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ARTICLE *in* THE JOURNAL OF PHYSICAL CHEMISTRY B · SEPTEMBER 2000

Impact Factor: 3.3 · DOI: 10.1021/jp001993q

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THE JOURNAL OF PHYSICAL CHEMISTRY B

 VOLUME 104, NUMBER 42, OCTOBER 26, 2000

LETTERS

Unraveling Exciton Relaxation and Energy Transfer in LH2 Photosynthetic Antennas

Kõu Timpmann,[†,‡] Neal W. Woodbury,[†] and Arvi Freiberg*[,†,‡]

Department of Chemistry and Biochemistry and Center for the Study of Early Events in Photosynthesis, Arizona State University, Tempe, Arizona 85287, and Institute of Physics, University of Tartu, 51014 Tartu, Estonia

Received: May 31, 2000; In Final Form: August 25, 2000

A long-standing uncertainty has been resolved concerning the early time evolution of light excitations in LH2 antennas of purple photosynthetic bacteria. Both isolated and native membrane-embedded LH2 antenna complexes were comprehensively studied using femtosecond time-resolved transient absorption spectroscopy together with conventional absorption and fluorescence emission spectroscopies at low temperatures. The results show that exciton relaxation within isolated LH2 complexes is characterized by subpicosecond time constants, while spectral evolution associated with energy transfer between the antenna complexes in photosynthetic membranes is characterized by time constants $\geq$ 1 ps.

Introduction

The purpose of this work is to unravel the different ultrafast energy relaxation channels active in photosynthetic bacterial membranes containing peripheral LH2 antenna complexes. This is vital in order to achieve a detailed understanding of light harvesting and energy trapping processes in photosynthesis. Exciton relaxation in bacterial antennas is known to occur with a time constant on the order of 10^{-13} s. In several cases, however, notably at low temperatures, relaxation kinetics as slow as 10^{-9} s has been observed in time-resolved spontaneous[1,2] and stimulated emission[3−5] spectra of different bacterial antennas. This slow (picoseconds and longer) relaxation is accompanied by a >10 nm spectral red shift, and its origin is a matter of ongoing debate. Originally, this was ascribed to energy transfer between spectrally inhomogeneous antenna complexes within large photosynthetic membranes.[1,2,6] Recent analysis revealing two types of spectral disorder that govern the shape of the linear and nonlinear exciton absorption spectra of the membrane-embedded LH2 complexes[7] lends further support to this interpretation. However, a number of alternative explanations for these observations have been put forth including intracomplex exciton relaxation[3] and, very recently, polaron formation.[8]

To separate experimentally the energy relaxation channels distinguishing lone LH2 complexes from their ensemble, in the present work we compare the femtosecond time-resolved pump−probe absorption spectra of isolated LH2 complexes with those of mutant photosynthetic membranes containing only LH2 antennas. A distinct aspect of this study is the use of spectrally narrow femtosecond excitation pulses in resonance with the low-energy exciton transitions at low temperature (10 K), facilitating selective excitation of a subpopulation of LH2 complexes within the total spectrally heterogeneous ensemble.

Sample Characterization. The samples from *Rhodobacter (Rb.) sphaeroides* were prepared as described earlier.[4,5,9] For the present studies, it is critical to know if the isolated LH2 complexes used were all present as separate complexes or whether larger aggregates or membrane fragments were present. To investigate this, the hydrodynamic radii (R_h) of the LH2 complex and membrane preparations at room temperature were determined by dynamic light scattering (DLS) technique.[10] As expected, the hydrodynamic radius found for the membranes

* Corresponding author.
† Arizona State University.
‡ University of Tartu.

10.1021/jp001993q CCC: $19.00
Published on Web 09/29/2000

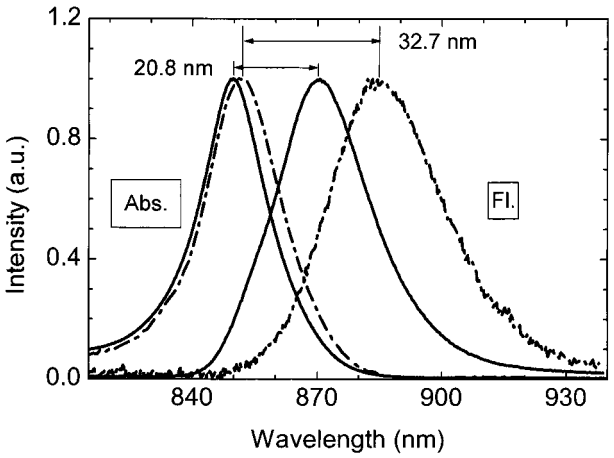

Figure 1. Peak-normalized absorption and fluorescence emission spectra of *Rb. sphaeroides* membranes (dash dot curves) and isolated LH2 complexes (solid curves) at 10 K.

