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Carotenoid triplet states in photosystem II: Coupling with low-energy states of the core complex

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ABSTRACT

The photo-excited triplet states of carotenoids, sensitised by triplet–triplet energy transfer from the chlorophyll triplet states, have been investigated in the isolated Photosystem II (PSII) core complex and PSII–LHCII (Light Harvesting Complex II) supercomplex by Optically Detected Magnetic Resonance techniques, using both fluorescence (FDMR) and absorption (ADMR) detection. The absence of Photosystem I allows us to reach the full assignment of the carotenoid triplet states populated in PSII under steady state illumination at low temperature. Five carotenoid triplet (^{3}Car) populations were identified in PSII–LHCII, and four in the PSII core complex. Thus, four ^{3}Car populations are attributed to β-carotene molecules bound to the core complex. All of them show associated fluorescence emission maxima which are relatively red-shifted with respect to the bulk emission of both the PSII–LHCII and the isolated core complexes. In particular the two populations characterised by Zero Field Splitting parameters $|D| = 0.0370–0.0373\ cm^{-1}/|E| = 0.00373–0.00375\ cm^{-1}$ and $|D| = 0.0381–0.0385\ cm^{-1}/|E| = 0.00393–0.00389\ cm^{-1}$, are coupled by singlet energy transfer with chlorophylls which have a red-shifted emission peaking at 705 nm. This observation supports previous suggestions that pointed towards the presence of long-wavelength chlorophyll spectral forms in the PSII core complex. The fifth ^{3}Car component is observed only in the PSII–LHCII supercomplex and is then assigned to the peripheral light harvesting system.

1. Introduction

Photosystem II (PSII) is a macromolecular light-dependent oxido-reductase which catalyses the oxidation of water to molecular oxygen and protons, and the reduction of plastoquinone to plastoquinol e.g. [1–3]. From a structural point of view PSII can be seen as composed of two moieties: i) the *core* that serves both as photo-catalytic centre and proximal light harvesting antenna to the reaction centre (RC), and appears to be substantially conserved throughout evolution e.g. [4,5], and ii) an external antenna, whose function is only that of light harvesting, and which varies greatly amongst species as an adaptation to the spectral quality of incident radiation in different ecological environments e.g. [6,7].

The core complex is composed of over 20 subunits [1–5], binds about 40 chlorophyll (Chl) *a* and 10 β-carotene (β-Car) molecules, together with the other cofactors required for electron transfer, including two pheophytin and two plastoquinone molecules, a cytochrome (Cyt b_{559}), a Fe atom and a 4Mn–1Ca cluster, which is the site of water splitting e.g. [8,9]. Four subunits bind the vast majority of the pigments: the CP43 and CP47 complexes that compose the proximal antenna and the D1D2Cytb_{559} complex, which harbours all of the electron transfer cofactors [1–5,8,9].

In the green lineage, the external antenna is composed of Chl *a*/*b*-binding complexes [3,11–14], which are the product of the nuclear gene family known as *lhcb* [6,7]. The PSII external antenna contains several components. The principal component, called Light Harvesting Complex II (LHCII), is a trimer and is bound with a stoichiometry of 2–4 trimers per core complex [3,15,16]. Each monomeric unit binds 8 Chl *a*, and 6 Chl *b* as well as four oxygenated carotenoids (xanthophylls), two lutein, one neoxanthin and one violaxanthin molecules [3,11–14]. The latter can be exchanged with zeaxanthin depending on the growth conditions [17–19]. The other PSII antenna complexes, CP24, CP26 and CP29, are all isolated as monomers and bind with 1:1 stoichiometry to

Abbreviations: PSII(I), Photosystem II(I); LHC, Light Harvesting Complex; RC, reaction centre; Chl, chlorophyll; Car, carotenoid; Xan, xanthophyll; ODMR, Optically Detected Magnetic Resonance; FDMR/ADMR, Fluorescence/Absorption Detected Magnetic Resonance; ZFS, Zero Field Splitting; MIF, microwave-induced fluorescence spectrum; T − S, Triplet minus Singlet; NPQ, Non-Photochemical Quenching; TTET, triplet–triplet energy transfer

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the core complex [3,15,16]. Like LHCII, albeit with a different stoichiometric ratio, the monomeric antennae bind, on average, 10–14 pigments per protein, both Chl *a* and Chl *b*, with ratios going from 1.2 to 2.2 and xanthophylls with ratios of 2–3 molecules per complex [3,11–14].

