EPJ Web of Conferences **41**, 08005 (2013)
DOI: 10.1051/epjconf/20134108005

Broadband 2D Electronic Spectroscopy Reveals Coupling Between Dark $1B_u^-$ State of Carotenoid and Q_x State of Bacteriochlorophyll

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Abstract. The study of LH2 protein of purple bacteria by broadband 2D electronic spectroscopy is presented. The dark $1B_u^-$ carotenoid state is directly observed in 2D spectra and its role in carotenoid-bacteriochlorophyll interaction is discussed.

1 Introduction

Chlorophylls and carotenoids are the main light absorbing pigments in light-harvesting proteins. Carotenoids have two different functions. They harvest the light energy and transfer it to chlorophylls. They also accept excess excitation energy from chlorophylls and dissipate it, thereby preventing formation of singlet oxygen which can damage the protein.

In the LH2 complexes of *Rps. acidophila* the light energy, absorbed by lowest allowed S_2 excited state of carotenoid (Car), either relaxes to dark Car S_1 state or is transferred to Q_x state of bacteriochlorophyll (BChl). The overall efficiency of energy transfer from Car S_2 state to BChl Q_x state is between 40-60% [1–4]. Theoretical study however predicted only 20% efficiency of Car-to-BChl transfer due to small spectral overlap [5]. Thus, the energy transfer between the two molecules is substantially contributes to the overall light-harvesting process. However despite active studies, no clear understanding of the underlying processes and mechanisms is present. The experimental obstacle is the strong overlap of the Car/BChl signals both spectrally and temporally. In several works on isolated carotenoids and light-harvesting proteins a signature of additional Car dark state ($1B_u^-$ or S_x state) was found, which increases the controversy of the interpretation of spectroscopic data [4, 6–8].

In the current study the 2D electronic spectroscopy was applied to the LH2 complexes of *Rps. acidophila*. The 2D spectroscopy gives more information than traditional transient absorption, providing spectral resolution along both excitation and emission energy scales. Broadband pulses in the spectral region of 500-630 nm were used in order to excite simultaneously both Car S_2 state and BChl Q_x state. The resulted 2D spectra revealed in addition to signals from Car S_2 state and BChl Q_x state, a contribution from previously unobserved intermediate state. The origin of this state and its function in LH2 protein is discussed.

2 Materials and Methods

The LH2 complexes from *Rhodopseudomonas acidophila* strain 10050 were prepared as previously described [9]. The sample solution of 0.3 OD/mm contained 0.1% LDAO. For measurement a 1 mm flow cell was used and the absorption spectra before and after the experiment were measured to exclude photo-degradation of the sample.

The 2DES setup is described in details elsewhere [10]. Briefly, the regenerative amplifier (Spitfire, Spectra-Physics) seeded by Ti:sapphire oscillator generates 150 fs pulses at 800 nm. A home-built NOPA converts the 10 uJ pump pulses into 10 mW broad-bandwidth pulses (<70 nm FWHM). The compressed to 13 fs pulses are split by diffractive optic into four beams and are arranged in the box geometry. The emitted signal is heterodyne detected by the spectrograph (Shamrock, Andor). The time axis t_1 (coherence time) is scanned from -45 to 45 fs (negative time range corresponds to non-rephasing signal, positive – to rephasing signal) and fourier transformed into excitation frequency axis (wavelength). The t_2 time axis was scanned in the 0 to 400 fs range with a time step of 1 fs. The dynamics of the absorptive 2D signal was monitored as a function of t_2 delays (waiting time).

