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Study of the carotenoid interaction in peridinin-chlorophyll-proteins

M Di Valentin, G Agostini, M Brustolon, D Carbonera, G Giacometti,

Dipartimento di Chimica Fisica, Università Degli Studi di Padova, Via Loredan, Padova, Italy, 35131. g.giacometti@chfi.unipd.it

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Introduction

The peripheral antenna from the *Dinoflagellate* alga *Heterocapsa pygmeae* is a pigment-protein complex (PCP) containing only one type of carotenoid, peridinin, and chlorophyll *a*. The stoichiometry of pigments is completely displaced (4:1) in favour of the carotenoid. The molecular structure of the analogous PCP protein from the species *Amphidinium carterae* has been provided by X-ray crystallographic investigation (Hofmann 1996). The chromophore disposition consists of two identical clusters made each of four peridinin molecules and one chlorophyll *a* molecule. In the PCP from *Heterocapsa* only one of two clusters is present and is supposed to be structurally identical to the former as inferred from the large sequence homologies of the corresponding polypeptides. Intracluster edge to edge distances between peridinins are in the range of 4-11 Å and the conjugated regions of all peridinins are in Van der Waals contact with the tetrapyrrole ring of chlorophyll. Low temperature circular dichroism and absorption spectra show evidence of exciton splitting in the singlet and upper triplet excited states of peridinins inside the cluster (Carbonera 1999 a, b).

We have used different magnetic resonance spectroscopy techniques to further investigate the carotenoid triplet state nature and the triplet-triplet energy transfer process among peridinin molecules in the PCP proteins. We have compared the magnetic behaviour with that of the peripheral light-harvesting protein complex LHCII from plants, for which carotenoid-carotenoid exciton interaction is not observed.

Materials and methods

Purified PCP from *Heterocapsa pygmeae* was a gift from R. Hiller and E. Hofmann. The ODMR apparatus is described by Carbonera et al. (1994). Direct detection time-resolved EPR experiments were performed using a Bruker ER 200D EPR spectrometer. Electron spin echo experiments were performed using a Bruker ESP 380 pulsed EPR spectrometer.

Results

Triplet-triplet energy transfer has been further investigated by Optically Detected Magnetic Resonance (ODMR) spectroscopy. ODMR spectra of the carotenoid triplet state are obtained by broad band continuous illumination, monitoring the fluorescence emitted by the interacting chlorophyll molecules. The carotenoid triplet state is populated by triplet energy transfer from the chlorophyll triplet state.

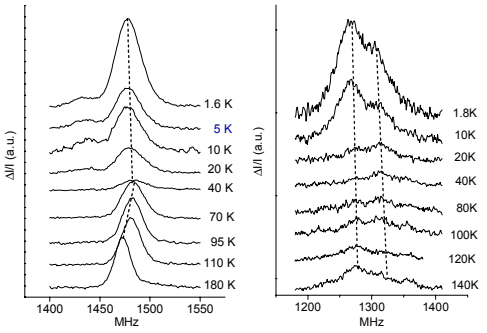

Fig.1. FDMR D+E transition of the carotenoid triplet detected at 670 nm (PCP) and 685 nm (LHCII). Mod.freq. 325 Hz, mw p. 1 W. Left: PCP, right: LHCII.

The ODMR signal temperature dependence has been followed in a wide temperature range in both antenna complexes (see Fig.1). For the *Heterocapsa* PCP complex the ODMR lines show a very strong temperature dependence in terms of frequency shift, linewidth narrowing and intensity variation. In the low temperature range the frequency shift goes toward higher frequencies while at temperatures over 70K it moves in the opposite direction and is accompanied by a continuous linewidth narrowing. From the point of view of the signal intensity, a reduction in intensity is effective only up to 40K, at higher temperature the behaviour is opposite to the expected one and the intensity starts to grow again up to 180K. The ODMR data corresponding to the LHCII complex have some common features with those from PCP, showing a continuous frequency shift toward higher frequencies and the recovery in signal intensity, which is not as strong as for the PCP complex, but is still present. The ODMR line heterogeneity can be ascribed to the presence of different carotenoid in LHCII.

