

## A new carotenoid-to-BChl energy transfer pathway in photosynthetic light harvesting

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### Introduction

Carotenoids play an important role in photosynthetic light-harvesting by absorbing blue and green sunlight and transferring the energy to nearby (B)Chl molecules (Ritz et al. 2000). The polyene backbone of carotenoids, which consists of alternating single and double carbon bonds, accounts for many of their excited state properties. At least two singlet excited states exist in the excited state manifold of carotenoids, generally referred to as  $S_1$  and  $S_2$  ( $2A_g^-$  and  $1B_u^+$ ), of which only the latter is optically allowed. Excitation to  $S_2$  is typically followed by fast internal conversion (IC) to  $S_1$  in  $\sim 100$  fs and then by IC of  $S_1$  to  $S_0$  on a picosecond timescale. It has been established that energy transfer to (B)Chl may occur from both the  $S_2$  and  $S_1$  states (Ritz et al., 2000).

In our recent work, (Gradinaru et al., 2001) we have shown that on the carotenoid spirilloxanthin, besides  $S_1$  a thus far uncharacterized excited state is formed upon optical excitation, which we tentatively referred to as  $S^*$ . When bound to the LH1 complex of the purple bacterium *Rs. rubrum*,  $S^*$  is the precursor state on the reaction pathway towards triplet formation, which occurs within a few picoseconds through an intramolecular homofission mechanism. On spirilloxanthin in solution,  $S^*$  returns to the singlet ground state on a picosecond timescale. We asserted that the  $S^*$  state most likely corresponds to the optically forbidden  $1B_u^-$  state, which was recently observed by Koyama and co-workers (Ritz et al. 2000).

To investigate the possible involvement of  $S^*$  in photosynthetic light harvesting, we studied an antenna complex with a highly developed carotenoid light harvesting function, the LH2 complex of *Rb. sphaeroides* 2.4.1. This complex binds spheroidene, which is  $\sim 95\%$  efficient in transferring excitations to BChl. We will show that a state similar to the  $S^*$  state of spirilloxanthin exists in the excited state manifold of spheroidene, and that it is actively involved in the energy transfer processes to BChl, thereby constituting a new mechanism of energy transfer in photosynthetic light harvesting.

### Materials and methods

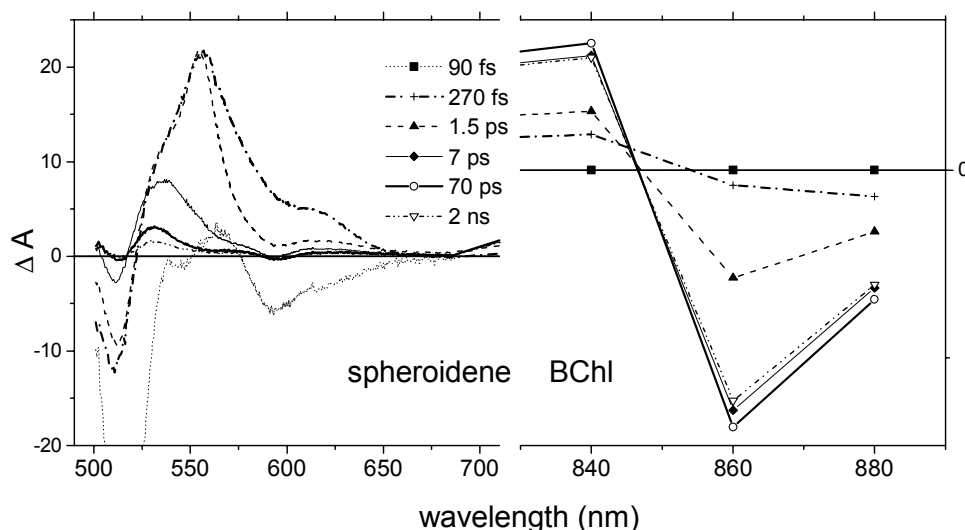
LH2 complexes of *Rb. sphaeroides* were prepared as in Cogdell et al., 1993. Femtosecond transient absorption measurements with excitation at 475 nm and probing in the visible were carried out with a 1 kHz amplified Ti:Sapphire laser system (Gradinaru et al., 2001). For probing in the infrared a 125 kHz amplified

Ti:Sapphire system was used. An OPA provided excitation at 490 nm and the pulse energy was reduced to 4.8 nJ to ensure annihilation-free conditions.

