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Femtosecond energy transfer processes in allophycocyanin and C-phycocyanin trimers

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Abstract. Ultrafast processes in C-phycocyanin and allophycocyanin trimers and monomers have been examined by means of polarization pump-probe technique. No femtosecond kinetics was observed in monomeric preparations. The absorption recovery kinetics with t=450±50 fs has been recorded in allophycocyanin trimers. The anisotropy decay kinetics was not obtained in femtosecond time domain. The conclusion about energy transfer between $\alpha 84$ and $\beta 84$ chromophores with different absorption spectra was made. The proposed model takes into account a stabilising role of linker peptide. Femtosecond anisotropy decay kinetics was obtained with C-phycocyanin trimers and explained by Förster energy transfer between $\alpha 84$ and $\beta 84$ chromophores with 65° angle between their orientations.

1. INTRODUCTION.

Allophycocyanin (APC) and C-Phycocyanin (C-PC) are photosynthetic antenna pigments of the light-harvesting complexes (phycobilisomes) of blue green bacteria and red algae. Our knowledge about the energy transfer in the phycobilisome has increased substantially during the last decade, see review works of Glaser (1985) and Holzwarth (1987). Important questions under investigations that still remain open are related to transfer of excitation energy between nearby chromophores in aggregates (mainly trimers) of C-PC and APC. One has, for instance, to discuss different models for the energy transfer including Förster and excitonic mechanisms. Of special interest is the APC trimer, since it contains only two kinds of chromophores, namely $\alpha 84$ and $\beta 84$ ones. The optical spectrum of the APC monomer has the maximum at 620 nm. In contrast to this, the spectrum of APC trimer has a sharp peak at 650 nm and a shoulder about 620 nm. In contrast to APC, C-phycocyanin monomer contains three chromophores, $\alpha 84$, $\beta 84$ and $\beta 155$, and absorption spectrum of the C-PC trimer with maximum near 620 nm shows close resemblance to that of the C-PC monomers.

The picosecond pump-probe technique has been applied to APC and C-PC monomers and trimers to investigate the excitation energy transfer (Sandström et al. (1988), Gillbro et al. (1988), Beck et al. (1990)). One can expect the fastest energy transfer processes between $\alpha 84$ and $\beta 84$, which form three small distance pairs upon formation of APC and C-PC trimers. The information about orientation of C-PC chromophores and distances between them (Schirmer et al., 1987), makes it possible to estimate rates of energy transfer processes in this pigment. We have examined the ultrafast processes in C-PC and APC monomers and trimers with femtosecond time resolution. Preliminary results obtained with APC were published in previous communication of Khoroshilov et al. (1991).

2.MATERIALS AND METHODS

The APC and C-PC trimers were isolated from *Mastigosladus laminosus*. We used two kinds of APC trimeric preparations. The absorption spectrum of the first one (Sample No 1) is very close to the spectrum of APC complex with 8.9 kD linker peptide (Holzwarth et al.,1990). The spectrum of the second preparation (Sample No 2) similar to the spectrum free of linker peptide APC with higher shoulder absorption. APC and C-PC monomers were obtained by adding, respectively, KSCN and NaSCN to 1.2 M directly before measurements. The measurements were made at 20° C in a rotating cell of 1 mm optical path length.

A 70-femtosecond pulses at 615 nm from CPM-laser were amplified at 10 kHz repetition rate in the multipass jet amplifier (Kryukov et al.,1988) and served both for pumping and probing. A pump-probe absorption measurements were made with polarization of probe pulse parallel or perpendicular to the polarization of the exciting pulse, and photoinduced anisotropy was calculated according to the standard formula.

3. RESULTS

Figure 1 shows the photoinduced optical density changes measured with the first APC preparation and Fig.2 exhibits results for the second APC sample. For both samples we have obtained 450±50 fs decay of the initial bleaching for both polarizations of a probing pulse. The photoinduced anisotropy is 0.4±0.1 for the first sample and 0.37±0.03 for the second one within two picoseconds after the excitation The anisotropy decay was not observed during this period. The main difference between two results is that for the second sample the rest bleaching measured after femtosecond decay is more than half of the bleaching measured immediately after the excitation, but approximately complete absorption recovery was observed for the first sample.