($R_h = 64 \pm 16$ nm; 16 nm is the standard deviation) is very different from that of isolated complexes ($R_h = 3.0 \pm 0.5$ nm) (detailed account of these measurements will be published elsewhere). The latter is close to the radius (~3.4 nm) determined[11] by X-ray structure analysis of the cylindrical LH2 antenna proteins from another purple bacterium *Rhodopseudomonas acidophila*, which is similar to *Rb. sphaeroides*. From this we conclude that our solution contains well-isolated LH2 antenna complexes.

Absorption and Fluorescence Emission Spectra. Figure 1 compares conventional absorption and fluorescence emission spectra of excitons near 850 nm (so-called B850 excitons) in membranes and isolated LH2 complexes at 10 K. Fluorescence was excited at 590 nm using a sample with an optical density of 0.05 measured at room temperature at 846 nm. The samples in a coldfinger type closed-cycle helium cryostat (APD Cryogenics) were prepared by adding glycerol (50/50 v/v) to the protein in buffer solution (TEN+0.2% LDAO).

The most distinct difference of the absorption spectra is a ~2 nm red shift of the maximum of the membrane spectrum with respect of that of the isolated complexes. Decreasing the detergent concentration to the level supporting aggregation of the LH2 complexes into larger oligomers also leads to a gradual red shift of the absorption spectra (data not shown) consistent with the red shift between isolated complexes and membranes demonstrated in Figure 1. The emission spectrum of the membranes is similarly red shifted with respect to the spectrum of the complexes. However, the relative shift observed in the emission spectrum is dramatically (almost 6-fold) larger than in the absorption spectrum (Figure 1). The related absorption and emission spectra, which are connected with arrows in Figure 1, demonstrate the resulting large difference between the Stokes shifts (shift between the maxima of the absorption and emission spectra) for the membranes (32.7 nm) and isolated complexes (20.8 nm). The absorption and emission spectra of the membranes are also generally broader than those observed for the isolated complexes (by ~11% and ~18%, respectively, at 10 K). All of these results suggest that there is an extra inhomogeneous broadening of the spectra of membranes compared to the spectra of isolated LH2 complexes.

Femtosecond Transient Absorption Spectra. Figure 2 shows transient differential absorption spectra of membranes and LH2 complexes at 10 K recorded at two different pump wavelengths and at two delay times (zero and 2.5 ps) between the 200 fs pump and probe pulses. The excitation wavelengths

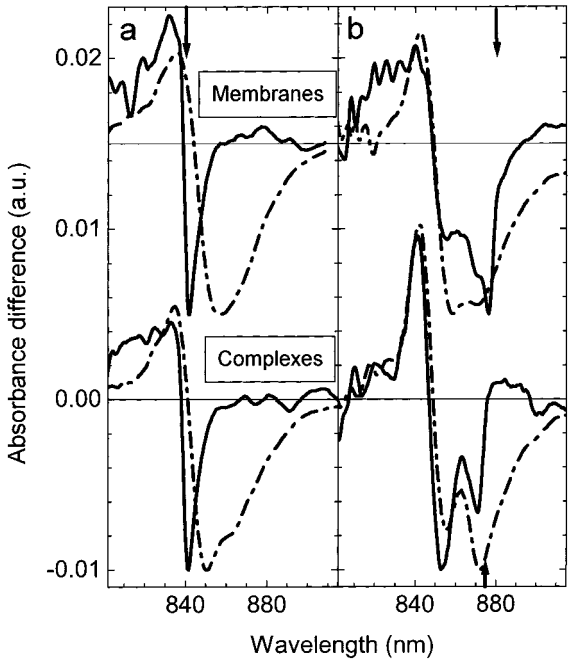

Figure 2. Peak normalized transient absorption spectra of membranes (upper part of the figure) and isolated LH2 complexes (lower part) from *Rb. sphaeroides* recorded at 10 K using zero delay (solid curves) and 2.5 ps (dash dot curves) between the 200 fs pump and probe pulses. For clarity, the spectra of the membranes are shifted upward relative to the spectra of the complexes by 0.015 units. Time zero is defined at the maximum of the bleach. Arrow at (a) 840 nm and (b) 874 nm (complexes) or 880 nm (membranes) indicates the excitation wavelength. The transient absorption spectrometer setup used has been described earlier.[5]

were chosen at the far-red edge (at 874 or 880 nm) and at the blue side of the maximum of the B850 steady-state absorption spectrum (840 nm).

