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Sequential assembly of photosynthetic units in *Rhodobacter sphaeroides* as revealed by fast repetition rate analysis of variable bacteriochlorophyll *a* fluorescence

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Abstract

The development of functional photosynthetic units in *Rhodobacter sphaeroides* was followed by near infra-red fast repetition rate (IRFRR) fluorescence measurements that were correlated to absorption spectroscopy, electron microscopy and pigment analyses. To induce the formation of intracytoplasmic membranes (ICM) (greening), cells grown aerobically both in batch culture and in a carbon-limited chemostat were transferred to semiaerobic conditions. In both aerobic cultures, a low level of photosynthetic complexes was observed, which were composed of the reaction center and the LH1 core antenna. Interestingly, in the batch cultures the reaction centers were essentially inactive in forward electron transfer and exhibited low photochemical yields F_V/F_M, whereas the chemostat culture displayed functional reaction centers with a rather rapid (1–2 ms) electron transfer turnover, as well as a high F_V/F_M of ~0.8. In both cases, the transfer to semiaerobiosis resulted in rapid induction of bacteriochlorophyll *a* synthesis that was reflected by both an increase in the number of LH1–reaction center and peripheral LH2 antenna complexes. These studies establish that photosynthetic units are assembled in a sequential manner, where the appearance of the LH1–reaction center cores is followed by the activation of functional electron transfer, and finally by the accumulation of the LH2 complexes.
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Keywords: Membrane development; Light-harvesting complex; Photosynthetic membrane; Fast repetition rate fluorescence; Reaction center kinetics

1. Introduction

Kinetic measurements of chlorophyll fluorescence have become routine in photosynthesis and plant physiology research [1]. In oxygenic phototrophs, chlorophyll fluorescence provides valuable information on the energetics of primary photosynthetic reactions, the extent and nature of photoprotective processes and electron transport rates. Similarly to photosystem II (PSII) in oxygenic phototrophs, variable fluorescence in anoxygenic phototrophic purple bacteria is related to the presence of pheophytin-

Abbreviations: bc_1 complex, ubiquinol-cytochrome c_2 oxidoreductase; BChl *a*, bacteriochlorophyll *a*; CM, cytoplasmic membrane; IRFRR, infrared fast repetition rate; F_0, minimal fluorescence yield; F_M, maximal fluorescence yield; F_V, variable fluorescence (difference between F_0 and F_M); ICM, intracytoplasmic membrane; LH1, core light-harvesting complex; LH2, peripheral light-harvesting complex; p, energy transfer between photosynthetic units; P_{870}, reaction center bacteriochlorophyll *a* dimer; PSII, photosystem II; σ, functional absorption cross-section

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quinone type reaction centers. It has been used to characterize the processes of light absorption, charge separation, and electron transport in various photosynthetic bacteria [2–7]. Although bacteriochlorophyll fluorescence has been historically recognized as a valuable tool in the study of bacterial photosynthesis, the number of studies of the variable fluorescence of BChl *a* is, in comparison with Chl *a* fluorescence, rather limited. This is likely to be connected with the numerous Chl *a* fluorescence applications in plant physiology, phycology or marine biology, together with the convenience of kinetic absorption measurements to monitor electron transfer reactions in photosynthetic bacteria, which has limited the need for alternative techniques.

