Energy transfer in a single self-aggregated photosynthetic unit

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Abstract The primary events of bacterial photosynthesis rely on the interplay of various specialized protein complexes organized in a supramolecular structure commonly termed the photosynthetic unit (PSU), which consists of the photochemical reaction center and of an associated antenna network. Employing single-molecule spectroscopic techniques we have been able to observe the excitation-energy transfer within a single PSU. From these findings we conclude that the building blocks of the PSU spontaneously form stable, functional aggregates in a non-membrane environment.

Key words: Light harvesting complex; Photosynthesis; Energy transfer; Self-aggregation; *Rhodobacter sphaeroides*

1. Introduction

In most purple bacteria, the photosynthetic unit (PSU) present in the membrane, contains besides the reaction centre (RC) two types of antenna complexes, the light-harvesting complex 1 (LH1) and the peripheral light-harvesting complex 2 (LH2) [1]. Depending on the growth conditions of the bacterium some species express another peripheral complex, LH3, which is a spectroscopic variant of LH2. LH1 and the RC are closely associated and form the so-called core complex, whereas LH2 is not in direct contact with the RC but transfers the energy to the RC via the LH1 complex [2].

The progress made in high-resolution structural studies of light-harvesting complexes of purple bacteria has strongly stimulated experimental and theoretical investigations to understand the efficient energy transfer in these antenna systems [3–10]. The X-ray structure of the LH2 complex, along with the lower-resolution structural information for LH1, shows a remarkable symmetry in the arrangement of the light-absorbing pigments in their protein matrix. The basic building block of LH2 is a protein heterodimer (αβ), which binds three bacteriochlorophyll (BChl) *a* pigments and one carotenoid molecule. Remarkably, all peripheral light-harvesting complexes form circular oligomers of the two hydrophobic α- and β-apoproteins that non-covalently bind the pigments, featuring a nonameric (*acidophila*, *sphaeroides*) or octameric (*molischianum*) quaternary protein structure.

Based on the homology between the LH1 and LH2 proteins, the basic LH1 subunit is believed to contain only two closely coupled BChl *a* molecules and presumably one carotenoid. From this knowledge together with theoretical modeling a scheme of the arrangement of the pigment–protein complexes within the PSU has been proposed in which the core complex is surrounded by several LH2 complexes in a two-dimensional structure [11,12]. Interestingly, the pigments show a hierarchical arrangement where pigments absorbing at higher energies are placed further away from the RC. It seems that the whole structure is highly optimized to capture light energy and, depending on the illumination conditions, either to funnel the excitation energy to the RC or to act as a reservoir to store the energy in order to avoid overexposure and damage of the RC. However, despite the tremendous progress in the field that has been achieved during the last decade important details, for example the structure of the LH1–RC complex or the supramolecular organization of the PSU, are unknown and currently an issue of hot debate.

The great difficulty to determine the various parameters that play a role in the description of the electronic structure of light-harvesting complexes and the process of energy transfer is the fact that the optical absorption lines are inhomogeneously broadened as a result of heterogeneity in the ensemble of absorbing pigments. In order to circumvent this problem we have applied single-molecule detection schemes to study the pigment–protein complexes individually, thereby avoiding ensemble averaging. Here we report on the observation of the energy transfer from LH2 to the core complex within a single PSU. Our findings suggest that the building blocks of the PSU form stable, functional aggregates in a detergent suspension.

2. Materials and methods

Core complexes of *Rhodobacter* (*Rb.*) *sphaeroides* (pseudo-wild-type ΔQX/g (pRKX)) were isolated as described previously [13], except that after the washing step in 2M NaBr, 0.2 M sucrose, membranes were directly resuspended at 10 mg ml^{-1} protein and extracted with 3% octyl-glucoside (OG) and 0.5% Na-cholate. Sucrose gradient isolated PMC_3 (photosynthetic membrane complexes), identified as the monomeric form of LH1–RC complex [13], were collected. The isolated

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Abbreviations: BChl, bacteriochlorophyll; LH1, light-harvesting complex 1; LH2, light-harvesting complex 2; PMC, photosynthetic membrane complex; RC, reaction centre; PSU, photosynthetic unit

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complexes were diluted down to 5×10^{-11} M in buffer (0.6% OG/0.2% Na-cholate/50 mM NaGlyGly/pH = 7.8) with 1.8% (wt/wt) purified polyvinyl alcohol (PVA; M_W 125 000) present. A drop (10 μl) of the solution was spin-coated on a LiF substrate by spinning it for 15 s at 500 rpm and 60 s at 2000 rpm, producing high-quality films with a thickness of less than 1 μm. The samples were mounted in a liquid-helium cryostat and cooled to 1.4 K.

