Spectral fine-tuning in excitonically coupled cyclic photosynthetic antennas

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Abstract

Photosynthetic organisms are famously adaptable to environmental variances, yet the underlying physical mechanisms are seldom known. Here, we study the mechanisms that are responsible for the very large (~50-nm) blue shift of the lowest-energy absorption band in the stressed LH3 antenna form from the photosynthetic bacterium Rhodopseudomonas acidophila compared to its position in regular LH2 antenna. By analyses of the antenna absorption and polarized fluorescence excitation spectra measured at 5 K, essential modifications of the antenna excitons have been revealed that involve both relative narrowing and shifting of the exciton band.

1. Introduction

The ways photosynthetic organisms, plants, algae, and bacteria, harvest solar energy and accommodate flexibly to the environmental extremes has been of considerable long-time interest. Apart from fundamental curiosity, this steady attention is fueled by the hope of finding new biomimetic principles applicable in devices of future technology. Modern studies of photosynthetic energy transduction can be traced back to the work of Franck and Teller already 70 years ago [1]. Subsequent investigations confirmed a concept of an energetic funnel formed by the transition energies of light absorbing pigment molecules, notably chlorophylls and carotenoids, in the specialized pigment–protein units that carry self-explanatory names of the light-harvesting (LH) and reaction center complexes. According to this notion the arrangement of the complexes is statistically ordered so that the pigments closer to the reaction center absorb progressively more red-shifted light from the solar spectrum [2]. In purple photosynthetic bacteria, for example, a core LH1 antenna complex closely encircles the reaction center while the cyclic LH2 complexes with blue-shifted spectra locate in periphery. Some strains of the bacteria, such as Rhodopseudomonas (Rps.) acidophila, develop unusual peripheral antennas called LH3 when grown under stressed (low light and/or low temperature) conditions [3]. While structurally highly homologous, the absorption spectra of the LH2 and LH3 complexes appear very different, as demonstrated in Figure 1. The spectra in Figure 1 measured at 5 K almost perfectly match the previously published spectra [4,5]. It is of notice that all the spectral peaks in Figure 1 are associated with the lowest singlet Qy electronic transitions in individual bacteriochlorophyll a (Bchl) molecules that are ‘solvated’ by the neighboring Bchls forming electronically coupled aggregates as well as by the surrounding protein matrix. From the former studies (see Refs. [6,7] for comprehensive recent reviews) it is known that the peaks around 800 nm represent the nine loosely packed (intermolecular separation >2 nm), thus weakly coupled, Bchl molecules as shown in the lower part of the inset of Figure 1. The lower-energy bands (customarily called the B820 and B850 bands) peak at 821.4 nm (LH3) or 869.9 nm (LH2) and are related to the tightly packed (intermolecular separation <1 nm) aggregates that involve 18 strongly excitonically coupled Bchl molecules (upper circle in the inset of Figure 1). The relative shift of these low-energy bands in the two complexes is rather huge – 49 nm (673 cm⁻¹), compared with the relative shift of the B800 bands, which is only 4.6 nm (72 cm⁻¹). Discussion of the latter band shift is outside the scope of the present Letter. A rather weak interaction between the Bchls from the two separate rings, B800 and B820/B850 (see inset of Figure 1), is commonly assumed.

