Dipole-Dipole Interaction in Phycobiliprotein Trimers. Femtosecond Dynamics of Allophycocyanian Excited State Absorption

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Difference absorption spectra of allophycocyanin trimers were measured in the 440 nm - 860 nm spectral range. Femtosecond kinetics measured in the spectral region of the ground state absorption support the model of the excitation energy transfer between the neighbouring α80 and β81 chromophores with different absorption spectra. Broad excited state absorption bands were observed around 500 nm and at wavelengths longer than 770 nm. A transient excited state absorption at 680 nm - 690 nm was observed at the earliest times. A general model of an excited state absorption resulting from a dipole - dipole interaction between a pair of molecules is given. The observed transient excited state absorption is probably due to the interaction between permanent dipoles of the neighbouring chromophores of allophycocyanin trimer.

I. Introduction

Phycobiliproteins are photosynthetic antenna pigments of cyanobacteria and red algae[1]. Like green plants, cyanobacteria and red algae contain two photosystems, and chlorophyll *a* with the absorption peaks near 430 nm and 670 nm is the main photosynthetic pigment of these organisms. Several spectrally different phycobiliproteins, namely C-phycocyanin (CPC), allophycocyanin (APC), phycoerythrin (PE) and phycoerythrocyanin (PEC), serve as accessory hight-harvesting pigments with strong absorption of sunlight in the spectral region between the main chlorophyll *a* absorption peaks. These pigments are chromoproteins containing linear tetrapyrrole chromophores covalently linked to the apoprotein via a cysteine linkage. Several hundreds bilin chromophores located in phycobilisomes (antenna complexes of blue-green bacteria and red algae) deliver the excitation energy mainly to the reaction center of Photosystem II.

The smallest functional blocks of the phycobilisomes are trimers of biliproteins. Each monomer unit of allophycocyanin consists of two protein subunits (α and β), each containing one phycocyanobilin chromophore (α80 and β81, respectively). APC monomer and trimer structures are schematically shown in Fig. 1. Three α80-β81 pairs are formed upon APC trimer formation from α80 and β81 chromophores of different monomer units. Similar pairs are found in other phycobiliproteins, e.g. CPC, PE and PEC. Together with APC they form a unique series of *in vivo* dimers located in phycobiliprotein trimers. The location of the neighbouring α and β chromophores in CPC, PEC and PE trimers

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is known from X-ray data[2-4]. The recent crystallographic data obtained for APC trimers[5] reveal similar distances between chromophores like in other phycobiliprotein trimers (Fig. 1).

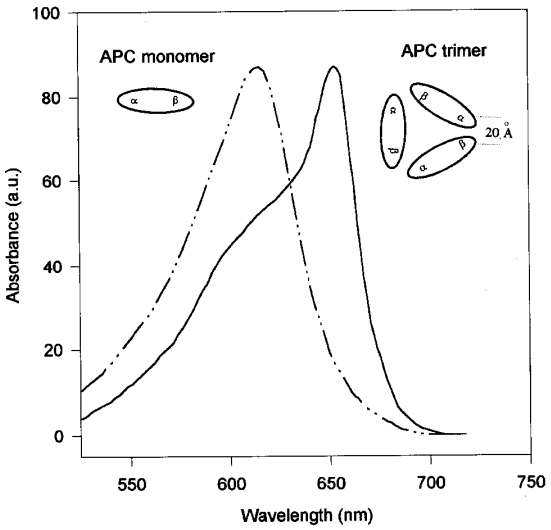

Figure 1. Mutually normalized absorption spectra of APC trimers in 100 mM potasium phosphate buffer, pH 7 (solid line) and APC monomers prepared by adding 1.2 NaSCN (dashed line), both measured at room temperature. The trimer absorption maximum is located at 653 nm. The monomer absorption maximum is located at 615 nm.

The absorption spectrum of APC trimer differs drastically from the monomer spectrum (Fig. 1). The excitonic interaction between chromophores in the dimer was invoked to explain this spectral change[6]. Femtosecond pump-probe measurements made on APC trimers exhibit 430 fs process attributed to the excitation energy transfer between a donor (absorbing around 620 nm) and an acceptor (absorbing around 650 nm)[7,8]. It was assumed that the acceptor absorption spectrum differs from the monomer spectrum because of new chromophore and/or protein conformations in trimer. Femtosecond kinetics was not observed for APC monomers[7,8]. Energy transfer between far distanced $\alpha 80$ and $\beta 81$ chromophores within a monomer unit takes tens of picoseconds[9,10]. Picosecond and femtosecond fluorescence techniques have also been applied to the investigation of ultrafast processes in APC trimers[11,12].