The carotenoids that are bound both to the core and to the external antenna of higher plants play several roles in the photosynthetic apparatus, particularly in PSII. They are involved in light harvesting of the portion of the incident solar spectrum in the blue-green region [19,20]. However, the spectral overlap with the so-called Soret band of Chl *a* and Chl *b* is significant and the increase in the antenna bandwidth is therefore limited overall [21]. Xanthophylls (Xan) are also involved in processes that regulate the efficiency of light harvesting in response to the increase in intensity of the incident radiation, such as Non-Photochemical Quenching (NPQ) (reviewed in Refs. [17–19]). One clear evidence is the enzymatic conversion of violaxanthin to zeaxanthin when the system is exposed to high irradiance regimes, concomitantly with the acidification of the thylakoid lumen [17–19,22]. Moreover, xanthophylls, either lutein or zeaxanthin, have been proposed to play a direct role in NPQ, representing the effective quenching site either through a singlet–singlet (Xan–Chl) energy transfer mechanism e.g. [23–25], or being partner in the formation of a Chl–Xan charge–transfer complex e.g. [26,27]. It has been also suggested that the carotenoid conformation (cis–trans isomerisation, bond twisting) can affect the chromophores binding within the LHC complexes and hence the chromophore–chromophore interactions, leading to the formation of singlet excited state quenchers (reviewed in Ref. [23]). Their involvement in controlling the fine "packing" of the antenna, mediating different interactions between adjacent complexes, has also been discussed [28]. Therefore, independently from the mechanism, which is much debated, the involvement of carotenoids in the NPQ process is generally accepted.

Carotenoids also have an important structural role, stabilising the folding of pigment binding proteins e.g. [29–33]. This has been demonstrated both in vitro by reconstitution experiments of LHC complexes [29,30], as well as in vivo in mutants affected in the carotenoid biosynthesis [31–33].

Certainly, a central role of carotenoids in the photosynthetic apparatus is the quenching of the Chl triplet state (^{3}Chl) through the triplet–triplet energy transfer (TTET) mechanism (reviewed in Refs. [20,34,35]). The direct population of the carotenoid triplet state (^{3}Car) by intersystem crossing (ISC) is a low probability event, due to the very short lifetime of the excited state of these molecules, that is dominated by internal conversion [20,34,35]. Energy transfer from the ^{3}Chl, which is populated with a yield of ~0.6 in the absence of other quenching mechanisms [36,37], is efficient in photosynthetic systems [38–40] because of the short average inter-pigment distances and because the ^{3}Car lays at an energy level which is below that of ^{3}Chl. It is established that the ^{3}Chl is an efficient sensitiser of singlet oxygen ($^1O_2$), which is an highly reactive species and one of the principal actors in photo-oxidative stress [41,42]. On the other hand, the energy level of the ^{3}Car present in photosynthetic complexes is too low to interact with molecular oxygen. Therefore, the quenching of the ^{3}Chl state by TTET to the ^{3}Car is of fundamental importance under physiological perspective. Indeed, the population of ^{3}Car under illumination is well documented both in relatively intact preparations, such as thylakoid membranes e.g. [38–40,43,44], as well as in isolated pigment–protein complexes where it has been investigated intensively e.g. [45–57].

However, the determination of specific Chl–Car interactions and the identification of the molecules involved in TTET are usually cumbersome due to the severe spectral "congestion" determined by the overlap in the absorption spectrum of different Chl forms, as well as different carotenoids, bound to oxygenic photosystems. Such difficulties can be in part overcome by the use of time-resolved magnetic resonance techniques, since the signal arising from the ^{3}Car is strongly polarized and the polarisation is extremely sensitive to the orientation of donor and acceptor molecules in the pair [58]. This allows the identification of the chromophores involved in the energy transfer process, provided that a structural model of the complex is known which sufficient accuracy. The approach has been successfully applied to the study of isolated LHCII and other antenna complexes e.g. [59–62].

