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COMMUNICATION

Ultrafast carotenoid band shifts correlated with Chl_z excited states in the photosystem II reaction center: are the carotenoids involved in energy transfer?

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We show a correlation between the electronic excitation of the peripheral chlorophylls ($Chls_Z$) of the photosystem II reaction center and a shift of the S_2 absorption bands of β-carotene, and suggest that the carotenoids may enhance the excitation energy transfer rate from these chlorophylls to the central cofactors.

The conversion of solar energy into chemical energy has been mastered in photosynthesis. The understanding of the molecular mechanisms underlying photosynthesis is crucial for achieving the efficient utilization of our largest energy source: the Sun.

In higher plants, algae and cyanobacteria a key step in photosynthetic energy conversion takes place in the photosystem II reaction center (PSII RC) where a series of energy and electron transfer reactions give rise to a charge separated state which ultimately powers the photosynthetic organism. The PSII RC contains six chlorophylls (Chls), two pheophytins (Phes) and two β-carotenes (Car). The X-ray crystal structure of PSII from cyanobacteria[1,2] shows that four Chls and two Phes arranged in two branches, D_1 and D_2, are situated in the center of the reaction center complex and two additional Chls ($Chls_Z$) are located at opposing sides at the periphery of the complex. Each of the two β-carotenes is located between $Chls_Z$ and the center of the complex with different orientations with respect to the thylakoid membrane, Car_{D1} is oriented perpendicularly to the membrane plane while the Car_{D2} orientation is parallel.

For charge separation in the PSII RC, it has been demonstrated that: (a) the central cofactors absorbing around 680 nm are excitonically coupled[3] which (b) leads to charge separation *via* two different ultrafast charge separation pathways[4] and that (c) the peripheral $Chls_Z$ absorbing at 670 nm transfer excitation energy to the central cofactors in about 20 ps.[5–7]

The absorption spectrum of plant PSII RC in the Car S_2 absorption region is shown in Fig. 1A and B. Linear dichroism (LD) experiments on PSII RC and larger PSII particles from spinach showed that the Car S_0–S_2 vibrational transitions at 442, 474 and 506 nm are oriented parallel to the membrane plane while the 458 and 490 nm transitions are approximately perpendicular to it.[8–10] Therefore, the broad band around 465 nm contains both 458 nm (Car_{D1}) and 474 nm (Car_{D2}) transitions and the 489 nm and 506 nm transitions correspond to Car_{D1} and Car_{D2}, respectively (Fig 1A).

It is known that Car perform a wide range of functions in photosynthetic organisms: they protect the photosynthetic machinery, absorb blue-green light not captured by the Chls, stabilize the pigment-protein structures and are involved in regulation of energy flow from and to Chl (for reviews see ref. 11 and 12). However, transient absorption studies show that the quantum efficiency of β-carotene-to-chlorophyll singlet energy transfer upon direct Car excitation is poor in spinach PSII RC and it involves mainly the S_2 state. The "hot" and relaxed S_1 states do not participate in excitation energy transfer (EET)[13] (it should be noted that in this study only Car_{D2} was excited).

In this study, transient absorption spectra of isolated PSII RC at 77 K have been recorded for: six narrow excitation wavelengths covering the Chl and Phe Q_Y region from 660 to 685 nm (5 nm fwhm), two broader excitation wavelengths at 662 and 682 nm (8 nm fwhm), and a non-selective excitation at 675 nm (12 nm fwhm). The aim of these experiments is to investigate the EET among the cofactors in the PSII RC. The data sets have been globally analyzed with a sequential model in order to follow the spectral evolution in time. The evolution associated difference spectra (EADS) obtained represent a mix of species whose population rises with the lifetime of the previous component and decays with its lifetime, *i.e.* the third EADS rises with the second lifetime and decays with the third lifetime.[14] An excellent description of the multi-exponential spectral evolution following those various excitations was obtained using the following set of lifetimes: 500 fs, 3 ps, 20 ps, 300 ps, 600 ps and 20 ns. Additionally, we have performed a target analysis according to a kinetic scheme. The target analysis generates the species associated difference spectra (SADS) which represent the spectra of the pure species described in the kinetic scheme (for details about the experimental conditions and dynamics related to Chl and Phe see ref. 4).