To perform fluorescence microscopy and fluorescence–excitation spectroscopy the samples were illuminated with a continuous-wave tunable Ti:sapphire laser (3900S, Spectra Physics) using a home-built microscope that can be operated either in wide-field or confocal mode. A fluorescence–excitation spectrum of an individual core complex was obtained in two steps. First a wide-field image of the sample was taken by exciting LH1 at 870 nm and detecting the LH1 fluorescence at 917 nm through a bandpass filter ($\Delta\lambda = 20$ nm) with a CCD camera (512 SB, Princeton Instruments). From this image a spatially well-isolated complex was selected. Next, a fluorescence–excitation spectrum of this complex was obtained by switching to the confocal mode of the set-up and scanning the excitation wavelength, while detecting the LH1 fluorescence at 917 nm with an avalanche photodiode (SPCM-AQR-16, EG&G). The spectra were recorded in rapid succession by scanning the excitation wavelength at a scan speed of 3 nm s^{-1} from 780 to 890 nm and storing the different traces separately. The spectral resolution is determined by the bandwidth of the laser and amounts to 1 cm^{-1}.

In order to examine the polarization of the emitted light a polarizer plate was introduced in front of the CCD camera and fluorescence images were recorded in rapid succession. Between two images the polarizer was rotated in steps of 9°. By integrating the total intensity of the fluorescence image of an individual complex on the CCD as a function of the read-out frame number we reconstructed the dependence of the emitted light on the angular position of the polarizer.

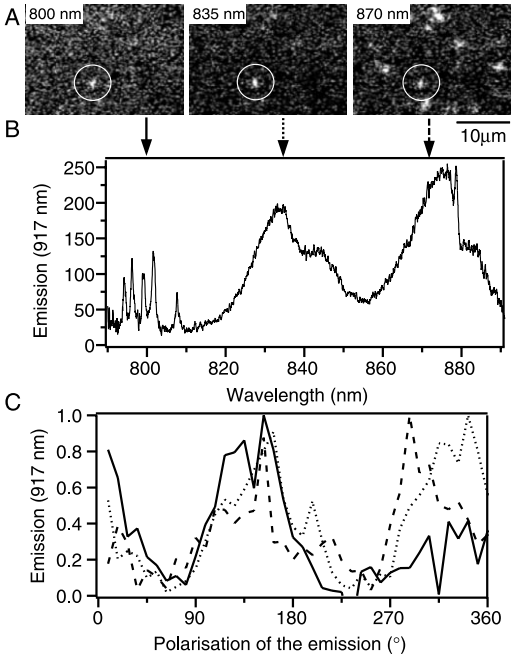

Fig. 1. Fluorescence-imaging and fluorescence–excitation spectroscopy of a single PSU from *Rb. sphaeroides*. A: Wide-field fluorescence images (20×25 μm^2) from LH1–RC dissolved in a polymer film at 1.4 K. From left to right the excitation wavelengths were 800, 835 and 870 nm, respectively, at an intensity of 250 W cm^{-2}. The fluorescence was detected at 917 nm. B: Fluorescence–excitation spectrum of the feature encircled in panel A. The sample has been excited at an illumination intensity of 20 W cm^{-2}. C: Intensity of the LH1 fluorescence as a function of the polarization of the emission for the feature encircled in panel A. The three traces correspond to excitation wavelengths of 800 nm (solid), 835 nm (dotted) and 870 nm (dashed) as indicated by the arrows in panel A.

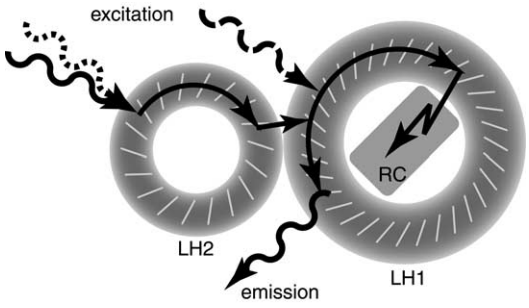

Fig. 2. Schematic sketch of the supramolecular arrangement of a PSU adapted from [25]. The arrows indicate the excitation emission and energy transfer pathways.

