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Evidence for water-mediated triplet–triplet energy transfer in the photoprotective site of the peridinin–chlorophyll *a*–protein

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ABSTRACT

Experimental and theoretical studies indicate that water molecules between redox partners can significantly affect their electron-transfer and possibly also the triplet–triplet energy transfer (TTET) properties when in the vicinity of chromophores. In the present work, the interaction of an intervening water molecule with the peridinin triplet state in the peridinin–chlorophyll *a*–protein (PCP) from *Amphidinium carterae* is studied by using orientation selective ^{2}H electron spin echo envelope modulation (ESEEM), in conjunction with quantum mechanical calculations. This water molecule is located at the interface between the chlorophyll and peridinin pigments involved in the photoprotection mechanism (Chl601(602)–Per614(624), for nomenclature see reference [1]), based on TTET. The characteristic deuterium modulation pattern is observed in the electron spin-echo envelopes for the PCP complex exchanged against ^2H_2O. Simulations of the time- and frequency-domain two-pulse and three-pulse ESEEM require two types of coupled ^{2}H. The more strongly coupled ^{2}H has an isotropic coupling constant (a_{iso}) of −0.4 MHz. This Fermi contact contribution for one of the two water protons and the precise geometry of the water molecule at the interface between the chlorophyll and peridinin pigments, resulting from the analysis, provide experimental evidence for direct involvement of this structured water molecule in the mechanism of TTET. The PCP antenna, characterised by a unity efficiency of the process, represents a model for future investigations on protein- and solvent-mediated TTET in the field of natural/artificial photosynthesis.

1. Introduction

Important electron transfer and energy transfer processes, including photoprotection via triplet–triplet energy transfer (TTET), occur in natural photosynthesis and provide the basis for the energy conversion process [2]. They have been widely studied in a variety of protein complexes, as well as in biomimetic systems. Mimicking nature by constructing artificial photosynthetic systems is an important research area where a detailed understanding of electron/energy transfer processes is invaluable [3–5]. The magnitude of the electronic coupling that governs the transfer reactions depends on several relevant factors, i.e., the electronic structure and the energies of the donor and acceptor groups, the distance and orientation between them. Among these factors, the electronic structure and geometry of the intervening medium between the partners involved in the transfer must be also considered. In proteins, the surrounding mediating residues and bridging molecules, including water, provide important pathways to finely optimise the electron/energy transfer efficiency [6].

Water enjoys a unique position as a medium for electron transfer. It can affect electron transfer rates by means of its electrostatic and quantum mechanical interactions with the redox partners. While several experimental and theoretical studies have focused on the efficiency of water in mediating electron-transfer processes in proteins and in bio-inspired supramolecular systems, with specific attention to its influence on the distance dependence of the electron transfer rates [7–13], only a small number of investigations have been carried out so far on solvent-mediated TTET [14–17].

The TTET process, based on Dexter's exchange mechanism [18], can be formalised as a simultaneous double electron transfer between the lowest unoccupied molecular orbitals (LUMOs) and the highest occupied molecular orbitals (HOMOs) and can be characterised by a non-adiabatic rate constant which depends on the exchange integral between the two electrons and thus on the electronic distribution over the molecular architecture involved in the process [19,20]. The overlap between wavefunctions may become critical at even shorter distances for TTET as compared to electron transfer. For this reason, superexchange models involving a bridge or the medium interposed between the donor and the acceptor molecules, which have been extensively discussed for electron transfer reactions [21], become even more important when describing TTET [22–27].

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The present paper is devoted to the spectroscopic characterisation of the TTET mechanism in the photosynthetic peridinin–chlorophyll *a*–protein (PCP), where the mechanism reaches unity efficiency [28] and an intervening water molecule between the partners involved in the transfer has been observed in the X-ray structure [1].

PCP is the peripheral water-soluble light-harvesting complex of most photosynthetic dinoflagellates, which constitute the main part of oceanic plankton. The PCP antenna complex of the dinoflagellate *Amphidinium carterae* can in many ways be considered as a model system for the study of energy transfer pathways in photosynthetic antenna complexes, due to its high symmetry, the availability of the X-ray structure at atomic resolution [1] and the high energy transfer efficiency [28]. A wealth of information not only on the singlet but also on the triplet transfer pathways in this complex is present in the literature [17,28–32].

The 2.0 Å X-ray structure reveals the presence of a non-crystallographic trimer of identical 32 kD subunits, each of which is constituted by a polypeptide forming a hydrophobic cavity containing the pigment molecules. The NH_2- and COOH-terminal domains of the monomer are characterised by a 56% sequence homology; each domain forms eight α-helices which bind a cluster of one chlorophyll *a* and four peridinin molecules (see Fig. 1A). The two domains are related by a pseudo-twofold symmetry axis, hence the two pigment clusters can be considered equivalent.

The PCP antenna is unique on account of the preponderance of carotenoid molecules, while in other light-harvesting complexes the chlorophylls predominate. The carotenoids in PCP are peridinins, highly substituted carotenoids, whose key structural features are a lactone and an allene group conjugated to the polyene chain, conferring special spectroscopic properties to the molecule [30]. The 4:1 ratio of peridinins to chlorophyll in PCP is explained by the necessity for efficient absorption of light in the blue-green region, which prevails in the marine habitat of dinoflagellates.