Upon 840 nm excitation and at zero delay time, the shape of the transient absorption spectrum of the isolated complexes is similar to that of the membranes (Figure 2a). By 2.5 ps, the negative part of the spectrum of the isolated complexes develops a two-peak (blue- and red-side) structure that is absent in the spectrum of the membranes. The blue-side peak is determined by the bleaching of the ground-state absorption, as is suggested by its spectral position. The position of the red-side peak is close to the maximum of the steady-state emission spectrum (Figure 1) and is assigned to stimulated emission from a subpopulation of the B850 excitons selectively excited by the 840 nm pulse. Spectral resolution of the blue- and red-side features is not possible in the spectrum of membranes because of ultrafast intercomplex energy transfer (see below).

Using long-wavelength excitation, both membranes and single complexes show a red-shifted spectrum with clear two-peak structure at zero picoseconds (Figure 2b). The red-side peak due to strong excited-state absorption,[7] which does not appear at zero picoseconds and 840 nm excitation, is very pronounced upon long-wavelength excitation. In the single complexes (lower spectra), exciton relaxation manifests itself during the subsequent 2.5 ps by the red-edge spectral evolution of the transient spectrum. In the membranes (upper spectra), not only is there similar red-edge evolution but also the resolution between the two peaks is greatly reduced, by 2.5 ps. In this case, a broadening of the ground state bleaching and stimulated emission spectra results due to transfer among spectrally inhomogeneous complexes. The combination of intracomplex exciton relaxation and intercomplex energy transfer thus gives rise to the intricate spectral evolution observed in membranes.

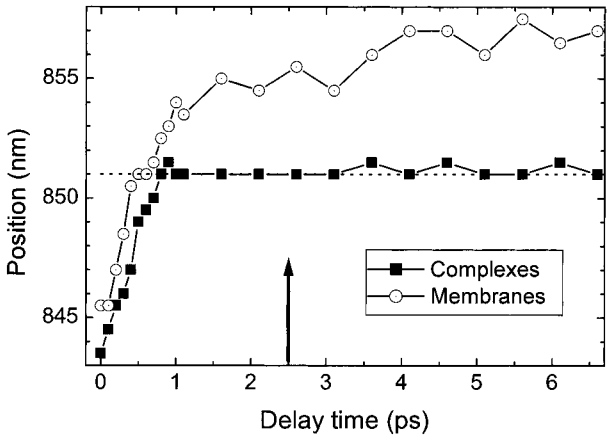

Figure 3. Position of the bleaching maximum in the transient absorption spectrum of membranes and isolated LH2 complexes from *Rb. sphaeroides* as a function of the delay time at 10 K. Excitation is at 840 nm. Arrow indicates the 2.5 ps time delay where the spectra in Figure 2 were recorded.

A comparison of the spectral dynamics in membranes and isolated complexes is presented in Figure 3. In the complexes, only ultrafast spectral dynamics can be seen. The maximum of the bleaching spectrum reaches its stationary position at ~851 nm in less than a picosecond (this position is displaced to the red by ~1 nm compared to the maximum of the ground-state absorption spectrum due to its overlap with the positive absorbance change in the excited state). In contrast, the difference spectrum of the membranes continues to red shift even beyond the 7 ps time scale of the present experiment, although its initial dynamics is almost as fast as that of the isolated complexes. This continued spectral evolution is another manifestation of the energy transfer between the spectrally inhomogeneous LH2 complexes within the membrane. This intercomplex energy transfer continues on even longer time scales, as evidenced by the large Stokes shift of the fluorescence spectrum of whole membranes (Figure 1).

Summarizing, for the first time, the ultrafast exciton relaxation inside a single antenna pigment−protein complex has been reliably separated from energy transfer processes between the complexes along large intracytoplasmic membranes of photosynthetic bacteria. While exciton relaxation in these antennas is characterized by subpicosecond time constants, incoherent energy transfer between different complexes within a heterogeneous ensemble of LH2 complexes along the photosynthetic membrane accounts for the long time evolution of the optical spectra. The rate of the intercomplex energy transfer is broadly distributed, with some of this transfer (presumably between nearest complexes) occurring on the single to few picosecond time scales. This agrees with the earlier reports showing that the rate-limiting step of energy transfer in intact photosynthetic bacterial membranes is transfer between the antenna and the photochemical reaction center rather than between the antenna complexes.[12]

Acknowledgment. This work was performed under the U.S. Department of Agriculture Grant No. 98-35306-6396. A. F. and K. T. acknowledge partial support from the Estonian Science Foundation, Grant No. 3865. The authors are grateful to J. Williams for donation of the *Rb. sphaeroides* strain with the puf operon deletion and to D. Dolak (Protein-Solutions Inc.) for performing the DLS measurements during the demo in Arizona State University (ASU). This is publication No. 440 from the ASU Center for the Study of Early Events in Photosynthesis.

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