In this paper we present the results of difference absorption spectra and kinetic measurements on APC trimers with femtosecond time resolution over a wide spectral region. Special attention is paid to the analysis of the excited state absorption (ESA) data.

Materials and methods

Transient absorption spectra were measured in the 440 nm - 860 nm spectral region. Amplified and compressed 300 fs pulses from a synchronously pumped dye laser were used for excitation at 587 nm. A femtosecond continuum was used for the probe (two-color pump-probe measurements at 1 kHz pulse repetition rate). A detailed description of the laser systems has been published elsewhere[13]. Spectra have not been recorded in the 575 nm -600 nm spectral range to avoid signals caused by scattered pump radiation. Difference spectra and femtosecond kinetics wae measured at parallel polarization of the probe light relative to the excitation light.

The APC trimers of *Mastigocladus laminosus* were prepared as reported[14]. The measurements were made at 298 K in a rotating cell of 1 mm optical path length. The optical density of the sample at 587 nm (excitation wavelength) was 0.5.

Results and discussion

Allophycocyanin transient absorption spectra

Fig. 2 shows difference spectra of APC trimers at 0.0 ps, 0.2 ps, 0.5 ps and 1.0 ps delays of the probe pulse relative to the pump pulse. The spectra exhibit a bleaching due to ground state depletion at 605 nm - 670 nm, and due to stimulated anission at 700 nm -770 nm. Broad excited state absorption bands were observed around 500 nm and at wavelengths longer than 770 nm. Transient excited state absorption at 680 nm - 690 nm is seen at the earliest times.

The difference spectra measured in the region of strong ground state absorption of APC trimers are similar to the spectra obtained at 635 nm - 670 nm when APC trimers were excited at 618 nm [8]. Fig. 3 shows kinetics measured at 620 nm, 645 nm and 670 nm. The initial bleaching obtained at 620 nm is followed by a recovery kinetics corresponding to the 430 fs process observed at 618 nm excitation[7,8]. It was assumed that the absorption recovery is due to excitation transfer from the excited donor chromophore absorbing at 620 nm to the acceptor chromophore absorbing at 650 nm.

The relaxation of the excited donor molecules to their ground state is accompanied by an absorption recovery at 620 nm (Fig.3).

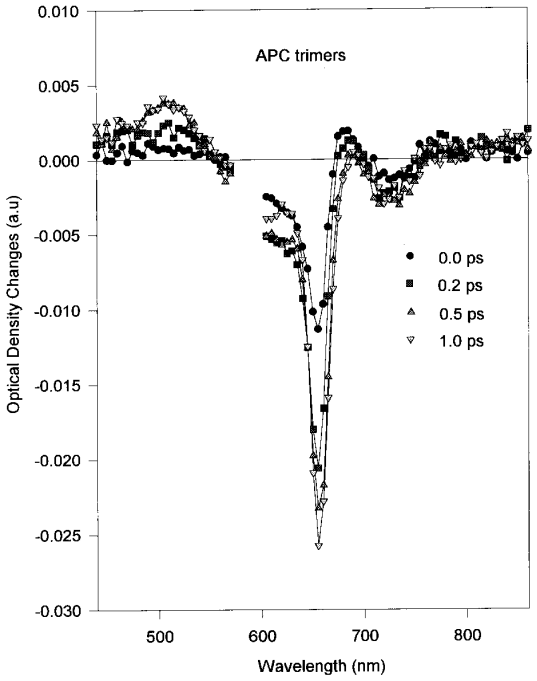

Figure 2. Photoinduced optical density changes measured in 440 nm - 860 nm spectral range at 0.0 ps, 0.2 ps 0.5 ps and 1.0 ps after the excitation of APC trimers with a 300 fs pulse at 587 nm. Polarization of probe light is parallel to polarization of excitation light.