3. Results and discussion

In Fig. 1A a series of wide-field images from a sample of LH1–RC complexes from *R. sphaeroides* is shown as a function of the excitation wavelength. Each bright dot represents the diffraction-limited image of an individual core complex. In order to obtain these images the excitation wavelength was switched between 800, 835 and 870 nm while for all images the detection wavelength was fixed at 917 nm, i.e. the maximum of the LH1 emission. Exciting the sample at 870 nm the wide-field fluorescence image (Fig. 1A, right) shows several individual LH1–RC complexes from *Rb. sphaeroides*. After changing the excitation to 800 or to 835 nm (Fig. 1A, left and center, respectively), only one of the features detected previously is still able to emit light at 917 nm. In order to investigate this finding in more detail we have recorded the fluorescence–excitation spectrum of this particular feature, Fig. 1B. It consists of several narrow absorptions at 800 nm and two broad bands at about 830 and 870 nm which feature further substructure. Fig. 1C displays the relative fluorescence intensity at 917 nm as a function of the polarization of the emission for excitation wavelengths at 800, 835 and 870 nm as indicated by the arrows in panel B. Within the experimental accuracy no difference in the orientation of the emitting transition-dipole moment is observable.

From these results we conclude that we have observed the excitation-energy transfer in a single PSU, as sketched in Fig. 2. This interpretation is supported by inspecting fluorescence–excitation spectra from individual, isolated LH2 and LH1–RC complexes (Fig. 3). The upper traces show the low-temperature fluorescence–excitation spectra from isolated LH2 (*Rhodopseudomonas* (*Rps.*) *acidophila*) [14] and isolated LH1–RC (*Rb. sphaeroides*) recorded from a large ensemble of proteins while the lower traces show the respective fluorescence–excitation spectra of an individual light-harvesting complex. For LH2 the BChl *a* molecules can be grouped in two distinct pigment pools labeled B800 and B850, according to their room-temperature absorption maxima in the near-infrared. In contrast, only a single absorption band at around 870 nm is observed for LH1. Apparently, this technique allows us to reveal details that are normally obscured by the process of ensemble averaging. Especially, a striking difference between the two absorption bands of LH2 becomes visible. The B850 is dominated by few broad absorptions which reflect the exciton character of the involved electronic excitations whereas the B800 band consists of several narrow lines which correspond to excitations that are (mainly) localized on

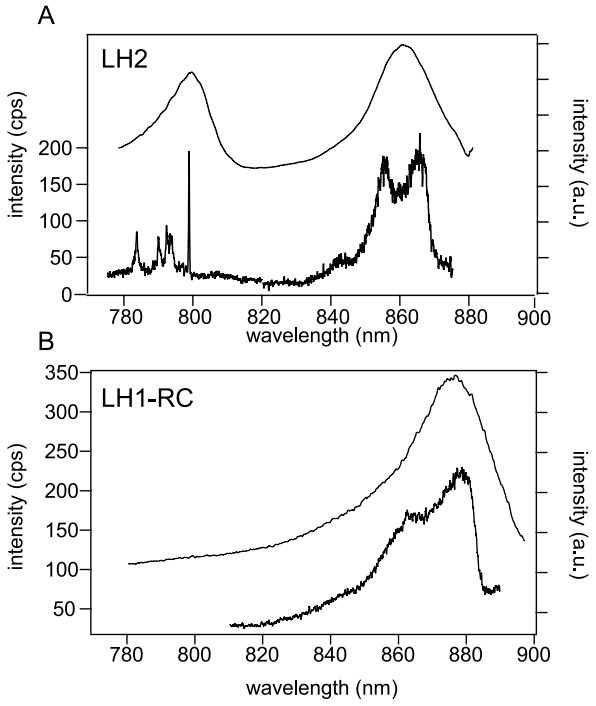

Fig. 3. Comparison of various fluorescence–excitation spectra from light-harvesting complexes recorded at 1.4 K. A: LH2 from *Rps. acidophila*. The upper trace has been recorded from a large ensemble of complexes and the lower trace shows the respective spectrum from an individual complex. For both spectra the LH2 emission has been detected at 890 nm. The left vertical scale is valid for the lower trace (adapted from [14]). B: LH1–RC from *Rb. sphaeroides*. The upper trace has been recorded from a large ensemble of complexes and the lower trace shows the respective spectrum from an individual complex. For both spectra the LH1 emission has been detected at 917 nm. The left vertical scale is valid for the lower trace.

individual BChl *a* molecules [15,16]. LH1–RC fluorescence–excitation spectra also show a limited number of broad bands with a characteristic polarization behavior, indicating that the excitations are delocalized over a large number of pigments as for the B850 band of LH2 [17,18]. Generally one might have expected a stronger quenching of the LH1 emission by the RC. But especially at low temperatures the lowest energy level of a significant fraction of the LH1 complexes is thought to be shifted to the red with respect to the energy level of the primary donor (P) of the RC, thus enhancing fluorescence from LH1 because of reduced trapping efficiency. [19,20]. Moreover, at cryogenic temperatures, light excitation induces charge separation only between the primary electron donor P and the primary quinone acceptor Q_A; electron transfer to Q_B is, in fact, blocked below 150 K [21,22]. As a consequence, once Q_A has been reduced following the first photon, no further photochemistry and concomitant quenching of the fluorescence can occur.

