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SINGLET ENERGY TRANSFER FROM CAROTENOIDS IN PURPLE BACTERIAL LIGHT HARVESTING ANTENNA

S. E. Bradforth, R. Jimenez, M. Ricci, S. N. Dikshit and G. R. Fleming
Department of Chemistry, University of Chicago, 5735 S. Ellis Avenue, Chicago, IL 60637, USA

ABSTRACT

Ultrafast fluorescence upconversion has been used as a probe of energy transfer in the photosynthetic pigment protein complexes LH-1 and LH-2. The internal conversion lifetime of spheroidene in the 1$^{1}B_u$ ($S_1$) state in solution has been determined to be 150 - 250 fs and depends on the polarizability of the surrounding environment. The internal conversion time in the protein complexes is estimated based on the solution measurements. The fluorescence lifetime of spheroidene in the intact light harvesting complexes is substantially shorter pointing towards direct and very rapid energy transfer from the $S_1$ state to bacteriochlorophyll, however the population on the lowest state of bacteriochlorophyll, as monitored by fluorescence at 910 nm, rises with a much longer timescale.

1. Introduction

Recently the 3D X-ray structure of the peripheral light harvesting antenna protein of the purple bacteria Rhodobacter sphaeroides has been solved by McDemott et al. A remarkable 9-fold symmetry for the pigment protein complex is revealed. Close interactions are indicated between the constituent pigments, bacteriochlorophyll (BChl) and the carotenoid spheroidene, within the antenna complex. Center to center distances for BChl molecules are found to be around 8-9 Å and the spheroidene molecules are shown to be in van der Waals contact with their associated bacteriochlorophylls. A lower resolution structure for the larger core light harvesting complex, determined from electron diffraction from two dimensional crystals of Rb. rubrum, has also just been published. This shows a similar structural arrangement for the BChl pigments and protein subunits as in the peripheral antenna; in this case there is 16-fold symmetry. A systematic study of the singlet energy transfer dynamics in the core (LH-1) and peripheral (LH-2) light harvesting antenna of the related purple bacteria Rhodobacter sphaeroides has been recently carried out in our laboratory utilizing ultrafast fluorescence upconversion. We have characterized the energy migration at room temperature between bacteriochlorophylls (BChl) in the B875 band of LH-1, and in the B850 band of LH-2 as well as between B800 and B850 in LH-2. Despite the small inter-chromophore spacings, we find that a Forster hopping description between BChl dimers can consistently describe the fluorescence depolarization and annihilation dynamics and that typical hopping times are ~100fs. Here we will describe our experiments exploring the mechanism of singlet transfer between spheroidene and BChl in both antenna.

The principal purpose of carotenoids in an antenna complex is to provide triplet protection for BChl, however, particularly in purple bacteria, they also perform an important energy harvesting role. The spectroscopy of carotenoids (approx. C$_{24}$, symmetry) is akin to that of polyenes: the strongly optically allowed transition is $1^1B_u - 1^1A_g$ and connects the
ground state to what has typically been called the S₂ state. The S₁ state (2^1A₂) is optically symmetry forbidden. Recent measurements that determine the energy of the “S₁” state also identify other low lying singlet states for spheroidene and suggest that several states may be involved in the internal conversion pathway. In addition the mechanism of energy transfer between photo-excited spheroidene and bacteriochlorophyll is currently unclear. Two experiments to address these issues will be described - time resolving the decay of fluorescence from the initial S₂ state of spheroidene within Rb. sphaeroides LH-1 / LH-2 complexes and the rise of fluorescence of the BChl acceptor Q₃ following excitation of the spheroidene. Accompanying experiments to determine independently the internal conversion dynamics of free spheroidene in a number of solvents are briefly described. Our goal is to develop a physical picture for energy transfer at very short ranges in the photosynthetic proteins and reveal the dynamics that underpin the function of the light harvesting complexes.

2. Experimental

Fluorescence upconversion experiments were performed with either the output of a Ti:S oscillator or via a 250 kHz regeneratively amplified Ti:S system. The excitation of the spheroidene pigment in the blue/green is achieved by either the second harmonic of the oscillator (460 nm) or from a portion of the white light continuum generated from the regenerative amplifier (505 nm). In both case the laser fundamental provides the upconversion gate pulse. An elliptical upconversion set up is employed which has been described elsewhere. The instrument function for experiments where second harmonic is used is 140 fs and 280 fs where the regen used. All experiments are performed at room temperature.