Figure 3 shows the optical density changes measured with trimers of C-PC within 1 ps period after the excitation. One can see the similar to APC femtosecond absorption recovery at parallel polarization of probing pulse and only the initial bleaching without any femtosecond recovery kinetics at perpendicular polarization. The anisotropy, calculated from experimental data, obtained within 5 ps, is shown in Fig.4. Immediately after the excitation the anisotropy is equal to 0.4 and decays to the level of 0.23±0.02. The anisotropy decay has 0.5+0.1 ps lifetime using one-exponential fitting (dashed line).

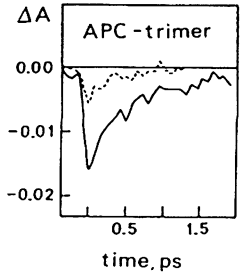

Figure 1. Absorption recovery of allophycocyanin trimers at 615 nm (Sample No 1) measured with probe pulse polarization parallel (solid line) and perpendicular (dashed line) to the exciting pulse.

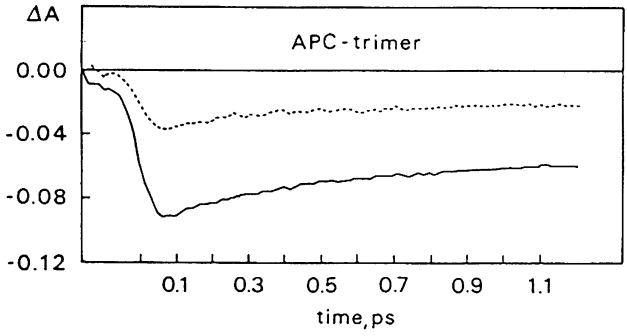

Figure 2. Absorption recovery of APC trimers at 615 nm (Sample No 2) with probe pulse polarization parallel (solid line) and perpendicular (dashed line) to the exciting pulse.

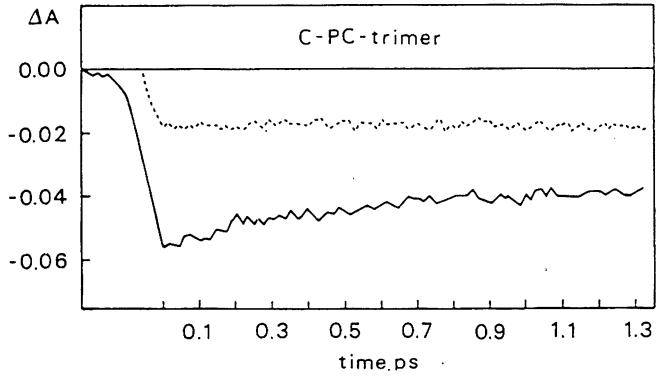

Figure 3. Absorption recovery of C-phycocyanin trimers at 615 nm with probe pulse polarization parallel (solid line) and perpendicular (dashed line) to the exciting pulse.

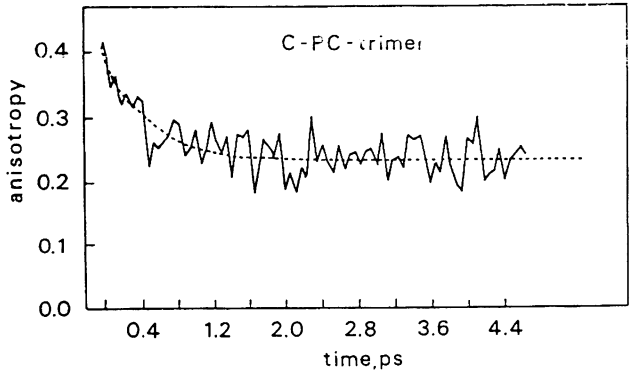

Figure 4. Anisotropy calculated for C-PC at 615 nm according to formula $r(t)=(\Delta A_{II}-\Delta A_{\perp})/(\Delta A_{II}+2\Delta A_{\perp})$ (solid line) and one-exponential fitting with 0.5 ps lifetime (dashed line).

No evident femtosecond kinetics was observed with APC and C-PC monomers within two picoseconds after the excitation for both polarizations of the probing pulse.