Further knowledge of the kinetic parameters and of the spin polarization patterns have been obtained by time-resolved EPR spectroscopy performed on the photoexcited carotenoid triplet state, on both antenna complexes. In this experiment the carotenoid triplet is populated via triplet-triplet transfer from the chlorophyll molecule excited by a laser pulse (585nm). 2D EPR experiments have been performed by detection of both the spectral and time

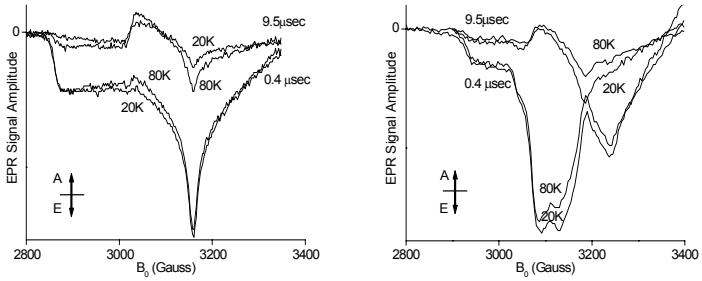

Fig.2. Transient EPR spectra of the carotenoid triplet at 0.4 μsec and at 9.5 μsec after the laser flash, at 20 K and 80 K. Mw freq. 9.4 GHz, mw p. 2 mW. Left: PCP, right: LHCII.

development of the EPR signal, in the complete temperature range at various microwave powers. For the LHCII complex both carotenoid and chlorophyll triplet signals are present, while for the PCP complex there is no chlorophyll contribution, suggesting high efficiency in the energy transfer.

The triplet state EPR spectra for *Heterocapsa* PCP and for LHCII detected at 0.4 μsec and 9.5 μsec after the laser flash have been compared at 20K and 80K (see Fig.2). The spectra taken at the initial time (0.4 μsec) show the original spin polarization of the carotenoid triplet. Only small variations of the pattern and signal intensity in the temperature range, were the ODMR spectra intensity is strongly changing, are detectable. The later spectra (9.5 μsec) show a spin polarization which is different from the initial one. The temperature effect on this later polarization is more pronounced, especially in the PCP antenna complex.

The EPR decay curves at the canonical orientations of the carotenoid triplet contain direct information on the triplet state dynamics. Unfortunately it is not possible to compare the transient signals corresponding to the same canonical transition for PCP and LHCII. While for PCP the triplet *x* transition is the most intense one, for LHCII this transition is obscured by the chlorophyll triplet and the *y* transition is the most unaffected one. Varying the microwave power, the time profiles go from an oscillatory to a non-oscillatory behaviour. In the

overdamping regime the decay curves can be simulated as biexponential functions depending primarily on the decay constants of the triplet state sublevels (data not shown). The transient EPR signal at high microwave power are shown in Fig.3. The oscillatory behaviour is temperature dependent for both antenna complexes. The variation with temperature is

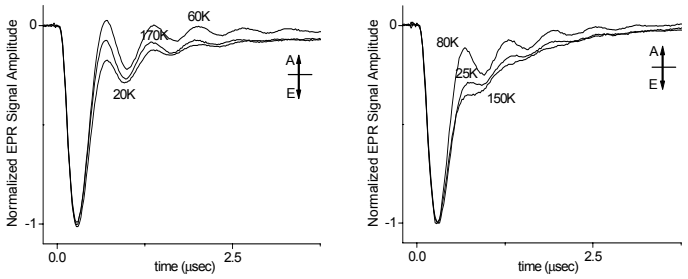

Fig.3. Time evolution of the EPR signal of the carotenoid triplet at different temperatures. Mw freq. 9.4 GHz, mw p. 200 mW, sampling interval 20 ns. Left: PCP, *x* transition at 3160 Gauss, right: LHCII, *y* transition at 3090 Gauss

different in the two system and can be ascribed to spin-spin relaxation time changes.