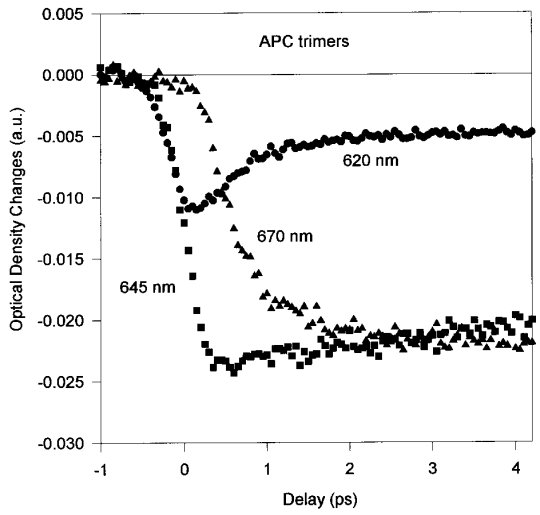

Figure 3. Photoinduced optical density changes measured at room temperature in APC trimers at 620 nm, 645 nm and 670 nm after the excitation with 300 fs pulses at 587 nm. The kinetics were measured at parallel polarization of the probe light relative to the polarization of the excitation light.

The bleaching observed at 670 nm (spectral region attributed to the acceptor absorption) is delayed relative to the excitation in the agreement with the donor - acceptor model. The kinetics recorded at 645 nm is intermediate between those obtained at 620 nm and 670 nm. One can assume that ground state absorption of donor and acceptor molecules is similar near 645 nm. Therefore, the energy transfer process is not accompanied by any clear decay or rise femtosecond kinetics at this wavelength.

An alternative explanation of the ultrafast (<2 ps) kinetics, obtained for APC trimers under picosecond excitation, was given by Beck and Sauer[10]. In this work the kinetics was attributed to the relaxation between two excitonic states formed as a result of the excitonic interaction between the $\alpha 80$ and $\beta 81$ chromophores. Anisotropy measurements are, however, in better agreement with the donor - acceptor model[8].

The transient excited state absorption kinetics measured at 680 nm is shown in Fig. 4. An excited state absorption is seen only during excitation and it decays immediately to negative optical density changes. One can assume that this excited state absorption is due to the excited donor molecules and that it does not derive from the excited acceptor molecules. It is, however, possible to give an altemative explanation of the observed transient excited state absorption. Below we present a general model of an excited state absorption resulting from a dipole - dipole interaction between a pair of molecules, and then we discuss the observed transient excited state absorption.

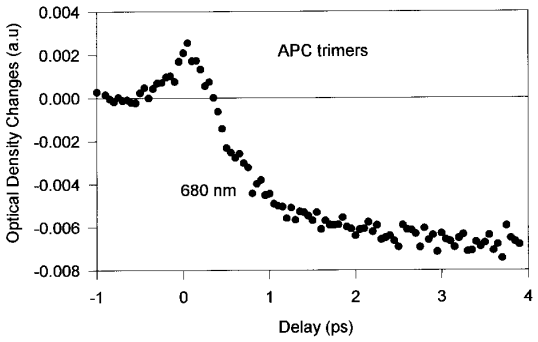

Figure 4. Photoinduced optical density changes measured at room temperature in APC trimers at 680 nm after the excitation with 300 fs pulses at 587 nm. The kinetics was measured at parallel polarization of the probe light relative to the polarization of the excitation light.

Dipole-dipole interaction between a pair of molecules and femtosecond transient difference spectra analysis

At first, we assume that two neighbouring molecules, α and β, do not interact with each other. Nevertheless, we apply the same formalism ("dimer representation") for the uncoupled pair of molecules as for a coupled pair of molecules. The uncoupled $\alpha-\beta$ dimer has the energy of the ground state equal to $E_\alpha + E_\beta$, and the energies of 1st and 2nd excited states, designated as S_β and S_α are $E_\alpha + E^*_\beta$ and $E_\beta + E^*_\alpha$ respectively, where E_α, E_β, E^*_α and E^*_β are energies of ground and 1st excited states of α and β molecules (Fig. 5). The dimer is in its ground state when both molecules are in their ground states, it is in the S_β state when β molecule is in its excited state and α molecule is unexcited, and the dimer is in the S_α state when α molecule is excited and β is unexcited. The next dimer excited state, designated as S_2, corresponds to the situation when both molecules in a pair are excited. This state is called a doubly excited state with the energy $E^*_\alpha + E^*_\beta$. The excitation of the second molecule in a pair is an excited state absorption in the "dimer representation". For noninteracting molecules the excited state absorption corresponding to the $S_\beta \rightarrow S_2$ transition is at the same energy $(E^*_\alpha - E_\alpha)$ as the $S_0 \rightarrow S_\alpha$ ground state absorption since both transitions correspond to the excitation of α molecule to its lowest molecular excited state. The same is valid for the $S_\alpha \rightarrow S_2$ and $S_0 \rightarrow S_\beta$ transitions (excitation of β). It is evident, that the difference spectra obtained for a noninteracting pair of molecules in a pump-probe experiment are molecular in nature regardless which representation we use. Nevertheless, it follows from the "dimer representation" that excited state absorption is always expected for a coupled (weakly or strongly) pair of molecules in the spectral region of the molecular ground state absorption.