When comparing the data presented so far one realizes two discrepancies between the spectra shown in Figs. 1 and 3. First, the spectrum in Fig. 1B shows a broad band at about 830 nm rather than a band at 850 nm and, second, in Fig. 1B a sharp peak is visible in the band at 870 nm which does not appear in the displayed isolated LH1–RC spectrum. Due to the lack of single-molecule spectra of isolated LH2 from *Rb. sphaeroides* we have displayed in Fig. 3A spectra from individual LH2 complexes from *Rps. acidophila*. However, it is known that at low temperatures the B850 band of LH2 from *Rb. sphaeroides* is shifted 18 nm to the blue with respect to the B850 band of LH2 from *Rps. acidophila* [23]. The sharp feature in the fluorescence–excitation spectra of individual light-harvesting complexes has been observed previously for both LH2 and LH1. It can be explained to result from the emission of the lowest excited state of the exciton manifold ($k = 0$) which has a long fluorescence lifetime of about 1 ns [14,17] or by the presence of a trap state in the ring of chromophores [18]. Whether it can be detected depends on the details of the electronically excited states of the particular pigment–protein complex under study. Taking these arguments into account we can state that the spectrum shown in Fig. 1 resembles a superposition of a spectrum of an individual LH2 and an individual LH1–RC complex. In Fig. 1C the trace that corresponds to an excitation wavelength of 800 nm shows beyond a polarization angle of 270° a significant decrease in intensity with respect to the other two traces. If we assign the narrow features at about 800 nm in Fig. 1B to the B800 absorptions of an individual LH2 complex this can be explained straightforwardly. In previous work it has been found that the pigment–protein complexes are susceptible to light-induced spectral fluctuations (see, for instance, [15]) resulting in slight changes of the spectral positions of the individual absorptions during the experiment [16]. This is of minor influence for the broad B850 and B870 spectral features but the relatively narrow absorption lines of the B800 band might get shifted out of resonance with the excitation laser during data acquisition.

From the relative intensity of the LH2 band with respect to the LH1 band in Fig. 1 we have to conclude that the transfer of excitation energy occurs very efficiently even in a non-membrane environment. Due to chromatic aberrations in our low-temperature microscope the effective excitation intensity (photon energy/time·area) is lower at 800 nm as compared with the intensity at 870 nm. As a consequence of this, the efficiency of the LH2–LH1 energy transfer is even underestimated by the above-mentioned criterion.

In summary, the data indicate clearly an energy transfer from the peripheral LH2 to an LH1–RC complex in a single supramolecular LH2–LH1–RC aggregate. The observation of narrow lines in the B800 region of the fluorescence–excitation spectrum suggests that one or, at most two LH2 are attached to the LH1–RC complex. A larger number of LH2 complexes would result in a significant change of the spectral shape of the B800 absorption due to 'ensemble' averaging. The detection of the fluorescence feature presented in Fig. 1, testifying energy transfer within an individual PSU, was a relatively rare event that could be observed only for a few percent of the studied complexes. Since the concentration of LH2 complexes in our LH1–RC preparations is very low, i.e. below the detection limit of a conventional ensemble absorption spectrum [13], it is reasonable to argue that the LH2 present in the preparation is all bound to RC–LH1 complexes. This suggests that the interaction between the peripheral and core antenna complex is strong enough to withstand partly detergent exposure during the purification procedure.

Previously, excitation-energy transfer from LH2 to LH1–RC has been observed at a reduced rate for assemblies reconstituted into liposomes [24]. Our work shows the formation of functional PSUs even in a non-membrane environment. These findings are supported by further experimental results. For

instance, mixing of two independent solutions which contained either isolated LH2 or isolated LH1–RC complexes resulted in PSUs fluorescence–excitation spectra similar to those shown in Fig. 1 (in preparation).

Finally, given the relatively low probability of LH2 binding to the core complex in detergent PMC suspensions, ensemble studies of the purified system are severely hampered. In contrast, the results of the present paper show that the single-molecule approach allows the investigation of energy transfer and protein–protein interactions in spontaneously reconstituted PSUs.

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