The main goal of this work is to understand qualitatively and to evaluate quantitatively the contributions of various physical mechanisms into the almost 50 nm up-shift of the B820 exciton absorption band in LH3 with respect to the B850 band in LH2. Considering complexity of the antenna systems, this appears a formidable task. There indeed are many sources to the bandshift [8–10], generally and somewhat arbitrarily, divided into specific (hydrogen bonding, axial ligation, steric conformational effects, etc.) and universal (van der Waals and dispersive) interactions. Yet several previous experimental results greatly simplify our task. Firstly, just two specific types of interactions have been recognized to be primarily responsible for tuning of the exciton spectra in the bacterial antennas: (i) electronic (exciton) couplings among the Bchl molecules [11] and (ii) hydrogen bonding of the C₃ acetyl carbonyl side-group of the...
Bchls with the surrounding protein [12,13]. Influence of axial ligation on the Bchl Qy transition energy is generally limited [14]. Secondly, near-atomic-resolution crystal structures have been determined for both the LH2 [15] and LH3 [3] antenna complexes. A few well-defined differences have been identified [3], which first and foremost concern the Cα acetyl carbonyl group hydrogen bonding patterns in the B850 and B820 aggregates. Consequently, the relative shift of the B850 and B820 absorption bands was in [3] entirely assigned to the shifts of the Qy transition energies of the participating Bchl sites. This latter conclusion, enforced in [16–18], served as the prime motivation for the present work. It is argued that any adjustment of the hydrogen bonds necessarily involves transformation of the ground and excited electronic state structures of the aggregates, hence their exciton properties. A straightforward way to demonstrate this is by direct comparison of the exciton state manifolds (or at least exciton bandwidths) of the B820 and B850 aggregates. Unfortunately, as it was demonstrated in [19], this information cannot be obtained from common ground-state absorption measurements. Here, a more responsive polarized fluorescence excitation spectroscopy technique is introduced to address the problem. The polarized fluorescence spectra reveal exciton band boundaries in cyclic molecular aggregates, thus allowing their exciton bandwidths to be determined [20,21].

2. Methods

Isolation and purification of individual LH2 and LH3 complexes was performed as described previously [3,4]. The concentrated stock solution stored at liquid nitrogen temperature was diluted with a 15 mM Tris–HCl buffer (pH 8)–glycerol mixture (1:2 volume ratio) before experiments to yield a desired optical density and a good optical quality glass sample at low temperatures. The buffer also contained 1% of the detergent (LDAO), necessary to maintain well-isolated complexes in the presence of glycerol and low temperatures. The absorption and fluorescence anisotropy measurements were performed at 5 K as described in [20]. The experimental anisotropy, r(\(\lambda\)), as a function of the excitation wavelength \(\lambda\) was defined as

\[
r(\lambda) = \frac{I_{vv}(\lambda) - I_{vh}(\lambda)}{I_{vv}(\lambda) + 2I_{vh}(\lambda)}
\]

where I_{vv} and I_{vh} are the emission intensities polarized parallel and perpendicular to the orientation of the electric vector of the linearly polarized excitation laser light, respectively. The exciton state-dependent anisotropy for individual diagonally disordered aggregates were computed as

\[
r = \frac{3}{5} \cos^2 \alpha - 1
\]

where \(\alpha\) is the angle between the transition dipole moments of the absorbing exciton and the emitting self-trapped exciton states. These individual anisotropies allow parallel and perpendicular fluorescence emission components to be calculated, the ensembles averages of which are directly related to the experimental intensities I_{vv} and I_{vh}. The self-trapped exciton model and methods of anisotropy calculations are in detail described in [20–25]. Compared with these references, however, physically more justified line shapes are applied in the present simulations based on dynamic exciton theory [26–28].