Dipole-dipole interaction between the molecules within a pair shifts the energy levels as shown in Fig. 5, and generally the four shifts are an different. The Hamiltonian operator of two molecules in the presence of dipole-dipole interaction can be written as:

$$\mathbf{H} = \mathbf{H}_\alpha + \mathbf{H}_\beta + \mathbf{V} \qquad (1)$$

where $\mathbf{H}_\alpha$ and $\mathbf{H}_\beta$ are the molecular Hamiltonian operators of isolated molecules and $\mathbf{V}$ is a term representing the dipole-dipole interaction. The corresponding shifts as shown in Fig. 5 are[15]:

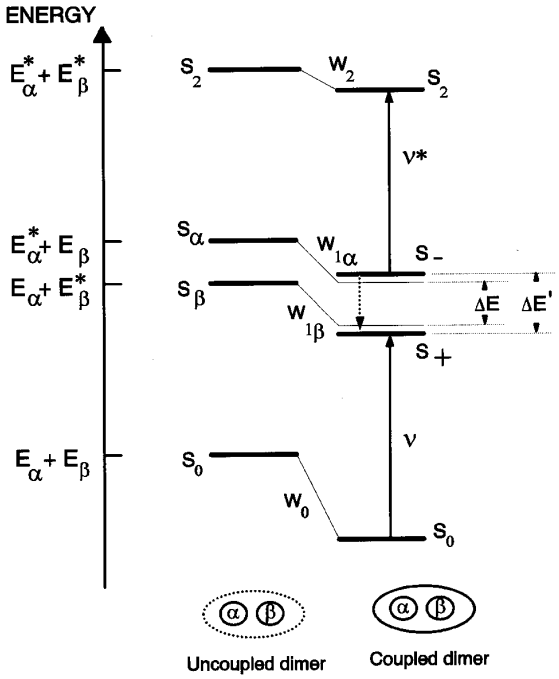

Figure 5. Energy level diagram for a pair of molecules considered in the "dimer representation" (explanation in the text).

$$W_0 = < \psi_\alpha \psi_\beta | \mathbf{V} | \psi_\alpha \psi_\beta > = n^{-2} R^{-3} |\mu^\alpha_g||\mu^\beta_g| r \qquad (2)$$

$$W_{1\alpha} = < \psi^*_\alpha \psi_\beta | \mathbf{V} | \psi^*_\alpha \psi_\beta > = n^{-2} R^{-3} |\mu^\beta_g||\mu^\alpha_e| r \qquad (3)$$

$$W_{1\beta} = < \psi^*_\beta \psi_\alpha | \mathbf{V} | \psi^*_\beta \psi_\alpha > = n^{-2} R^{-3} |\mu^\alpha_g||\mu^\beta_e| r \qquad (4)$$

$$W_2 = < \psi^*_\alpha \psi^*_\beta | \mathbf{V} | \psi^*_\alpha \psi^*_\beta > = n^{-2} R^{-3} |\mu^\beta_e||\mu^\alpha_e| r \qquad (5)$$

where ψ_α and ψ_β are electronic wavefunctions of α and β molecules in their ground states, ψ^*_α and ψ^*_β are wavefunctions of their excited states, μ_g, and μ_e are the permanent dipole moments of the molecules in their ground and excited states, respectively, R is the distance between molecules, n is the refractive index of the medium and r is the orientation factor:

$$r = (e_\alpha e_\beta) - 3(e_\alpha e_{\alpha\beta}(e_\beta e_{\alpha\beta}) \ , \qquad (6)$$

where $\mathbf{e}_\alpha$ and $\mathbf{e}_\beta$ are unit vectors along the dipole directions of α and β molecules (we suppose for simplicity that $\mu_g^i || \mu_e^i$), and $\mathbf{e}_{\alpha\beta}$ is the unit vector in the direction joining the two molecules. We assume the orientation factor to be negative (as e.g. for the pair of neighbouring $\alpha 84$ and $\beta 84$ chromophores in CPC and PEC trimers[2-3]).