An alternative approach is that of using techniques that allow the correlation of parameters characterising the electron spin of the chromophore with the optical properties of either the chromophore itself (i.e. ^{3}Car) or the sensitiser molecule (^{3}Chl). One such technique is Optically Detected Magnetic Resonance (ODMR), which is extremely selective for the detection of photo-excited triplet states (reviewed in Refs. [63–65]; a description of the basic principles of the ODMR technique is presented in Supporting information, Appendix 1). ODMR allows precise estimation of the zero-field splitting (ZFS) parameters that determine the energy split between the triplet sublevels and depend on the unpaired spin distribution with respect to the chromophore molecular frame. In recent years, the use of this technique has allowed the investigation of both ^{3}Car and ^{3}Chl in intact systems, such as the thylakoid membranes [65–69]. Using this spectroscopic method, particularly by fluorescence detection (FDMR), it was possible to identify the ^{3}Chl states populated in both Photosystem I (PSI) and PSII under ambient redox conditions [66–68]. These ^{3}Chl states were suggested to be involved in photo-oxidative stress [70]. It was also possible to detect a ^{3}Chl associated with the reaction centre of PSII under non-reducing conditions [66,67,71]. This ^{3}Chl shows a remarkably fast relaxation [66,67,71] with respect to the ^{3}Chl observed under reducing conditions typically employed to induce charge recombination e.g. [67,72–74].

Although the fluorescence yield of carotenoids is extremely low and therefore almost undetectable, especially in crowded chromophore–protein complexes, it is still possible to monitor ^{3}Car by FDMR, detecting the fluorescence emission of Chl molecules [45–48,65]. This is because the change in the mean rate of the ^{3}Car decay, induced by resonance conditions, affects the steady-state population of the singlet excited states of the Chl fluorescent molecules to which Cars are coupled by energy transfer (Fig. 1). Employing the FDMR technique it was then possible to observe, for the first time, that the ^{3}Car associated with the external antenna of PSI embedded in the thylakoid membranes [69]. The assignment was later confirmed in isolated LHCI complexes [75]. The ^{3}Car associated with PSII have also been investigated by FDMR in thylakoids [76]. However, due to the significant overlap of PSII and PSI

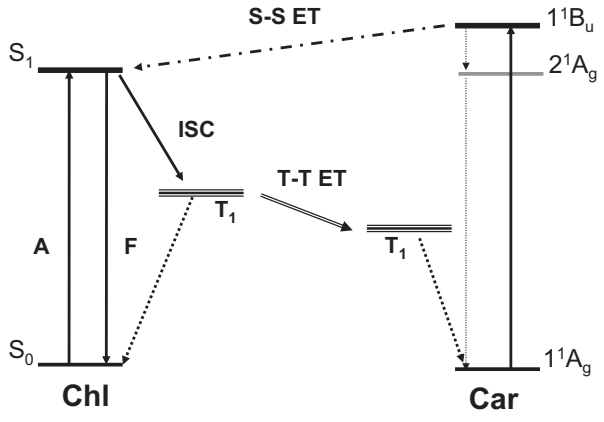

Fig. 1. Scheme describing the principle of FDMR detection of ^{3}Car by monitoring Chl fluorescence emission in photosynthetic systems. On the left it is shown the energy diagram for a Chl molecule, considering its ground (S_0), first singlet excited (S_1) and triplet excited (T_1) states. The Chl T_1 state is populated by intersystem crossing (ISC). On the right, the energy diagram for a Car molecule, considering its ground (1^1A_g), first (2^1A_g) and second (1^1B_u) singlet excited and triplet excited (T_1) states. The Car T_1 state is populated by triplet–triplet energy transfer (TTET) from the Chl triplet. The 1^1B_u singlet excited state of Car is coupled by singlet–singlet energy transfer (SSET) to the S_1 state of Chl. FDMR measurements are possible because the applied microwave field in resonance with a pair of sublevels of the Car T_1 alters the population of the Car ground and singlet excited state. Because of SSET between the Car and Chl molecules, the Chl excited state population is also affected and monitored on its fluorescence emission.