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Part III Spectroscopy and Advances in Measurements

Femtosecond Excitation Transfer in Allophycocyanin

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Allophycocyanin (APC), C-phycocyanin (C-PC) and other biliproteins (phycoerythrin and phycoerythrocyanin) of cyanobacteria and red algae harvest solar energy in regions of the visible spectrum having weak chlorophyll absorption. This excitation energy is then efficiently transferred to chlorophyll in the photosynthetic membrane [1]. Light-harvesting complexes of cyanobacteria (phycobilisomes) consist of several hundred chromophores. The smallest (monomeric) subunit of C-PC consists of three (α-84, β-84 and β-155) chromophores. The APC monomeric subunit consists of two (α-84 and β-84) chromophores. In phycobilisomes APC and C-PC monomeric subunits are organised as trimers. Absorption spectrum of APC monomeric subunit changes drastically upon trimeric formation. Absorption maximum shifts from 620 nm for monomeric preparations to 650 nm with a 620-nm shoulder for trimeric preparations.

Isotropic absorption recovery kinetics with τ=440 fs was recently observed in APC trimers at 615 nm [2-3]. The corresponding anisotropy was constant and close to 0.4 during the first few picoseconds. The results are consistent with a model of APC trimer in which α-84 and β-84 chromophores have different absorption spectra and femtosecond process corresponds to Förster energy transfer from 620-nm chromophore to neighbouring 650-nm one. Now new results from measurements in the 650-nm absorption band is presented here for further understanding of the ultrafast processses in APC trimers.

70-fs pulses at 620 nm from a CPM-laser were amplified at 100 Hz repetition rate in a multipass jet amplifier (pumping at 308 nm) and served as exciting pulses. A part of the amplified light was used for continuum generation. Pump-probe measurements were made with probe pulse polarization parallel or perpendicular to the polarization of the exciting pulse. APC was isolated from *Mastigosladus laminosus*.

Figure 1 shows difference spectra obtained in the 635-690 nm spectral region at parallel polarization of probe relative to the pump pulse. One can see that the bleaching of the 650-nm band is delayed relative to the bleaching of the 620-nm band. The strong red-shifted bleaching at 660 nm, due to stimulated emission, is further delayed relative to the bleaching of the 650-nm band. Fast recovery kinetics (corresponding to the 440-fs process observed at 615 nm [2-3]) was measured at 635 nm after initial bleaching (not shown). In contrast, only rise term was observed at 658 nm within the first picosecond after excitation. Decay of anisotropy from 0.4 to 0.2, however, occurs at that wavelength during this period. Femtosecond processes were not observed in monomeric preparations.

There are two possible explanations for the absorption spectrum of APC trimer. Due to the first one, 650-nm band and 620-nm shoulder both belong to α-84 and β-84 chromophores. One may believe that excitonic coupling between neighbouring α and β chromophores of different monomeric subunits is very strong (620-nm and 650-nm excitonic bands). For C-PC trimers the excitonic coupling (112 cm^{-1}) was calculated on the base of X-ray crystallography data [4]. Förster energy transfer between neighbouring excitonically coupled α and β chromophores with nearly similar absorption spectra was recently observed in C-PC trimers [5].

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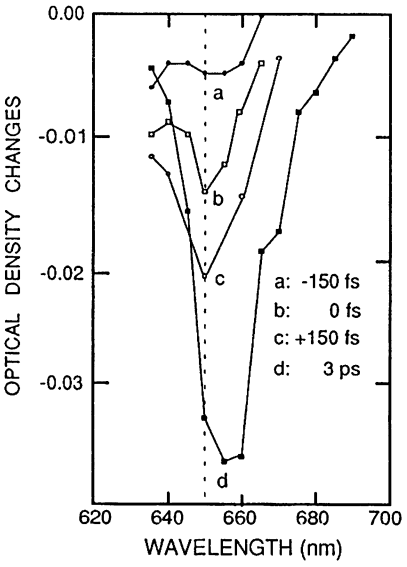

Fig.1 Difference absorption spectra measured at -150 fs (a), 0 fs (b). +150 fs (c) and 3 ps (d) delay after excitation at 620 nm with polarization of probe pulse parallel to the polatization of exciting pulse. Vertical line corresponds to APC absorption maximum

Unfortunately X-ray data are not available for APC trimers, but the excitonic splitting similar to C-PC could be assumed [6]. The 620-nm band in this case is a vibronic band of 650-nm electronic transition of α and β chromophores. An alternative explanation [3] is that one chromophore in a pair absorbs at 620 nm and the other (most likely β-84) at 650 nm due to a conformational change.

Our results strongly support the latter model. If the 650-nm band and the 620-nm shoulder belong to both chromophores, one should observe simultaneous bleaching of both bands due to ground state depletion. The delay in the bleaching of the 650-nm band (0 fs spectrum in comparison to the -150 fs spectrum) corresponds to Förster energy transfer from a 620-nm donor to the 650-nm acceptor. Absorption recovery observed at 615 nm [2-3] and at 635 nm is due to relaxation of donor molecules to the ground state as a result of excitation energy transfer. Acceptor molecules absorbing mainly at 650 nm participate to a small extend to the isotropic or anisotropic kinetics measured at 615 nm [3]. the strong bleaching appearing near 660 nm is red-shifted relative to the bleaching of the 650-nm band (3 ps spectrum in comparison to 150 fs and 0 fs spectra). This shift is probably due to vibrational relaxation in the acceptor's excited state. Anisotropy decay observed at 658 nm is a result of Förster energy transfer between differently oriented donor and acceptor chromophores (as predicted from C-PC data).

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