3. Results and discussion

We first focus on the absorption spectra drawn in Figure 2a and b with chained black squares (experiment) and blue lines (simulation). Standard methods [8,7,11.29–31] have been used to calculate the exciton state manifolds for individual B850 antenna aggregates (Figure 3, top panel) as well as the density of the exciton states...
The experimental fluorescence polarization anisotropies as a function of excitation wavelength for the LH2 and LH3 complexes from *Rps. acidophila* are shown with black circles in Figure 2a and b, respectively. In qualitative agreement with the previous measurements on LH2 complexes from another purple bacterium *Rhodobacter sphaeroides* [20], the anisotropy, being generally low (<0.1) at short wavelengths, rises steeply with the excitation wavelength across the B850 and B820 absorption bands. Theoretical limit of the anisotropy (0.4) is approached at the low-energy edge of the spectrum. More significant in the context of the present work, however, are the two depressions seen at the edges of the anisotropy spectrum. Comparison with the simulated ground-state absorption spectra confirms that these minima are well correlated with the \( k = \pm 1 \) and \( k = \pm 8 \) exciton states. A more detailed analysis that will be presented elsewhere evidences also a contribution of the \( k = 9 \) state into the high-energy polarization dip. A slower experimental polarization rise at low energies and a shallower high-energy dip in LH3 should be noticed. Both effects can be explained by the fact that LH3 is not a pure sample but a mixture of different LH2 complexes, as indicated in the caption of Figure 1. Thus Figure 4b clearly suggests that the reduced visibility of the high-energy anisotropy feature of LH3 is due to overlap with residual LH2 complexes that deform (lower) its low-energy side. In neither complex the fluorescence anisotropies shows any interference with the B800 absorbance of the quasi-monomeric Bchl molecules. This is another advantage of the present technique that favorably distinguishes it from the above alternative approaches.

The red curves in Figure 2 represent simulated fluorescence anisotropy excitation spectra. Although slightly more structured, the calculated spectra reasonably well reproduce not only general trends of the measurements but also the positions and relative depths of the anisotropy minima at the excitation band boundaries. A rapid drop of the calculated anisotropy at red edge is an artifact of the long Lorentzian tails of the engaged line shapes that are re-

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Figure 3. (top panel) Exciton level diagrams for a single idealized (central thin bars) and diagonally disordered (flank bold red bars) B850 aggregate. A single disorder realization of site energies from a Gaussian distribution is represented. The arrows indicate transition dipole moment orientations for the disorder-split \( k = \pm 1 \) and \( k = \pm 8 \) exciton states. (bottom panel) The absorption spectrum (red continuous curve) and the corresponding DOS (thick black curve) for an ensemble of 2000 B850 aggregates. The shapes drawn with thin line represent ensemble distributions of 18 individual exciton states. The filled colored areas highlight the distributions for the disorder-split \( k = \pm 1 \) and \( k = \pm 8 \) exciton states. (For interpretation of the references in color in this figure legend, the reader is referred to the web version of this article.)
spectra, are presented in Table 1. In this table, \( E_0 \) is the effective mean site energy (in Figure 2 denoted by downward arrows); \( \sigma_{\text{int}} \) and \( \sigma_{\text{ext}} \) are the standard deviations of Gaussian internal and external diagonal disorders, respectively, as defined in [30,37]; \( \Delta E \) is the exciton bandwidth, being operationally defined as the energy separation between the low- and high-energy exciton minima; \( V \) and \( V' \), respectively, are the nearest neighbor intra- and inter-dimer exciton coupling energies. The two energies emerge because of a dimeric build-up of the B850 and B820 aggregates [3,15]. It also assumes different transition energies of the dimer sites. Symmetric splitting (by absolute value close to \( V \)) of dimer site energies around \( E_0 \) was applied, as suggested by circular dichroism studies of the LH2 complexes [31].

