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Energy transfer pathways in the peridinin chlorophyll-a protein complex as revealed by the near-infrared time-resolved spectroscopy

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Introduction

The peridinin chlorophyll-a protein (PCP) is a water-soluble light harvesting complex from dinoflagellates, with a pigment stoichiometry of 8 carotenoids (peridinin) and 2 chlorophyll-a (Chl-a) molecules. The crystal structure of PCP (Hofmann et al. 1996) has provided a good basis for both experimental and theoretical studies of energy transfer pathways within the complex (Bautista et al. 1999a, Damjanović et al. 2000, Krueger et al. 2001). These studies demonstrated high efficiency (88 ±2%) of peridinin-chlorophyll energy transfer with a characteristic time constant of 2.3-3.2 ps. It was suggested that energy transfer took place predominantly from the peridinin S_1 state to the chlorophyll Q_y state (Bautista et al. 1999a), although more recent results were interpreted as partial energy transfer via peridinin S_2 state (Krueger et al. 2001). However, the lowest excited state of peridinin exhibits rather unusual behaviour, which was attributed to a state with charge-transfer (CT) character in the excited state manifold lying close to the S_1 state (Bautista et al. 1999b). Dynamics of the peridinin CT state in solution was recently studied by near-infrared femtosecond (Zigmantas et al. 2001), but a role of the CT state in energy transfer between peridinin and Chl-a in PCP remains unclear. Here we apply near-infrared femtosecond spectroscopy to study dynamics of the lowest excited states of peridinin in the PCP complex. We address the important question whether the CT state of peridinin plays a role during energy transfer between peridinin and Chl-a in the PCP complex.

Materials and methods

The PCP complexes from *Amphidinium carterae* were prepared as described previously (Hofmann et al. 1996) and stored in dark at –55^{o}C. Samples were dissolved in a buffer (25 mM Tris pH 7.5, 2 mM KCl), in which H_2O was replaced by D_2O in order to avoid water absorption in the IR region. Samples were adjusted to an optical density of about 0.2/mm at the excitation wavelength and placed in a 2 mm rotating quartz cell. For the near-IR transient absorption measurements we utilized an amplified Ti:sapphire femtosecond laser system operating at a 5 kHz repetition rate. Two optical parametric amplifiers were used for the generation of pump and probe pulses at various wavelengths (see Zigmantas et al. 2001 for a detailed description of the apparatus). To avoid sample degradation and annihilation processes, the excitation intensity was kept below 40 nJ/pulse.

Results and Discussion

The PCP complexes were excited at 535 nm, a wavelength corresponding to the low energy edge of the peridinin S_0-S_2 absorption, to avoid any contribution from the vibrational dynamics in the S_2 state. This excitation wavelength also assures that only the peridinin molecules are excited, since Chl-a absorbs minimally in this spectral region.
Transient absorption spectra of the PCP complexes at two time delays of 0.5 ps and 30 ps