3. Results and Discussion

A series of measurements on spheroidene extracted from whole cells of Rb. sphaeroides and dissolved in range of solvents has been performed. These studies indicate that the S₂ internal conversion times for

Figure 1: Time resolved fluorescence from the S₂ state of spheroidene. Data shown are for experiments with spheroidene extract in n-pentane, and native in detergent isolated LH-1 and LH-2 complexes. Excitation is at 460 nm, and fluorescence at 525 nm (or 535 nm for the proteins) is upconverted. The signals are fit with a dominant decay (> 95%) of 60fs and 80 fs time constant in the case of LH-1 (fastest decaying trace) and LH-2, respectively, and with a single exponential decay τ = 250 fs for n-pentane (slowest trace).
spheroidene in vitro depend on the polarizability (and to a lesser extent the polarity) of the solvent. Decay times range between 250 fs for n-pentane (fig. 1) and 160 fs for carbon disulfide. For spheroidene in pentane solution we determine a significantly shorter internal conversion time than Shreve et al., but a value similar to those determined for β-carotene. The major effect of increasing the solvent polarizability is to lower the $S_2$ state energy and therefore decrease the $S_2 - S_1$ energy gap; the energy of the $S_1$ state is expected to only weakly vary with solvent polarizability. The energy gap law for radiationless transitions appears to apply fairly well to the data if we assume that the lower state is actually the $S_1$ (i.e. $2^1A_g$). Using the energy gap law we may estimate the internal conversion time for spheroidene embedded in the protein environment from the position of the $S_2$ band in the absorption spectrum. In this manner, we determine that the $S_2$ internal conversion lifetime in a medium of equivalent polarizability to the LH-1 protein will be ~ 170 fs and ~ 160 fs for LH-2. Figure 1 shows that for the antenna proteins, the $S_2$ fluorescence decays are in fact substantially shorter. This leads us to suspect that energy transfer directly from the $S_2$ state appears to significantly shorten the state’s lifetime. For example, the observed 60 fs fluorescence lifetime for LH-1 taken together with the estimated internal conversion time would suggest ~100fs energy transfer out of the spheroidene $S_2$ state.

The mechanism of energy transfer between carotenoids and bacteriochlorophyll has in general been assumed to be via the dipole forbidden $S_1$ state (after fast internal conversion of the carotenoid $S_2$ state) through an exchange type rather than Coulombic ( Förster type) coupling. Shreve et al. have proposed that (Coulomb allowed) energy transfer directly from the $S_2$ state to the $Q_a$ of BChl may compete with internal conversion and account for a large part of the yield (see Figure 2). Our result for the very fast decay of the $S_2$ state fluorescence certainly seems to be consistent with this assertion. However, in separate
experiments in our lab, when the S₂ spheroidene band is excited at 505 nm, the appearance of fluorescence at 910 nm (BChl Qₚ) occurs on a significantly longer timescale. Figure 3 shows that the population on the Qₚ state of BChl (the final singlet energy acceptor) in LH-1 can be fit by a bi-exponential rise. The same experiment in LH-2 yields even slower timescales (~ 60%, 800 fs and ~ 40%, 3 ps). This rise time is slower than that observed by Shreve et al.¹⁰ in pump-probe measurements. It should be possible to describe the experimental signals in Figure 1 and 3 with the same four state kinetic scheme (Figure 2). The dashed line in Figure 3 represents an attempt to model the BChl rise consistent with the internal conversion time and direct energy transfer time found in the spheroidene fluorescence decays. The characteristic times used in the kinetic scheme for this curve are 170 fs and 9.1 ps for the spheroidene S₂ and S₁ internal conversion, 200 fs for the BChl Qₓ⁻Qᵧ internal conversion and 90 fs and 2.0 ps for the energy transfer rates from the spheroidene S₂ and S₁ states respectively. Quite clearly this is unsuccessful. Rather long internal conversion times for the Qₓ⁻Qᵧ process are required to make the model fit; we find it unlikely that this internal conversion is slower than 200 fs. A determination of the BChl Qₓ⁻Qᵧ internal conversion timescale and a direct experiment to determine the S₁⁻Qᵧ energy transfer rate are in progress to resolve the mechanism of this singlet energy transfer.

We have argued that the lifetimes of the S₂ state of spheroidene in vivo are much shorter as a result of energy transfer occurs from the S₂ state to BChl directly (~ 100 fs). However the rise time kinetics of BChl emission are not consistent with these conclusions. We cannot rule out the alternative argument that the spheroidene internal conversion is significantly speeded up by deformation of the spheroidene backbone by the protein host (as seen in the LH-2 X-ray structure).¹

4. References