In further investigation on the triplet state relaxation times, we combine the time-resolved EPR with Electron Spin Echo spectroscopy experiments. We use in this case laser excitation at 532nm to populate the carotenoid triplet state. The temperature dependence of the phase memory time T_M has been extracted from the Hahn Echo decay curves corresponding to the *x* transition of the peridinin triplet and to the *y* transition of the xanthophyll triplet. In Fig. 4 the decay curves at three temperatures are compared. The Hahn Echo decay curves for the PCP antenna complex show a continuous decrease in phase memory time at temperatures higher that 80K, while in the low temperature region there is a change in the decay law from a linear to a stretched exponential function with no evidence of any change of the phase memory time.

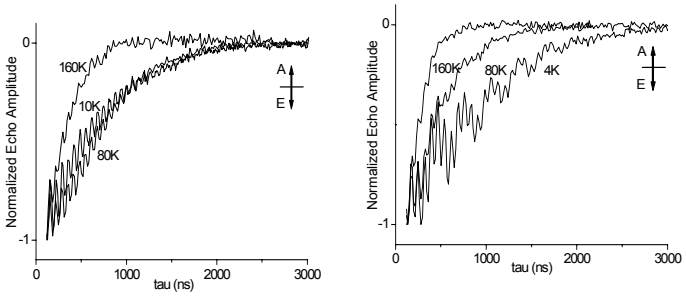

Fig.4. Two-pulse electron spin echo envelope modulation of the carotenoid triplet at different temperatures. MW freq. 9.7 GHz, initial τ 120 ns, time increment 16 ns. Left: PCP, *x* transition at 3270 Gauss, right: LHCII, *y* transition at 3180 Gauss.

The Hahn echo decay curves for LHCII show substantial differences from the latter: the decay law is a linear exponential in the complete temperature range, and the T_M temperature dependence shows a continuous decrease starting from the lowest temperature.

Discussion

The ODMR signal temperature dependence, for the *Heterocapsa* PCP complex in the low temperature region, has been successfully simulated in the framework of exciton interaction among peridinin molecules (Carbonera 1999 b). Some common features with the ODMR data for the LHCII complex, such as the inversion in the temperature dependence of the line intensity, can not be explained in terms of exciton interaction and seem to be specific unexplained features of the triplet state of the carotenoid molecules (Ullrich 1990). The ODMR signal intensity reflects in general the behaviour of the population, decay rates and spin lattice relaxation of the carotenoid triplet state; further knowledge on the spin polarization pattern and on the kinetic parameters has been provided by the time-resolved EPR experiments. No significant variation with the temperature of the initial spin polarization pattern has been detected but a clear effect is visible on the later polarization, especially for

the PCP antenna complex. This could be ascribed to variation either of the triplet population mechanism or of the kinetic parameters. Spectral simulations are in progress in order to ascertain the possible correlation of these changes to the ODMR intensity variation in zero field.

The variations with the temperature in the oscillatory behaviour of the EPR decay curves, and the acceleration of the Hahn Echo decays, must be ascribed to changes of the spin-spin relaxation time. The T_M temperature dependence, for the peridinin triplet state, has been analysed in terms of the effects on spin memory that may result from energy migration among triplet levels: no evidence from this source has been found in the temperature range analysed. These effects could however be masked by spectral diffusion processes or, else, changes due to exciton interaction may occur in the very low temperature region, which has not been explored. The different changes in the time evolution of the EPR signal with the temperature, in the two complexes, must then be ascribed to different activation energies for the relaxation processes for the different carotenoid molecules contained.

The variation of the triplet state sublevel populations and the anomalous dependence of the homogeneous relaxation times on the temperature, are evidence of still unexplained features of the global magnetic resonance picture of carotenoid triplets. The complete analysis of the temperature dependence of the different magnetic resonance observables, which is still under way, combined to the study of various antenna systems and model compounds should allow to solve some of these open questions.

Acknowledgments

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