Let us suppose that molecules have similar nature and they differ mainly due to their surroundings which effects their absorption bands. This assumption allows us to suppose equal absolute values of the ground state (excited states) permanent dipoles of both molecules, i.e. $|\mu_g^\alpha| = |\mu_g^\beta|$ and $|\mu_e^\alpha = |\mu_e^\beta|$. Consequently, $W_{1\alpha} = W_{1\beta} = W_1$. Additionally, the S_β and S_α states are symmetrically shifted due to the excitonic interaction between molecules, and S_+ and S_- excitonic states are formed as shown in Fig. 5. If we denote the difference between S_β and S_α states as ΔE, then separation $\Delta E'$ between the excitonic states is[16]:

$$\Delta E' = 2\sqrt{(\Delta E/2)^2 + |V|^2}\ , \qquad (7)$$

where V is given by:

$$V = < \psi_\alpha^* \psi_\beta |\mathbf{V}| \psi_\alpha \psi_\beta^* > = n^{-2} R^{-3} |\mu_{ge}|^2 r \qquad (8)$$

μ_{ge} is the transition dipole moment of the molecular transition which we assume to be equal in absolute value for both molecules, regardless of the fact that they can absorb at different wavelengths. The state S_+ is located lower than S_- in the case of the negative orientation factor, and vice versa. The corresponding wavefunctions of S_+ and S_- states are[17]:

$$\psi_\pm = sin\gamma \psi_\alpha^* \psi_\beta + cos\gamma \psi_\alpha \psi_\beta^* \qquad (9)$$

where γ is given by:

$$\tan\gamma = (\Delta E/2V) \pm \sqrt{1 + (\Delta E/2V)^2} \qquad (10)$$

In the case of a strongly excitonically coupled pair of nearly identical molecules we have $|V| \gg \Delta E/2$, and S_+ and S_- are delocalized states, i.e. the excitation is delocalized over both molecules when the dimer is in the S_+ or S_- state. If $\Delta E/2 \gg |V|$, the excitation is mainly localized on the β molecule when dimer is in the S_+ state and the excitation is mainly localized on the α molecule when the dimer is in its S_- state.

A strong excitonic coupling means that delocalized states are formed. The value of the coupling, V, determines the dipole-dipole interaction between transition dipoles. It should always be compared to ΔE and, moreover, to the homogeneous bandwidth, $\Delta\nu$, of the ground state molecular absorption band. Two molecules are strongly excitonically coupled only when $V \gg \Delta\nu$ [18]. S_+ and S_- states are virtually degenerate when $\Delta\nu \gg V \gg \Delta E/2$. They are always excited in phase and the excitation will be localized on the α or β chromophore[19]. The excitation of the upper excitonic state (S_- for the negative orientation factor) can be followed by interexciton state relaxation to the S_+ state in the case of strong excitonic coupling (Fig. 5, dashed arrow). For weakly excitonically coupled molecules this interexciton state relaxation means a transfer of the excitation from α to β because S_- and S_+ states are almost localized on α and β, respectively. The donor - acceptor pair with well separated spectra is a typical case of very weakly excitonically coupled molecules, and the same four levels diagram is applicable in this case as well as for strongly coupled dimer. It is seen from the "dimer representation" that Förster energy transfer and interexciton state relaxation is obviously one and the same process for weakly excitonically coupled molecules. Nevertheless, the excitonic interaction modifies to some extend also the dimer absorption spectrum for a weakly coupled pair of molecules, because the excitonic coupling changes the initial level separation ΔE.

APC trimers can be considered as an example of a rather strong excitonic coupling if we assume that the main absorption peak at 650 nm and the shoulder at 620 nm are the two excitonic bands. This is, however, rather unlikely because the corresponding splitting would be in this case about 700 cm^{-1} - 800 cm^{-1}. This value should be compared to $V = 56$ cm^{-1} calculated for CPC trimers[20]. The same phycocyanobilin chromophores at similar distance form pairs in APC trimers as well as in CPC trimers[5]. The orientations of the β chromophores are almost the same for APC and for CPC, although the α chromophores have slightly different conformations[5]. According to (8), this difference seems to be insufficient to significantly change the V value. The spectrum of the α chromophore, however, most likely will be changed and, therefore, the donor -

acceptor model is in better agreement with the recent crystallographic data than the excitonic model.