The long wavelength laser light used to excite the chlorins in the PSII RC is too low in energy to excite the Car to the S_2 state; therefore no changes in the absorption due to Car are

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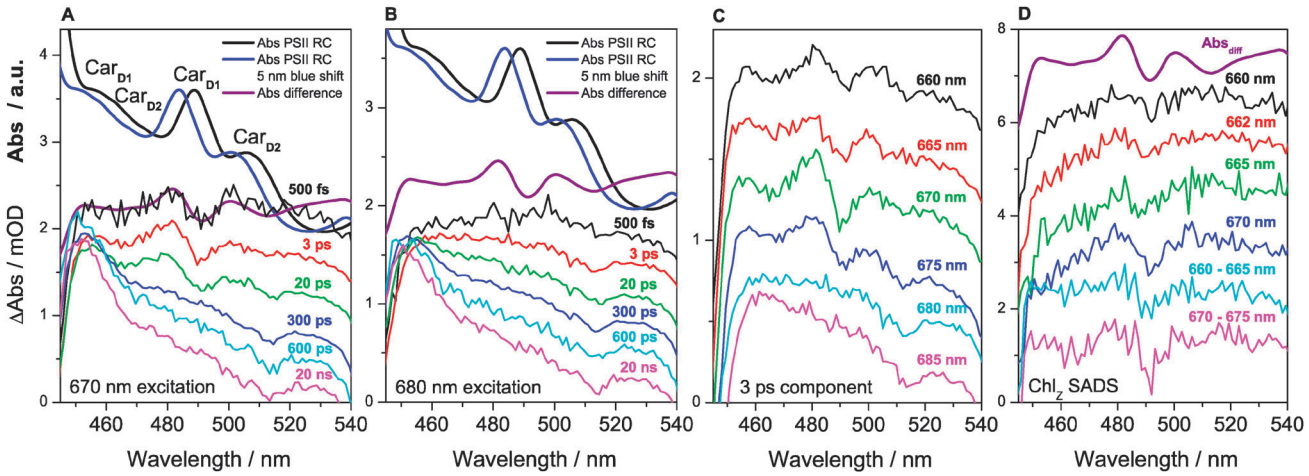

Fig. 1 PSII RC ground state and transient absorption spectra at 77 K in the carotenoid region. All the spectra have been vertically translated arbitrarily for better comparison. (A and B) PSII RC ground state, 5 nm blue shifted absorption and their difference spectra (*thick lines*); Evolution Associated Difference Spectra (EADS) for (A) 670 nm and (B) 680 nm excitation. (C) EADS for the 3 ps component upon different excitation wavelengths. (D) Species Associated Difference Spectra (SADS) for $Chls_Z$ from the target analysis of six linked data sets (660, 665, 670 and 675 nm (5 nm fwhm); 662 nm (8 nm fwhm); and 675 nm (12 nm fwhm) excitation wavelengths) (660–665 nm and 670–675 nm SADS), and from a simplified target analysis of four linked data sets (660, 662, 665 and 670 nm) together with the absorption difference spectra (*thick line*).

expected. However, the EADS display two negative features around 465 and 492 nm (Fig. 1A, C and D). Interestingly, these features are only present in the 500 fs, 3 ps and 20 ps EADS. The small differences in the shape of the negative features are due to the band overlap with contributions from other signals: the Phe anion band at 455 nm, the Phe vibrational $Q_X(0–1)$ band at 512 nm and the featureless excited state absorption from Chl and Phe in the 500 fs and 3 ps components. We note that the same negative Car features are also observed in the Chl_Z SADS obtained by target analysis[4] which represent the spectra of the pure Chl_Z excited states plus their effect on the Car S_2 states. The Chl_Z SADS decays in about 20 ps.

