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Fluorescence-emission spectroscopy of individual LH2 and LH3 complexes

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Abstract

The lowest exciton states of individual LH2 and LH3 complexes have been studied by fluorescence-emission spectroscopy at low temperature (1.2 K). The spectra from the two complexes are broader than expected, which we attribute to spectral diffusion. In LH3 the absence of a hydrogen bond to the C_3-acetyl group of the β-B820 pigments leads to conformational freedom of the chromophore which translates into enhanced fluctuations in spectral position. A dependence of the degree of disorder on the solvent matrix was discovered by comparing the summation of the single-complex with bulk fluorescence spectra in combination with simulations of the data.

Keywords: Single-molecule; Light harvesting; LH2; LH3; Excitons; Energy transfer; Fluorescence

1. Introduction

The first step in photosynthesis is the absorption of photons by pigment–protein complexes that are specialized in light harvesting (LH). They efficiently transfer the excitation energy to a reaction center (RC), where charge separation takes place [1]. Collective properties of the pigment molecules play an important role in the excitation dynamics and spectral properties of such systems, which are usually described in terms of exciton models. In cases where the structure of the pigment–protein complexes is available from X-ray crystallography, model calculations and predictions can be based on detailed structural information, which can be compared with data from experimental studies of the intrinsic photo-physical properties of light harvesting complexes.

Optical spectroscopy of these systems at the level of individual complexes provides direct insight into their electronic properties, avoiding the inhomogeneous broadening that is typical for ensemble spectra, while providing easy access to disorder parameters [2–9]. In previous publications we reported the optical absorption spectra of individual light harvesting complexes LH2, LH3 and LH4, respectively, from *Rhodopseudomonas* (*Rps.*) *acidophila* and *Rps. palustris* [6–8]. Here we address the fluorescence-emission spectra of single LH2 and LH3 complexes. In these systems the fluorescent state differs from the states that determine the absorption spectra. Therefore, the emission spectra provide information that is not readily accessible in fluorescence detected absorption spectra. The emitting state of single complexes is furthermore an excellent reporter on the stability of the protein and its local environment [9].

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LH3 is a spectroscopic variant of the extensively studied LH2 complex. The structures of LH3 and LH2 have both been resolved at high resolution by X-ray crystallography [10,11]. They show that the overall structure of the two complexes is very similar. In fact, their pigment arrangements are practically indistinguishable. Both LH3 and LH2 have a nine-fold circular repetition of subunits consisting of an αβ-protein pair, each containing three bacteriochlorophyll *a* (BChl *a*) molecules and one carotenoid molecule. The 27 BChl *a* molecules in the complexes are arranged in two concentric rings, the B800 and B820 rings in the case of LH3 and the B800 and B850 rings in LH2. The nomenclature for the rings refers to the spectral position of their respective absorption bands [12]. The band absorbing at 800 nm consists of nine monomeric BChl *a* pigments with their macrocyles in the membrane plane, while the remaining 18 BChl *a* pigments in the B820 band of LH3 are oriented perpendicular to the membrane plane, like the B850 BChls in LH2. The pigments in the latter ring are strongly coupled due to the near anti-parallel alignment of their dipole moments and the short distances (about 9 Å center-to-center) between adjacent pigments.

In this paper, we present the results of fluorescence-emission spectroscopy on individual LH2 and LH3 complexes at low temperature. Downward relaxation in the exciton manifold of light harvesting pigment–protein complexes typically occurs on a sub-picosecond time scale [13–15]. Under the low temperature conditions of our measurements, this implies that fluorescence originates from the lowest state in the exciton manifold, whereas most of the oscillator strength is contained in higher exciton states.

Our data reveal large differences between the spectra of the two LH complexes. We attribute these differences to spectral diffusion processes that are characteristic for each of the complexes. Also the summations of the single-complex spectra display distinct behavior. We elaborate on the structural differences between the complexes that may contribute to the observed effects. Numerical calculations based on the structural models of both complexes are used to analyze the results in more detail. We have analyzed the fluorescence properties of LH2 and LH3 at the single-molecule level to further extend the exciton model, and to investigate effects of static and dynamic heterogeneity.

2. Materials and methods

Isolation and purification of LH2 (strain 10050) and LH3 (strain 7050) from *Rps. acidophila* was performed as described previously [16,17]. Samples for single-complex studies were prepared as in earlier work from our group [7,8,18]. Briefly, the stock solution was utilized for bulk as well as single-complex experiments. For the single-complex experiments, the stock solution was diluted with a buffer containing 20 mM Tris, 0.1% LDAO and 1.8% PVA at a pH of 8. The concentration of the complexes was decreased to approximately 10^{-11} M after which a droplet of 10 μl was spin-coated onto a lithium-fluoride sample plate.

Bulk experiments were performed using a single beam spectrophotometer with a spectral resolution of 0.5 nm. Cryogenic temperatures (10 K) were maintained with a helium flow cryostat (Oxford Instruments). To obtain clear samples, and as a cryoprotectant, glycerol was added (66%, v/v). The optical density of the sample was 0.1 cm^{-1} at 800 nm. The excitation wavelength in fluorescence measurements was 790 nm, which is on the blue side of the B800 band of LH3.

Fluorescence-emission spectra of individual LH2 and LH3 complexes were obtained with a home-built confocal microscope setup operating at low temperature (1.2 K). The sample was scanned confocally at a wavelength of 795 nm for a systematic search for LH complexes by fluorescence detection using an avalanche photodiode (APD) (Perkin–Elmer SPCM-AQR-16). The excitation wavelength was optimized by a spectral search around 795 nm for the maximum fluorescence intensity. This is necessary because of the relatively narrow absorption line width of transitions of LH2 and LH3 in the 800 nm region [19]. Signal levels were typically between 100 and 600 photon counts per second, the background level was approximately 40 counts per second.

For the acquisition of fluorescence-emission spectra, the collimated fluorescence was dispersed by a blazed grating (Richardson Gratings, 235 lines/mm). The diffracted beam was projected through a lens onto a CCD camera (Liquid nitrogen cooled, Roper Scientific). The spectral resolution was 2 nm.

Individual complexes were excited with circularly polarized light at an intensity of approximately 500 W/cm^2. Each spectrum was integrated for 10 min. The filters consisted of a series of three identical band-pass filters (Dr. Anders, Germany) for detection and a holographic notch filter (Kaiser Optical Systems, USA) in the excitation beam. The transmission of the band-pass filters ranged from 820 nm through 915 nm. All spectra were corrected for the transmission spectrum of the band-pass filters as well as for the sensitivity curve of the CCD camera.

The bulk emission and summed single-complex spectra of LH2 and LH3 were simulated using an exciton model with an effective Hamiltonian approach in a point-dipole approximation. This approach has been used successfully in studies on LH2 [20–22] LH3 [7], and LH4 [8]. The applied site energies for the simulations were $E_\alpha = 12{,}300\ cm^{-1}$ and $E_\beta = 12{,}060\ cm^{-1}$ for LH2 and $E_\alpha = 12{,}860\ cm^{-1}$ and $E_\beta = 12{,}600\ cm^{-1}$ for LH3. Coupling between pigments is included up to the second-nearest neighbor. Inhomogeneous broadening, which is caused by stochastic variations in the protein environment, was introduced by adding random elements from a Gaussian distribution to the site energies. In our simulations we assume that all fluorescence is emitted by the lowest exciton state, the $k = 0$ state, and that the fluorescence spectrum is composed of a zero-phonon line (ZPL) and a phonon-side band