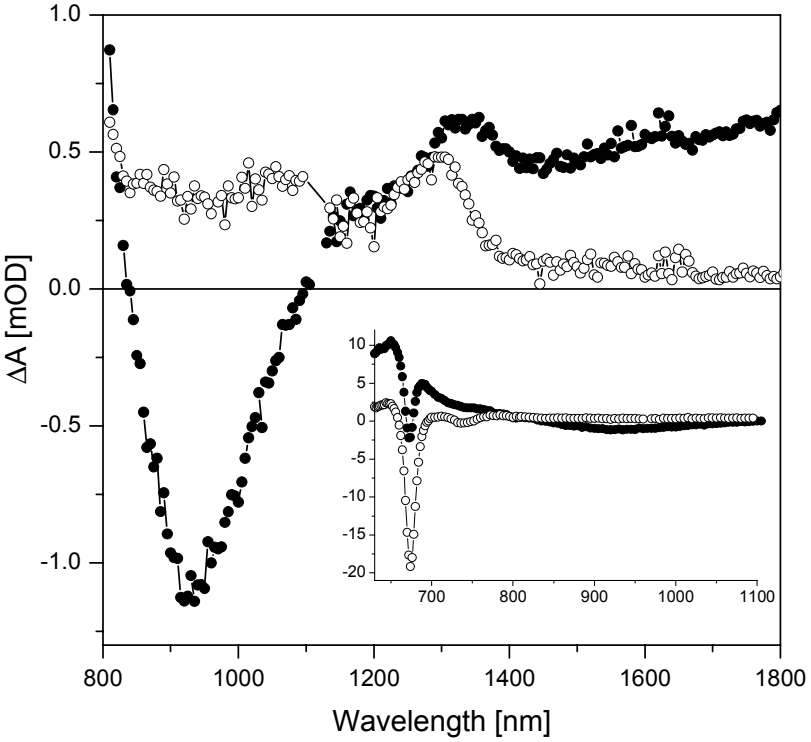

Fig. 1. Near-infrared transient absorption spectra (800-1800 nm) of the PCP complex recorded at 0.5 ps (full circles) and 30 ps (open circles) after excitation at 535 nm. Inset shows part of the transient spectrum including Chl-a bleach (630-1000 nm).

after the excitation are shown in Fig.1. Given the known peridinin lifetime of about 3 ps in the PCP complex, these transient spectra allow us to separate contributions from peridinin and Chl-a. At 30 ps most of the excitations are trapped on Chl-a molecules, while at 0.5 ps a major part of excitations still resides on the peridinin molecules. The 30 ps transient spectrum is dominated by a Chl-a bleaching centered at 674 nm (shown in inset). In addition, a weaker negative band at 740 nm attributable to a stimulated emission of the Chl-a to higher vibrational levels of the ground state and a rather featureless excited state absorption (ESA) of Chl-a,can be observed. On the other hand, the transient spectrum recorded at 0.5 ps exhibits a few additional features. First, a clear ESA corresponding to the well-known S_1-S_N transition of carotenoids is seen at wavelengths below 750 nm overlapping with the Chl-a bleaching, which is also distinctly pronounced on the top of this ESA even at early time delays. However, the most interesting feature of the 0.5 ps transient spectrum of the PCP complex is the negative band centered at around 930 nm, which is due to a peridinin, since this band is not observed at longer time delays. A similar negative band was observed for peridinin in methanol and was attributed to a stimulated emission from an intramolecular charge transfer (ICT) state occurring in the vicinity of the peridinin S_1 state (Zigmantas et al. 2001). Detailed

inspection of the 0.5 ps transient absorption spectrum also reveals an ESA in the spectral region 1100 – 1800 nm, which is attributed mainly to the ESA corresponding to the S_1 - S_2 transition of peridinin. Since this transition extends beyond the detection limit of our apparatus, we can observe only transitions occurring into higher vibrational states of the S_2 state. Exact assignment of the vibronic bands in this region is rather complicated, due in part to an overlap with the Chl-a ESA, which exhibits a pronounced band at 1200 nm as is obvious from the transient spectrum recorded at 30 ps delay.

Kinetics measured at wavelengths corresponding to the Chl-a bleach (670 nm) and to the stimulated emission from the peridin ICT state (930 nm) are shown in Fig.2. To obtain reasonable fits, both kinetic traces must be fitted with four time constants: 0.7 ± 0.1 ps, 2.5 ± 0.2 ps, 35 ± 4 ps and 3.4 ns. At both wavelengths, the dominating component is