In addition, the amplitudes of the negative features around 465 and 492 nm are highly dependent on the excitation wavelength (Fig. 1C): they increase from 660 nm to a maximum at 670 nm, decrease at 675 nm and are completely absent at 680 and 685 nm excitations. This effect is also observed in the SADS obtained by target analysis (Fig. 1D).

These facts and the proximity of the Car to the $Chls_Z$ strongly indicate that the negative features are related to the population of Chl_Z excited states ($Chls_Z$*). A similar effect was reported for the LH2 antenna complex from photosynthetic bacteria[15] in which ultrafast Car band shifts were observed which correlated with energy transfer between the B800 and B850 bacteriochlorophyll rings. The ultrafast carotenoid response was interpreted as an electrochromic shift due to the changes in the local electric field near carotenoid molecules upon photoexcitation of the bacteriochlorophyll molecules. This interpretation was further supported by quantum chemical calculations.[16] Time dependent density functional theory (TDDFT) strongly suggests that the mutual pigment orientation determines the extent of electrochromic shift.[17]

Along the same line, in the PSII RC the shape of the negative features can be reproduced by a five nanometer blue shift of the absorption spectrum (Fig. 1A, B and D). Surprisingly, both negative features, at 465 and 492 nm, are reproduced in the absorption difference spectra ($Abs_{5nm\ blue\ shifted} - Abs_{ground\ state}$) despite the fact that the two Car have a completely different orientation with respect to the tetrapyrrol ring and the Q_Y transitions of $Chls_Z$.

Then the question arises: is this Car absorption blue shift a simple effect of the local electric field generated by the $Chls_Z$ excited states or, in addition, does it have physiological significance? The data clearly show that both Car sense the excitation on $Chls_Z$, manifested as an electrochromic shift of their $S_0–S_2$ transition. This shift could just be a result of the close proximity of the Car and $Chls_Z$ with no influence in the EET dynamics among the cofactors in the PSII RC. However, at this point, we would like to move a step further and hypothesize an additional energetic implication for this phenomenon: the shift of the Car $S_0–S_2$ transition could imply the presence of mixing between the electronic states of $Chls_Z$ and the Car. Due to the location of the Car (between $Chls_Z$ and the central cofactors) this mixing may increase the coupling between $Chls_Z$ and the exciton states of the central cofactors and thereby enhance the EET rate between $Chls_Z$ and the central cofactors.

This proposition is based on several evidences. On one hand, the discrepancy between experiment and theory on the energy transfer rate from $Chls_Z$ to RC central cofactors (also found in LH2).[18] From the experiment, the energy transfer rate is $(20\ ps)^{-1}$,[5–7] while from modeling of spectroscopic data using the modified Redfield/modified Förster theory, both approaches treat the electronic states of the central cofactors as excitonic, energy transfer rates of up to $(100\ ps)^{-1}$ [19,20] were found (note that these theoretical studies do not take into account the presence of Car). On the other hand, quantum mechanical calculations on LH2 from purple bacteria showed that interaction of the B800 and B850 bacteriochlorophylls transition densities with the Car molecules had an effect on B800 and B850 electronic couplings, increasing them up to

≈30%. As a consequence, the Car appear to be capable of enhancing the energy transfer rate (by *ca.* 50–70%) from B800 to B850.[21]

Accordingly, in our view the Car in the PSII RC could act as electronic coupling bridges between the peripheral $Chls_Z$ and the RC central cofactors increasing the rate of energy transfer from $Chls_Z$ to the central cofactors estimated from the modified Redfield/modified Förster theory. Nevertheless, further experiments combined with quantum mechanical calculations including the coupling between Car and $Chls_Z$ are necessary to verify this hypothesis.

Conclusions

We have demonstrated that the two β-carotene molecules present in the PSII RC from higher plants feel the excitation on $Chls_Z$ manifested as a five nanometer blue shift of their S_0–S_2 transition. We propose that the Car may increase the electronic coupling between $Chls_Z$ and the central Chls. To our knowledge, this is the first time that experimental evidence pointing to a possible role of the carotenoid molecules in enhancing the excitation energy transfer rate between Chls in the PSII RC has been reported.

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