A global analysis scheme was used to analyze the recorded time resolved spectra (van Stokkum et al., 1994). Species associated difference spectra (SADS) were determined by applying an irreversible sequential model i.e.  $A \rightarrow B \rightarrow C \rightarrow D$  where the arrows indicate successive monoexponential decays of increasing time constants which can be regarded as the species' lifetimes.

## Results and Discussion

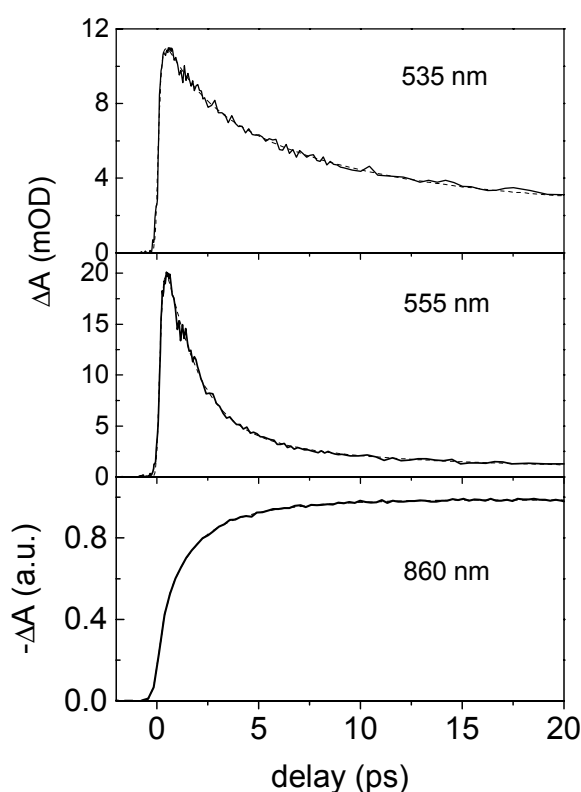
We investigated the dynamics of carotenoids and BChls in the LH2 complex of *Rb. sphaeroides* 2.4.1 by performing transient absorption experiments with excitation of spheroidene and probing in the visible and near-infrared, where spheroidene and BChl have their specific absorptions, respectively. Simultaneous analysis of the data indicated that 6 components were required to achieve a satisfactory description. The resulting SADS are portrayed in Fig. 1 together with the corresponding lifetimes, while selected kinetic traces at 535, 555 and 860 nm are presented in Fig. 2.



**Fig. 1:** SADS and associated lifetimes for spheroidene (left) and BChl (right) in the LH2 complex of *Rb. sphaeroides* 2.4.1 upon spheroidene excitation. Note that the indicated lifetimes correspond to both sides of the figure.

The instantaneously appearing SADS (Fig. 1 left side, thin dotted line) has a lifetime of 90 fs. It features a sharp bleach at 515 nm and broad negative signals on the red part of the visible and reflects the  $S_2$  state of spheroidene. The 90 fs decay of  $S_2$  is followed by the appearance of the 2<sup>nd</sup> SADS (dot-dashed line) which has a lifetime of 270 fs. It shows the characteristic excited state absorption (ESA) of the spheroidene  $S_1$  state, which peaks at 555 nm, indicating that IC from the  $S_2$  to  $S_1$  state has occurred. The 3<sup>rd</sup> SADS (dashed line) features the  $S_1$  ESA at 555 nm but is narrowed with respect to the 2<sup>nd</sup> SADS, indicative of a vibrational relaxation process of the  $S_1$  state with a time constant of 270 fs. This SADS has a lifetime of 1.5 ps, and the spectral changes upon going to the 4<sup>th</sup> SADS (thin solid line) indicate that this process corresponds to the disappearance of the  $S_1$  state. This lifetime is significantly shorter than that of spheroidene in solution, 9 ps, demonstrating that efficient energy

transfer to BChl takes place from the  $S_1$  state (Zhang et al., 2000). The 4<sup>th</sup> SADS has a lifetime of 7 ps and represents a significant ESA feature at 535 nm. This SADS, which has spectral and temporal characteristics clearly distinct from those of the  $S_1$  state, represents a state of spheroidene that bears close resemblance to the  $S^*$  state of spirilloxanthin in the LH1 complex of *Rs. rubrum*; Its peak at 535 nm is already existent as a shoulder on the blue side of the ESA of the 2<sup>nd</sup> and 3<sup>rd</sup> SADS, indicating that this state is populated directly from  $S_2$ , in parallel with the  $S_1$  state, as in *Rs. rubrum* (Gradinaru et al., 2001). These observations strongly indicate that the 4<sup>th</sup> SADS actually corresponds to  $S^*$ , which, judging from its amplitude, is formed with a significant yield in the LH2 complex of *Rb. sphaeroides*. In 7 ps,  $S^*$  largely disappears (5<sup>th</sup> SADS, thick solid line), and at still longer times the spheroidene triplet state T is formed from  $S^*$  (6<sup>th</sup> SADS, dash-dot-dotted line), similar to our observations of spirilloxanthin in *Rs. rubrum* (Gradinaru et al., 2001).