Using the "dimer representation" we can predict the excited state absorption resulting from the dipole - dipole interaction between a pair of molecules. This excited state absorption can be clearly observed in pump -probe measurements and it does not occur for an isolated molecule. We will consider two cases. In the first case the permanent dipoles are equal in the molecular ground and excited states ($\Delta\mu = \mu_e - \mu_g = 0$). In the second case $\Delta\mu \neq 0$.

The wave numbers of the $S_0 \rightarrow S_+$ and the $S_- \rightarrow S_2$ transitions are ν and ν^*, respectively. It is seen from (2-5) that $W_0 = W_1 = W_2$ and $\nu = \nu^*$ when $\Delta\mu = 0$. For very weakly excitonically coupled molecules the $S_- \rightarrow S_2$ and the $S_0 \rightarrow S_+$ transitions correspond to the excitation of β molecules, and the $S_- \rightarrow S_2$ excited state absorption is completely superimposed on the bleaching of the $S_0 \rightarrow S_+$ band provided that the upper S_- state is populated. Evidently, the experimentally measured difference spectrum corresponds in this case to the excitation of the α molecules, and it will exbibit the $S_0 \rightarrow S_-$ band bleaching only. The excited state absorption due to the $S_- \rightarrow S_2$ transition can be observed, however, in a pump - probe experiment for strongly excitonically coupled molecules, when the dipole moments of the $S_0 \rightarrow S_+$ and the $S_- \rightarrow S_2$ transitions are different. The absorption to the doubly excited excitonic state has been discussed for APC trimers[8,10] and for other photosynthetic antenna systems[21,22].

When $\Delta\mu \neq 0$, the excited state absorption can be also observed in pump - probe measurements for weakly excitonically coupled dimers with $\Delta E/2 \gg |V|$. According to (2-5), the $S_- \rightarrow S_2$ excited state absorption band should appear in this case at the position:

$$\nu^* = \nu + n^{-2}R^{-3}h^{-1}c^{-1}(\Delta\mu)^2 r \qquad (11)$$

Here h is Planck's constant and c is the speed of light. This excited state absorption is located near the acceptor ground state absorption band and the equation (11) is very similar to the formula (8) for the excitonic splitting. In (11), however, ground and excited state permanent dipoles contribute to the interaction instead of the transition dipole for the excitonic interaction. In the "molecular representation" the expected excited state absorption is due to Stark shift of the acceptor ground state absorption band in the presence of the excited donor chromophore. The decay of this excited state absorption accompanies the excitation energy transfer process. An excited state absorption shifted relative to the donor ground state absorption should finally appear.

Equation (11) can be applied to the analysis of APC trimer transient excited state absorption spectra (Fig. 2) and the kinetics obtained at 680 nm (Fig. 4). Assuming that distance between the molecules, R, and the orientation factor, r, are similar to the corresponding values for CPC trimer, we can estimate the excited state absorption red shift relative to the acceptor ground state absorption band. The shift is $\nu - \nu* \approx 200$ cm^{-1} for $\Delta\mu = 20D$. One can see that an observable shift occurs only for the molecules with very large $\Delta\mu$. A large permanent dipole moment was calculated for the $\alpha 84$ ground state of PEC ($\mu_g = 25D$) [23]. Moreover, the quantum chemical calculations show that the charge transfer states, where an amino acid electron is transferred to the chromophore, are mixed with the local chromophore excited states of PEC[23]. Charge transfer from His $\alpha 90$ reduces PEC $\alpha 84$ permanent dipole value to 16 D, and the permanent dipole is even smaller when the charge is transferred from Asp $\alpha 87$ ($\mu_e = 5D$) [23]. An interaction between the protonated nitrogen atoms of two pyrrole rings of the tetrapyrrole chromophore with aspartate residue of the protein is a common principle of chromophore-protein interaction in phycobiliproteins[3], which is expected also in APC, and influences its spectral properties. Therefore, we can predict a strong change in the permanent dipole upon excitation of APC chromophores, and "dipole" nature of the observed transient excited state absorption cannot be ruled out. ln contrast, the broad excited state absorption bands around 500 nm and at the wavelengths longer than 770 nm have "pure molecular" nature.

Acknowledgements

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