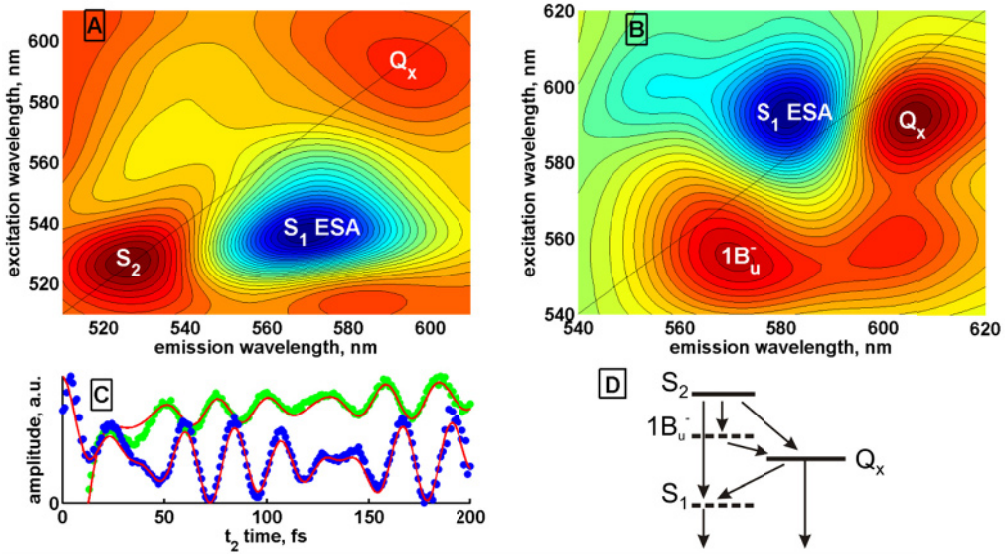

Fig. 1. 2D ES data after excitation in two spectral regions. A – two-dimensional spectrum with excitation in 530-590 nm taken at t_2=170 fs in, B – two-dimensional spectrum with excitation in 550-630 nm taken at t_2=100 fs, C – decays and fit of data sampled at S_2 (blue dots) and $1B_u^-$ (green dots) peaks, D – preliminary electronic level scheme.

3 Results and Discussion

Figure 1 shows 2D spectra at selected t_2 delays for two different excitation spectral ranges. When sample is excited with 530-590 nm pulses, both Car S_2 state and BChl Q_x state are excited (Figure 1A). In addition to the two diagonal peaks, corresponding to the bleach and stimulated emission signals of the two states, a negative off-diagonal peak is observed in the spectrum. This signal is due to the excited state absorption (ESA) from Car S_1 state, a feature of carotenoids, well known from transient absorption studies [4]. The ESA signal has maximum after excitation into Car S_2 state, indicating the $S_2 \rightarrow S_1$ internal conversion. A weak tail of the Car S_1 ESA at longer excitation wavelengths indicates presence of BChl $Q_x \rightarrow$Car S_1 back energy transfer (in Figure 1B this feature is more pronounced). The S_2/Q_x positive cross peak overlaps with the ESA signal (Figure 1A) and represents the $S_2 \rightarrow Q_x$ energy transfer. Figure 1B shows a 2D spectrum obtained with 550-630 nm

excitation pulses. A new diagonal peak (denoted as $1B_u^-$) is observed at 560 nm wavelength. The decay sampled at the $1B_u^-$ peak (Figure 1C green dots) shows very similar oscillations to the oscillations observed at the S_2 peak (Figure 1C blue dots). These decays can be fitted with a sum of exponentials and three frequency modes: 1000 cm^{-1}, 1250 cm^{-1} and 1600 cm^{-1} (see red line in Figure 1C), known to be a signature vibrational frequencies of carotenoids [11]. Since the S_2 state is not excited in Figure 1B, the $1B_u^-$ peak is due to an additional carotenoid state, which has features very similar to the features of the dark $1B_u^-$ (or S_x) state, predicted theoretically [12] and extensively discussed in the literature (for review see [13]). As follows from the 2D spectrum in Figure 1B, Car $1B_u^-$ state strongly interacts with the BChl Q_x state. The Car $1B_u^-$/BChl Q_x cross peak indicates substantial $1B_u^- \rightarrow Q_x$ energy transfer. At the same time no Car S_1 ESA is observed after excitation of the $1B_u^-$ state, indicating no $1B_u^- \rightarrow Q_x$ energy transfer. The summarised electronic level scheme and observed energy pathways are shown in Figure 1D. Appearance of the dark $1B_u^-$ is ascribed to the borrowing of the dipole moment strength from the BChl Q_x state. Presence of $1B_u^- \rightarrow Q_x$ energy transfer can explain the disagreement between experiment and theory in the energy transfer efficiency since the Car $1B_u^-$ state was not accounted for in the theoretical studies [1–5].

4 Conclusions

In this work we report 2D spectra with clear presence of the Car $1B_u^-$ dark state. This state has been controversially discussed both in experimental and theoretical studies for the last decade, however no direct observation (as a ground state bleach) of that state in the spectrum has been reported before. Due to the very low dipole moment the Car $1B_u^-$ state is invisible in the stationary spectra and can only be observed under selective excitation conditions. The substantial contribution of the $1B_u^-$ state to the interaction between carotenoid and bacteriochlorophyll molecules is demonstrated. Taking into account the Car $1B_u^-$ state is essential for understanding of the energy dynamics in light-harvesting proteins.

Acknowledgements

Authors thank Daniel B. Turner for assistance with 2DES setup. This work was supported by the Natural Sciences and Engineering Research Council of Canada and DARPA (QuBE).

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