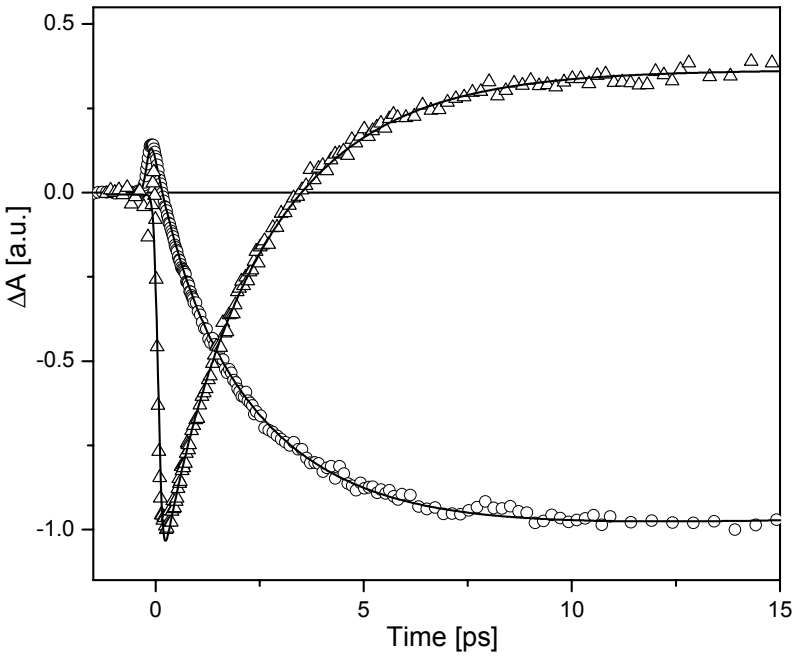

Fig. 2. Kinetics of the PCP complex recorded at 670 nm (circles) and 930 nm (triangles). Solid lines represents multiexponential fits of the kinetics.

characterized by a time constant of 2.5 ps seen as a decay of the 930nm band and as a rise of the Chl-a bleach, matching the energy transfer process between peridinin and Chl-a. The amplitudes of other components are significantly lower. The 3.4 ns component is the Chl-a lifetime, while the 35 ps process is most likely connected with annihilation and/or equilibration processes among Chl-a molecules. In addition to the traces taken at 670 nm and 930 nm, the kinetics were examined at various wavelengths spanning the spectral region of the transient absorption spectrum shown in Fig. 1. These yielded the same time components as for the 670 nm and 930 nm traces. At wavelengths above 1000 nm (data not shown), one more time component with time constant less than 100 fs was required. This time component is due to ultrafast dynamics of the peridinin S_2 state, exhibited as a decay of the S_2-S_N transition in the near-infrared region (Zigmantas et al. 2001).

The fact that the decay of the stimulated emission band in the transient absorption spectrum matches the rise of the Chl-a bleach strongly suggests that the peridinin ICT state plays an important role in the energy transfer between peridinin and Chl-a in the PCP complex. Similarity of the transient absorption spectra of PCP and that of peridinin in the polar solvent methanol (Zigmantas et al. 2001) also suggests that the protein environment surrounding the

peridinin molecules in PCP is indeed very polar. This in turn increases the possibility for population of the ICT state, which cannot be populated in non-polar solvents. For peridinin in methanol, the ICT emission exhibits a clear rise time of about 1 ps, but in the PCP complex no rise component of the 930 nm band longer than 200 fs was detected. This suggests that in the PCP complex the ICT state is populated directly from the initially populated S_2 state. We cannot unequivocally assign an energy to the ICT state from the data shown here but comparing with our previous results on peridinin in methanol, the energy of the ICT state in the PCP complex is most likely lower than the S_1 state. Thus the overlap of the ICT emission with the absorption of the Chl-a Q_y state (~14900 cm^{-1}) will be poorer than that with the S_1 state. This somewhat contradicts the efficient peridinin to Chl-a energy transfer, but the poorer spectral overlap can be compensated by a large transition dipole of the ICT state, which facilitates energy transfer. The time constant of this energy transfer is 2.5 ps, which is significantly shorter that the peridinin lifetime in methanol (10 ps). Since this lifetime is strongly dependent on solvent polarity, the exact peridinin lifetime in the PCP complex in absence of energy transfer is not known and thus prevents any feasible estimation of the energy transfer efficiency via the ICT state. In addition to the 2.5 ps time constant, application of multiexponential global fitting of the kinetics reveals an 0.7 ps component with spectral profile very similar to the 2.5 ps component. Although the amplitude of the 0.7 ps does not exceed 20 % in any of the measured kinetics, omitting this component led to a considerably worse fits. The origin of this component is not clear. A possible explanation could be more efficient transfer from one of the peridinins, which is in a more favourable position with respect to a Chl-a acceptor, resulting in a faster energy transfer rate than that observed for other peridinins in the PCP complex.

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