4. DISCUSSION

We can conclude from the experimental results that femtosecond processes take place only in APC and C-PC trimeric prepapations. We attribute the observed kinetics to energy transfer processes between adjacent $\alpha 84$ and $\beta 84$ chromophores in trimers. The results obtained with C-PC trimers are in a good agreement with Förster energy transfer calculations (Sauer and Scheer,1988) based on X-ray crystal structure data of Schirmer et al.(1987). .The lifetime of the experimentally observed anisotropy decay is determined by the sum of rate constants of direct ($\alpha 84 \rightarrow \beta 84$) and back ($\beta 84 \rightarrow \alpha 84$) energy transfer reactions. The rate constants were estimated by Sauer and Scheer (1988) from the distance between chromophores and their orientations and also from the absorption spectra of the individual chromophores published by Sauer et al.(1987). This sum is 3039 ns^{-1} for *M.laminosus* and corresponds to 330-fs kinetics. This kinetics is faster than depicted in Fig.4 using one-exponential fitting. But taking into account that 30-ps energy transfer processes were measured by Sandström et al.(1988) and calculated by Sauer and Scheer (1988) for C-PC trimers ($\beta 155 \rightarrow \beta 84$ process), it is possiple to describe the experimentally observed anisotropy decay by two-exponential fitting with t_1 =0.33ps and t_2 =30ps. The final anisotropy , which was obtained when the equilibrium between $\alpha 84$ and $\beta 84$ has been reached, depends on the angle between these two chromophores. Taking 65° for this angle (Schirmer et al.,1987) and using absorption cross sections of $\alpha 84$, $\beta 84$ and $\beta 155$ chromophores at 615 nm and

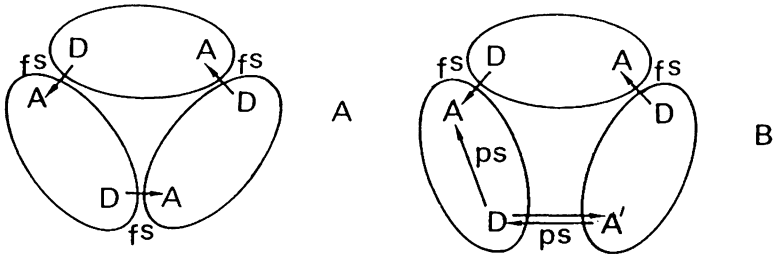

Figure 5. A model for fast energy transfer pathways in intact trimers (A) and in partly disintegreted trimers (B).

rate constants for direct and back reactions, we calculated the final anisotropy as 0.225 which is in agreement with the experimentally obtained value.

The explanation of the fast isotropic decay in APC trimers, which is not accompanied by a change of the anisotropy, would be a transfer of energy from a chromophore (α84 or β84) absorbing at 615 nm to another chromophore absorbing at 650 nm. Upon aggregation of monomers into trimers only one of the chromophores acquires a strongly red-shifted spectrum with maximum at 650 nm because of the new chromophore surrounding, while the other one might contribute to the shoulder at 620 nm. If the absorption of the 650-nm chromophore contributes negligibly to the absorption at 615 nm, one would expect no change in anisotropy during the energy transfer, but a complete recovery of the ground state absorption at 615 nm as the excited 615-nm chromophore transfers its energy to the other chromophore. This situation was realised with the first APC trimeric preparation and is described by the energy transfer scheme with C_3 -symmetry (Fig.5a). To explain the rest optical density changes obtained with the second APC preparation we invoked trimer heterogeneity (Fig.5b). This heterogeneity, probably, deals with the partial desintegration of the Sample No 2 because of the loss of the stabilizing role of linker peptide. As a result, the distance between donor and acceptor in one of three pairs is increased, and acceptor (A') acquires 620-nm spectrum instead of 650-nm one of another acceptors (A). This leads to the increasing of the absorption in 620-nm shoulder of the spectrum free of linker peptide APC. Excited at 615 nm donor D (or acceptor A') of this pair transfers its energy in picosecond time domain, and the rest bleaching, observed after femtosecond kinetics, is due to these chromophores in the excited state.

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