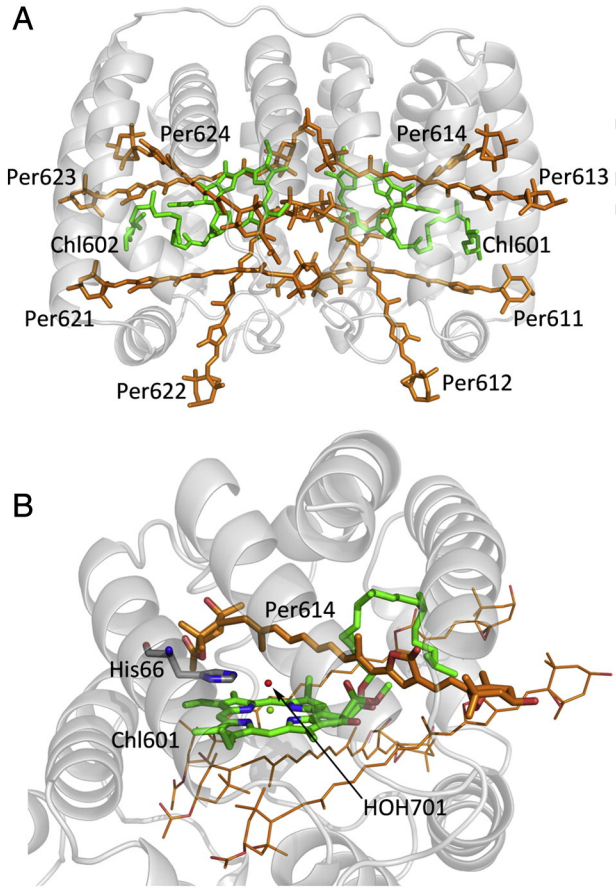

Fig. 1. (A) Pigment cluster associated with the basic unit of the PCP complex from *A. carterae* (PDB entry 1PPR)[1]; (B) highlight of the molecules of the photoprotective site in the NH_2-terminal domain of PCP: peridinin 614, chlorophyll 601, the water molecule HOH701 coordinated to chlorophyll 601 and hydrogen-bonded to the histidine residue His66.

In addition to the light-harvesting function, peridinins fulfil the important function of protecting the system against photo-oxidative damage by quenching chlorophyll triplet states, formed under excess light conditions. Chlorophyll triplets sensitise the formation of singlet oxygen, which is a powerful oxidising agent capable of damaging the whole photosynthetic apparatus. By virtue of their low-lying triplet state, carotenoids are able to quench the chlorophyll triplet state as well as the singlet oxygen directly if it is formed.

Stringent structural conditions must be realised in the light-harvesting complex architecture in order to achieve high efficiency in the TTET process. In PCP, the chlorophyll molecules within each pigment cluster (Chl601 and Chl602) are arranged between two pairs of mutually orthogonal peridinins (Per611–Per612 and Per613–Per614 in the NH_2-terminal domain and Per621–Per622 and Per623–Per624 in the COOH terminus). The structure of PCP reveals two highly conserved histidine residues (His66 and His229), which are hydrogen-bonded to a water molecule (HOH701 and HOH678) acting as the fifth ligand of the chlorophyll's Mg ion. These water molecules are at the interface between Chl601 and Per614 and Chl602 and Per624, respectively, as highlighted for one of the two pseudo-symmetric couples in Fig. 1B. The identification numbers for amino acids, water molecules and pigments are in accordance with the X-ray nomenclature reported in reference [1].

The TTET in PCP has been extensively studied by means of advanced optical spectroscopies [28,31] and by Electron Paramagnetic Resonance magnetic (EPR) spectroscopies, exploiting the presence of the endogenous probe, the pigment triplet state [17,33–37]. Time-resolved EPR experiments, in conjunction with spectral simulations based on the theory of TTET, have allowed identifying the specific path for triplet quenching [17]. It has been shown that the two pigment pairs Chl601–Per614 and Chl602–Per624, related by pseudo-symmetry, are responsible for photoprotection in the PCP antenna complex. The conclusion that the triplet state generated by TTET is localised on a single peridinin molecule, in each subcluster, is further supported by results of ENDOR (Electron Nuclear DOuble Resonance) experiments [33,35,36].

The identified peridinin molecule is distinguished by a smaller centre-to-centre distance to chlorophyll with respect to the other peridinin molecules of the pigment cluster, however all four peridinin molecules are at Van der Waals distance from the chlorophyll ring. The unique feature of the Chl601–Per614 (and equivalently Chl602–Per624) pair is the presence of the water molecule interposed between the two pigments (Fig. 1B) while no bridging molecules are present at the interface between the chlorophyll and the other peridinins. For this reason we raised the hypothesis that the interfacial water molecule might favour TTET by extending the overlap of the donor and acceptor wavefunction. This interfacial water molecule is also conserved in the high-salt PCP complex, a variant of the main form PCP, in the in vitro reconstituted PCP with different pigments and in the N89L-mutant, where the mutation of Asn-89 to Leu in the vicinity of Per614 has been introduced to study the effects of the environment on the spectroscopic properties. For all the variants this structural information is provided by high-resolution X-ray data [32].

In the present work, electron spin echo envelope modulation (ESEEM) spectroscopy is applied to yield information on small proton hyperfine couplings that could not be resolved in the ENDOR spectra of the peridinin triplet state. The aim is to characterise the structure of the photoprotection site in PCP and in particular to study the interaction between the interfacial water molecule and the carotenoid triplet state. This is achieved by combining ESEEM with hydrogen–deuterium exchange in order to highlight the exchangeable water protons in proximity to the paramagnetic centre. ^{2}H-ESEEM is experimentally convenient