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Efficiency of light harvesting in a photosynthetic bacterium adapted to different levels of light

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ABSTRACT

In this study, we use the photosynthetic purple bacterium *Rhodobacter sphaeroides* to find out how the acclimation of photosynthetic apparatus to growth conditions influences the rates of energy migration toward the reaction center traps and the efficiency of charge separation at the reaction centers. To answer these questions we measured the spectral and picosecond kinetic fluorescence responses as a function of excitation intensity in membranes prepared from cells grown under different illumination conditions. A kinetic model analysis yielded the microscopic rate constants that characterize the energy transfer, trapping inside the photosynthetic unit as well as the dependence of exciton trapping efficiency on the ratio of the peripheral LH2 and core LH1 antenna complexes, and on the wavelength of the excitation light. A high quantum efficiency of trapping over 80% was observed in most cases, which decreased toward shorter excitation wavelengths within the near infrared absorption band. At a fixed excitation wavelength the efficiency declines with the LH2/LH1 ratio. From the perspective of the ecological habitat of the bacteria the higher population of peripheral antenna facilitates growth under dim light even though the energy trapping is slower in low light adapted membranes. The similar values for the trapping efficiencies in all samples imply a robust photosynthetic apparatus that functions effectively at a variety of light intensities.

1. Introduction

The photosynthetic unit (PSU) of purple bacteria consists of two general types of pigment–protein complexes — the reaction center (RC) and the light-harvesting (LH, also referred to as antenna) complexes [1,2]. The main function of the LH complexes is to capture sunlight and to transfer the excitation energy to the RCs where it serves to initiate a charge separation process [3–7]. The LH complex thus plays a vital role in the energy transduction process in photosynthetic organisms, plants, algae, and bacteria, by increasing the effective cross section for solar light absorption of each RC, and by ensuring the photosynthesis function over a wide range of incident light intensity [5,8,9].

In most purple bacteria, the photosynthetic membranes contain two types of LH complexes: the core light harvesting complex 1 (LH1) and the peripheral/distal light harvesting complex 2 (LH2). While LH1 is firmly bound to RCs, LH2 transfers the excitation energy to the RCs via LH1. The major photoactive pigment in all these LH complexes is bacteriochlorophyll-a (BChl). In LH2 and LH1 complexes multiple BChl molecules are tightly packed against each other, inferring that the photoexcitation transport in bacterial membranes takes place by means of collective excitons rather than by individual excitations hopping along arrays of molecules [6,10].

To ensure that photosynthesis is effective under vastly varying environmental conditions such as light intensity and its spectral distribution, the structural composition and the related spectral properties of the PSU have to be versatile. High resolution atomic force microscopy studies of the intracytoplasmic photosynthetic membranes of bacteria have disclosed variations of the structural organization of PSU that parallel growth conditions [11–13]. In membranes from the cells grown under high light intensities (henceforth called high light (HL) adapted membranes), the number of peripheral antenna complexes available in a PSU is relatively small, whereas in low light (LL) adapted membranes, which have been harvested from the cells grown under dim light, the PSU is dominated by the peripheral antenna complexes [12,14–16]. In some other species of photosynthetic purple bacteria light adaptation involves significant modification of spectral properties as well as the structure of the distal antenna [13,16,17]. These structural and spectral adaptations allow the bacteria to accommodate different ecological niches.

It has long been observed that the fluorescence from photosynthetic bacteria, a waste when assessed from the point of view of photosynthesis efficiency, responds to the state of the photochemistry taking place

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within the RC [18–21]. There are two well defined phases of photosynthesis in purple bacteria related to the excitation light intensity. At low excitation intensity all the RCs will be open, ready to accept excitons from the antenna; the special pair of the RC (commonly assigned to P) is reduced and available for charge separation upon photoexcitation. This phase, characterized by low antenna fluorescence yield, is named here as the "active region of photosynthesis". At high excitation intensity another phase termed the "saturated region of photosynthesis" is reached with the majority or all of the RCs in a closed state; in the closed state the special pair of the RC is oxidized (P^+) and the antenna fluorescence yield is maximal. When the excitation light intensity is kept between the active and saturated regimes, a mixed (actinic) state with some open RCs and the remaining closed RCs is created.

The fluorescence yield from the antenna can be formally evaluated as

$$\varphi_f = \frac{k_f}{k_2}, \quad (1)$$

where k_f is the fluorescence decay rate constant and $k_2 = k_f + k_t + k_{IC} + k_{ISC} + k_q$ is the total quenching rate constant via various decay routes including the fluorescence, trapping at the RC (k_t), internal conversion (k_{IC}), intersystem crossing (k_{ISC}), and quenching by still other routes (k_q). Assuming constant k_{IC}, k_{ISC}, and k_q, the variable fluorescence is due to a change of the trapping rate k_t according to the state of the RCs (see below).

The variable fluorescence is essentially related to the antenna exciton lifetime, thus providing a complementary diagnostics tool for the different photosynthesis phases by measuring the fluorescence decay time. The active regime of photosynthesis is expected to be characterized by the shortest lifetime, because of the prevailing quenching of antenna excitons by mostly reduced RCs. Under increasing excitation intensity the exciton lifetime will gradually rise, reaching a maximum at saturated regime.