The data in Figure 2 and Table 1 clearly imply that excitons in the stressed LH3 complexes are significantly modified compared to them in regular LH2 complexes. The main modifications according to the applied model include (i) a blue shift by \( 510 \) cm\(^{-1} \) of the effective mean site energy, \( E_0 \), (ii) shrinking by \( 160/180 \) cm\(^{-1} \) (measured/calculated) of the exciton bandwidth, \( \Delta E \), and (iii) a decrease by \( 86 \) cm\(^{-1} \) of the average exciton coupling energy estimated as \( (V+V')/2 \). All these exciton model parameters contribute in various degrees into the experimental shift of the B850 and B820 absorption bands. In relative terms the shift of \( E_0 \) consists about 4% of its original value, while the changes of \( \Delta E \) and \( (V+V')/2 \) are much greater, \( \sim 11/12\% \) (measured/calculated) and \( \sim 25\% \), respectively. Therefore, the former idea [3,16,17] associating the B850-to-B820 spectral shift just with the site energy changes requires qualitative revision. The shift positively has largely exciton origin. However, within the simplified molecular exciton model applied here, it is quite impossible quantitatively uniquely evaluate how much the site energy and the exciton coupling energy variations separately contribute into the experimental shift of the exciton absorption band. It is clear from the crystallographic studies [3] that changes of the hydrogen bonds that coordinate the two Bchl molecules in dimeric building block of the B820 aggregate are not symmetric. This may cause a big part of the observed exciton bandwidth variation, provided that spatial orientations and conformations of the pigments are conserved. That this indeed might be the case is indicated by different degree the exciton rule of thumb, \( \Delta E = 2(V+V'/2) \), is obeyed: almost precisely in the B850 aggregates, where \( \Delta E = 1480 \) cm\(^{-1} \) and \( 2(V+V') = 1394 \) cm\(^{-1} \), whilst rather poorly in the B820 aggregates (\( \Delta E = 1380 \) cm\(^{-1} \); \( 2(V+V') = 1054 \) cm\(^{-1} \)). Involved quantum chemical techniques should be applied for precise quantitative evaluations, still impractical in case of large molecular assemblies like the antenna aggregates.

The exciton coupling energies, \( V \) and \( V' \), evaluated from low-temperature experiments for the B850 aggregates of LH2 complexes from *Rps. acidophila* in the present work and in [17,34] are different, being significantly greater in this work. In search for possible reasons we also noticed that the absorption spectra of the LH2 complexes reported in [17,34] are several nanometers blue shifted compared to the spectra in Figure 2, suggesting smaller \( \Delta E \), blue-shifted \( E_0 \) or both. It seems likely that the noted discrepancies are related to different host solid matrices used: a regular Tris-HCl buffer with some addition of detergent (LDAG) and cryoprotectant (glycerol) in this work and a polymer (PVA) film in [17,34]. Solvent effects on protein structure (and spectra) are commonplace. The blue-shifted absorption spectrum in polymer surroundings along with weaker exciton couplings is consistent with a swallowed (denatured) protein structure. A reverse effect (i.e., a spectral red shift) has been observed in mutant LH2 complexes that miss the B880 pigment ring and, therefore, allow increased exciton coupling [20,38,39].

It is finally of interest to compare \( E_0 \) with the mean \( Q_y \) transition energy of the quasi-isolated B800 molecules. Compared with the
solubilized Bchls, which show a broad distribution of transition energies around 787 nm [19], the B800 molecules in the protein surroundings are stabilized by a combined effect of axial ligation [10], hydrogen bonding, and relatively weak [40] exciton couplings. The relative positions of the B800 absorption bands and downward arrows indicating $E_0$ appear to suggest that in LH3 almost the entire B820 absorption band red shift at 5 K has exciton origin (the conclusion already reached in [16]), whilst in LH2 the exciton mechanism might be responsible for not less than half of the total shift of the B850 band. The rest could then be assigned to the site energy shift. Although somewhat speculative, this estimate almost certainly reflects the reality.

Summarizing, a long-standing problem – what are the elementary physical mechanisms involved in spectral adaptation of photosynthetic organisms to environmental changes – has been tackled in the specific case of LH2 and LH3 cyclic antenna complexes of purple bacteria prepared under different environmental stress conditions. Significant modifications of antenna exciton properties are first revealed along with varying pigment transition energies that accompany changes of the hydrogen bond pattern that coordinate the Bchl pigments to the surrounding protein. Quantitative evaluation of these mechanisms is achieved by model analysis of experimental low-temperature fluorescence anisotropy and absorbance data. Insights into how disorder in site energies and electronic couplings affect the spectroscopy has been reported. In stressed LH3 complexes, it is confirmed that almost the entire exciton absorption band red shift relative to the absorption of individual Bchls has exciton origin. In regular LH2 complexes the exciton mechanism is responsible for more than half of the absorption band shift.

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