm acceptor as well as stimulated emission of both molecules are the main processes forming the difference spectra. The results obtained with picosecond resolution [16] are also in agreement with this model. Almost complete recovery of the transient bleaching at 620–633 nm was observed during < 2 ps [16].

The obtained 430-fs time constant seems too long for relaxation from the upper excitonic state to the lower one. Recent investigation of exciton dynamics in the light harvesting complexes of *Rhodobacter sphaeroides* indicates a time constant of approximately 20 fs for interexciton state relaxation [19]. However, we cannot exclude the possibility of excitonic state relaxation for APC trimers completely because femtosecond kinetics and difference spectra obtained in the present work could be described by an excitonic model, taking into consideration a strong excited state absorption due to formation of a doubly excited state of the $\alpha$-80/$\beta$-81 dimer. The polarization results are very important for further understanding of the mechanism of interaction between $\alpha$-80 and $\beta$-81.

The absence of anisotropy decay measured at 615 nm (Fig. 5) is in agreement with the donor-acceptor model. The relaxation of excited donor molecules to the ground state observed as absorption recovery at 615 nm should not change the anisotropy because only the initially excited $S_0 \rightarrow S_1$ transition of donor molecules is involved in this process.

Anisotropy decay, however, should be observed at 615 nm during the first picosecond after the excitation if we accept the excitonic model. Excited state absorption will appear at 615 nm as a result of the 430-fs excitonic-state relaxation process. The anisotropy value depends on the orientation of the transition dipole corresponding to the excited state absorption, relative to the dipole of the initially excited transition. It will be close to the theoretical maximum value of 0.4 only if both transitions are parallel. However, the transition from the relaxed excitotonic state to a doubly excited state should be polarized perpendicularly to the transition initially excited at 615 nm [16]. Therefore, absorption recovery kinetics will be accompanied at 615 nm by anisotropy decay. However, such a decay was not observed (Fig. 5). Anisotropy decay was observed at 620–635 nm at times longer than several picoseconds [16]. Although a negative anisotropy value is the result of this decay, a process other than subpicosecond formation of relaxed excitonic state should be responsible for the formation of a perpendicularly polarized state. Assuming a donor-acceptor model, one can propose that energy transfer between acceptors in a trimer will produce a negative anisotropy on the picosecond time scale.

For a 3 ps delay the anisotropy in the 635–680 nm spectral range is determined by the acceptor orientation. Neglecting excited state absorption, anisotropy values obtained at a 3 ps delay, $r(t) = 0.10–0.25$ (Fig. 8A) correspond to an angle of 30°–45° between donor and acceptor orientations according to the formula:

$$r = 0.4(3\cos^2\theta - 1)/2 \quad (2)$$

The high anisotropy obtained at 658 nm during the excitation (Fig. 7B) is probably determined by stimulated emission of the initially excited 620-nm chromophore. In a recent fluorescence work by Fleming and coworkers [20] a subpicosecond fluorescence depolarization was observed at 650 nm (excitation at 605 nm), in agreement with our results.

Anisotropy results, however, could be in better agreement with a vibrational relaxation process rather than with an excitonic relaxation, if we suppose strong absorption from vibrationally relaxed state in the spectral region around the APC absorption maximum. Strong excited state absorption in the spectral region of the long-wavelength absorption peak was observed for a number of cyclic tetrapyrroles including chlorophyll *a* [21,22].

More detailed answers to still open questions concerning ultrafast processes in APC trimers might be obtained in experiments at different wavelengths with a time resolution better than 100 fs and with a better signal-to-noise ratio. To allow for comparison with the results of different investigations the APC samples should be better characterized. We have paid special attention to the ratio of absorption at 653 nm to

absorption at 620 nm which varies from 2.0 [10,18] to 1.3 [16,20]. Such variation was attributed to partial distortion of trimers assuming that the higher ratio is a characteristic of the more native sample [10], but particular attention has to be paid to the influence of linker polypeptides, which strongly affect the peak-to-shoulder absorption ratios. Finally, monomers can also partially participate in absorption, in particular in the absence of linkers, and at lower concentrations than used here.

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