When the excitation intensity is increased beyond the saturating level such that there are two or more excited states simultaneously available in the accessible radius for energy transfer, then the excited states may get annihilated and both the fluorescence yield and lifetime will drop. This excitation intensity range is further called the non-linear excitation quenching range. The first clear demonstration of this kind of behavior using picosecond time-resolved fluorescence measurements was published in [20,22] (reviewed in [1,23], see also [24]). Still, the evidence was not exhaustive, because of limited low excitation intensity range covered by these early experiments. Also, the previous work did not establish a clear, quantitative link between the fluorescence yield and the picosecond rate of fluorescence decay.

In the present work, we revisit these issues by studying membranes prepared from the *Rhodobacter sphaeroides* wild type strain 2.4.1 that has different ratios of peripheral LH2 and core RC–LH1–PufX light-harvesting complexes. As a limiting case, the set holds a mutant membrane that contains just core complexes. To the best of our knowledge, there are so far only a few and mostly theoretical studies of energy transfer in photosynthetic purple bacteria grown under different light intensities [25,26]. We use a much expanded excitation intensity range that spans the active to the saturated regimes of photosynthesis, even to the annihilation regime, with the help of different sample holding cuvettes, as detailed in Materials and methods section.

We specifically address the following fundamental question — how does the addition of peripheral antenna under low light growth influence the rate of delivery of excitation energy to the RCs and the quantum efficiency of charge separation? We also exploit the expanded excitation intensity range to explore the energy transfer time in the active and saturated states of photosynthesis, i.e., the states corresponding to mostly open and mostly closed RCs, respectively. Parallel monitoring established a direct correlation between the steady-state fluorescence yield and picosecond time-resolved fluorescence decay.

2. Materials and methods

2.1. Samples

The wild type *R. sphaeroides* strain 2.4.1 was used for the preparation of intracytoplasmic photosynthetic membrane vesicles (also called chromatophores) containing all the components of photosynthesis machinery — the LH2, LH1, and RC chromoprotein complexes. Gene expression for photosynthesis complexes in the strain 2.4.1 is dependent upon oxygen levels and light intensity, with full expression reached under anaerobic conditions under low light intensity [27,28]. The wild type chromatophore samples used in the present work (see Table 1) were prepared from cells grown anaerobically using light intensities between 100 μE/m^2/s and 1500 μE/m^2/s. The core complexes from the wild type organisms incorporate the PufX polypeptide, i.e., they (mostly) have dimeric structure [12,29–31]. PufX enables quinones/quinols to cross the LH1 barrier [32,33] and diffuse to the cytochrome bc_1 complex, a key requirement for the cyclic electron transport and for the efficient photosynthetic growth [34]. The membranes containing just LH2, LH1, or dimeric core RC–LH1–PufX complexes were prepared from a series of genetically modified mutants of *R. sphaeroides* [35]. They are indicated by adding "-only" to the name of the complex (e.g., LH2-only membrane). These mutants were grown under oxygen-limited conditions in the dark, as specified in [36].

All the membranes were prepared according to the method in [36]. Following cell disruption by French pressing the cell extracts were loaded onto 15%/40% (w/w) sucrose step gradients and centrifuged for 10 h at 27,000 rpm. Each intracytoplasmic membrane fraction was harvested from the 15%/40% interface, concentrated by centrifugation and stored in freezer at −78 °C until used. The defrosted, concentrated HL and LL samples were diluted with 20 mM TRIS (pH 7.8), the LH2-only membranes with 20 mM HEPES (pH 7.5), the LH1-only membranes with 10 mM TRIS–HCl (pH 7.9), and the RC–LH1–PufX-only membranes with 20 mM HEPES (pH 7.8) buffer solutions to obtain the optical density around 0.1 or 0.3 in the cuvette. The denser samples were usually required in fluorescence lifetime measurements to improve signal to noise ratio. The small differences in pH are unlikely to have any effect upon the pigments buried within a complex within the membrane. To mimic the fluorescence decay times in the active RC condition, the membranes were mixed with 5 mM sodium ascorbate and 25 μM phenazine methosulfate (PMS) along with the buffer solution. Reduced PMS serves as an external electron donor, supporting the cytochrome bc_1 complex in avoiding the accumulation of oxidized (P^+) states of RCs under intense illumination. The presence of ascorbate is required in order to generate reduced PMS [37]. No special precaution against environmental oxygen was taken.

Absorption spectra of the samples in buffer solution were measured using a UV/VIS spectrometer (Model V-530, JASCO Corporation) with a spectral resolution of 0.5 nm. All the measurements were performed at ambient temperature, 295 ± 3 K.

2.2. Steady state and transient picosecond fluorescence measurements

The steady state fluorescence spectrometer consists of a continuous-wave Ti: sapphire laser (3900S, Spectra Physics) pumped by a Millennia

Table 1
LH2 to LH1 ratio in the wild type chromatophore samples of *R. sphaeroides*.

Sample	LH2/LH1 absorption ratio[a]	LH2/LH1 ratio
HL116	0.89 ± 0.02	2.15 ± 0.05
HL133	1.21 ± 0.03	2.79 ± 0.07
LL181	2.02 ± 0.04	4.79 ± 0.10
LL196	2.56 ± 0.04	6.06 ± 0.10

[a] Evaluated as the ratio of integral (area) spectra of the components between 700 and 950 nm; the uncertainties here and elsewhere are the standard deviation of several independent measurements.

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