**Fig. 2:** Kinetic traces taken in the LH2 complex of *Rb. sphaeroides* 2.4.1 upon spheroidene excitation, detected at the wavelengths indicated (solid lines). The dashed lines indicate the results of a global analysis with 6 components (see text). Note that the 860 nm trace has been inverted and in fact represents a bleaching signal.

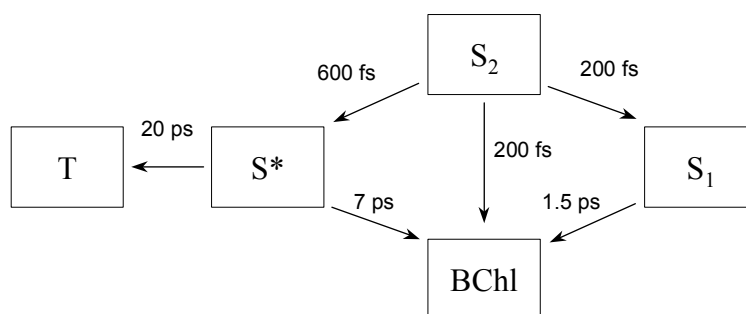
To illustrate the different temporal behaviors of the  $S_1$  and  $S^*$  states, the traces at 535 and 555 nm are shown in Fig. 2. The signal measured at 555 nm, at the  $S_1$  absorption maximum, has a major decay component of 1.5 ps, while the slower phase with a small amplitude corresponds to the red tail of the ESA that originates from the  $S^*$  state (see 4<sup>th</sup> SADS of Fig. 1). The 535 nm trace, which mainly probes  $S^*$ , clearly decays slower than the 555 nm trace, and does not relax to zero in the timeframe of our experiment, indicating formation of the spheroidene triplet state T.

After having established that  $S_1$  and  $S^*$  have distinctly different temporal characteristics, we are in a position to discriminate their possible contributions to the energy transfer process to BChl by measuring the rates at which the excitations arrive on B850. To this end, we have measured the rise of the transient absorption signal at 860 nm, shown in Fig. 2. At this wavelength, bleaching and stimulated emission (SE) of B850 is probed. We observe that the rise of the signal is multiphasic, with very fast initial rise in ~200 fs, and slower components in the picosecond range. It is clearly

seen that the signal is still rising well beyond 5 ps, indicating that excitation energy is still being transferred to BChl long after 1.5 ps, which is the lifetime of the spheroidene  $S_1$  state. This implies that spheroidene states other than  $S_1$  and  $S_2$  are involved in energy transfer to BChl. Given its 7 ps lifetime, the  $S^*$  state is a very likely candidate.

The correlation between the disappearance of  $S^*$  and the arrival of excitations on BChl is demonstrated in the SADS of Fig. 1, where the right side describes the BChl dynamics in the IR region. Three IR wavelengths of detection are shown, 840, 860 and 880 nm, where B850 shows ESA, bleaching/SE, and pure SE, respectively (see e.g. Kennis et al., 1997). The amplitudes of the SADS in the IR increase during the energy transfer processes that are associated with the  $S_2$  and  $S_1$  states, i.e. 90 fs (squares to crosses), 270 fs (crosses to up triangles) and 1.5 ps (up triangles to diamonds), as expected from previous studies (Zhang et al., 2000). The 7 ps component, which in the visible part of the spectrum denotes a significant loss of  $S^*$ , represents the final rise step of the BChl signal (diamonds to open circles), accounting for roughly 10% of the final amplitude. This observation provides strong evidence that  $S^*$  is a donor state in the energy transfer process from spheroidene to BChl.

On the basis of our results we propose the kinetic scheme depicted in Fig. 3, which includes not only the established pathways of energy transfer via the  $S_2$  and  $S_1$  states of carotenoids to BChl, but also a pathway mediated by the newly discovered  $S^*$  state.



**Fig. 3:** Kinetic scheme to portray energy transfer pathways in the LH2 complex of *Rb. sphaeroides* 2.4.1.  $S_2$ ,  $S_1$ ,  $S^*$  and T denote the various electronic states of spheroidene described in the text.

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