Here, we used a newly designed near-IR fast repetition rate (IRFRR) fluorometer to monitor photosynthetic unit development in the facultative photoheterotroph *Rhodobacter* (*Rba.*) *sphaeroides*, a member of the α-3 subclass of the *Proteobacteria*, which provides an ideal experimental system for membrane biogenesis and assembly studies. When grown under anaerobic conditions in the light, *Rba. sphaeroides* forms a system of intracytoplasmic membranes (ICM) that house photosynthetic units consisting of core structures in which the reactions center is surrounded by the light-harvesting (LH) 1 complex, with the LH2 complex arranged at their peripheries. Under these growth conditions, levels of LH2 relative to LH1–reaction center core complexes are related inversely to light intensity [8]. Although ICM formation is repressed by high oxygen tension under chemoheterotrophic conditions, lowering the oxygen partial pressure results in a gratuitous induction of ICM assembly in the dark (greening) by invagination of the cytoplasmic membrane (CM), together with the synthesis and assembly of light-harvesting and reaction center complexes [9]. These developmental changes are under the control of a global two-component oxygen sensing, signal transduction system [10–13], and additional regulatory components that include overlapping aerobic repressor circuits for the repression of bacteriochlorophyll *a* (BChl *a*), carotenoid and LH2 synthesis [14,15], as well as a photoreceptor that integrates both redox and light signals [16,17].

The ability to isolate putative sites of CM invagination permits membrane development in *Rba. sphaeroides* to be conveniently followed by biochemical and biophysical techniques. These membrane domains are obtained in an upper-pigmented band which sediments more slowly than the ICM-derived chromatophore vesicle fraction during rate-zone centrifugation on sucrose density gradients. Pulse-chase [18–20], membrane fractionation [20] and spectroscopic studies [3,21] have shown that, in addition to containing respiratory CM, the upper pigmented fraction is enriched in nascent LH1–reaction center core complexes, which represent “developing centers” that mature into photosynthetic units after further addition of LH2 [18], which drives vesicularization of the ICM [22–25].

In this paper, we demonstrate the applicability of variable BChl *a* fluorescence transients, generated by IRFRR fluorometry, for readily elucidating functional aspects of the photosynthetic unit assembly process. The basic concept of this analysis is that the initial rapid pulse sequence (150-μs time scale) elicits single charge separation in all the reaction centers [26]. The registered variable fluorescence signal reflects the redox status of the reaction center, where low fluorescence yield indicates an open reaction center (no charge, reaction center is ready to perform photochemistry) and high fluorescence yield indicates a closed reaction center (charged reaction center, transiently non-functional photochemistry). The difference between the minimum fluorescence F_0 and maximum F_M serves as an estimate of the quantum yield of the primary charge separation (estimated from the F_V/F_M ratio). The analysis of the induction kinetics provides information of functional absorption cross-section (σ) of the photosynthetic complexes and connectivity (p). Later in the protocol, slow relaxation kinetics are recorded which are assumed to reflect reopening of the reaction center. Our results from both isolated membrane fractions and low-aeration cell suspensions cultured in batches and in a chemostat further demonstrate that the components of the photosynthetic unit are assembled in a sequential rather than simultaneous manner.

2. Materials and methods

2.1. Growth, ICM induction (greening) and membrane isolation procedures

Induction of ICM formation in batch cultures of *Rba. sphaeroides* NCIB 8253 was performed as described previously [27] on a gyratory shaker in a medium with DL-malate as electron donor [8]. Cells grown chemoheterotrophically at high aeration (350 rpm) were washed and resuspended as concentrated suspensions at low aeration (200 rpm) in the dark, and photosynthetic pigment formation was followed for 22–24 h.

For the induction of ICM formation in steady-state cells cultured in a chemostat, malate at a concentration of 5 mM served as the limiting nutrient and the concentration of casamino acids was halved (0.5 g/L). The chemostat was inoculated from a dense batch culture. Cells were grown in a bleached state aerobically for 2 days by pumping air through the chemostat; low-aeration conditions were then established by lowering the oxygen tension to 3% (balanced with N_2). The culture was sampled over a 30-h period for IRFRR measurements, near-IR absorption spectra, pigment analyses and transmission electron microscopy on thin-cell sections.

For membrane isolation, cells grown photoheterotrophically at high light intensity (850 W m^{-2}) were disrupted in a French pressure cell and cell-free extracts were subjected to rate-zone sedimentation on sucrose density gradients [25]

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