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Organization in photosynthetic membranes of purple bacteria *in vivo*: The role of carotenoids

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ABSTRACT

Photosynthesis in purple bacteria is performed by pigment–protein complexes that are closely packed within specialized intracytoplasmic membranes. Here we report on the influence of carotenoid composition on the organization of RC–LH1 pigment–protein complexes in intact membranes and cells of *Rhodobacter sphaeroides*. Mostly dimeric RC–LH1 complexes could be isolated from strains expressing native brown carotenoids when grown under illuminated/anaerobic conditions, or from strains expressing green carotenoids when grown under either illuminated/anaerobic or dark/semiaerobic conditions. However, mostly monomeric RC–LH1 complexes were isolated from strains expressing the native photoprotective red carotenoid spheroidenone, which is synthesized during phototrophic growth in the presence of oxygen. Despite this marked difference, linear dichroism (LD) and light-minus-dark LD spectra of oriented intact intracytoplasmic membranes indicated that RC–LH1 complexes are always assembled in ordered arrays, irrespective of variations in the relative amounts of isolated dimeric and monomeric RC–LH1 complexes. We propose that part of the photoprotective response to the presence of oxygen mediated by synthesis of spheroidenone may be a switch of the structure of the RC–LH1 complex from dimers to monomers, but that these monomers are still organized into the photosynthetic membrane in ordered arrays. When levels of the dimeric RC–LH1 complex were very high, and in the absence of LH2, LD and ΔLD spectra from intact cells indicated an ordered arrangement of RC–LH1 complexes. Such a degree of ordering implies the presence of highly elongated, tubular membranes with dimensions requiring orientation along the length of the cell and in a proportion larger than previously observed.

1. Introduction

Purple photosynthetic bacteria possess a modular photosynthetic apparatus in which a photochemical reaction center (RC) is fed with energy by a closely associated LH1 light harvesting pigment–protein [1]. In many species these so called RC–LH1 complexes are in turn surrounded in the photosynthetic membrane by one or more types of peripheral light harvesting complexes, such that an extensive pool of bacteriochlorophyll (BChl) and carotenoid pigments provides the light harvesting capacity to power charge separation within each RC [2]. Light harvesting complexes in these bacteria have a common general architecture in which concentric cylinders of two types of membrane-spanning polypeptides encase rings of BChl and carotenoid pigments [3,4]. In the case of LH1 the dimensions of this hollow cylinder are sufficient to accommodate a RC in the center [5], and in some species an additional polypeptide, PufX, is present that disrupts the continuity of the LH1 cylinder [6]. In the most heavily studied purple photosynthetic bacterium, *Rhodobacter* (*Rba.*) *sphaeroides*, the PufX protein [7] limits aggregation of LH1 subunits around the RC to 14 pairs of membrane-spanning polypeptides, 28 BChls and 28 carotenoids [1]. PufX is necessary for the assembly of RC–LH1 into dimers in which two RCs are related by an axis of two fold symmetry, the associated LH1 forming an S-shape when viewed perpendicular to the plane of the membrane [1,5,6,8–11].

In addition to light harvesting, a key function of some carotenoid pigments in the bacterial photosystem is protection against damage caused by the photogeneration of singlet oxygen. This role was established around 60 years ago, when Griffiths and co-workers reported that a mutant strain of *Rba. sphaeroides* unable to synthesize colored carotenoids was susceptible to photo-oxidation leading to cell death [12,13]. Growth of this mutant under anaerobic, illuminated conditions ceased upon the introduction of air into the culture, with resultant cell death and breakdown of BChl. It was subsequently shown that the formation of relatively long-lived BChl triplet excited states in an aerobic

Abbreviations: LH1 and LH2, light-harvesting complex 1 and 2; RC, reaction center; P, primary electron donor; BChl, bacteriochlorophyll; BPhe, bacteriopheophytin; LD, linear dichroism; ICM, intracytoplasmic membrane; EM, electron microscopy; AFM, atomic force microscopy; *Rba*, *Rhodobacter*

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environment sensitized the formation of highly reactive singlet oxygen [14]. Carotenoids prevent damage caused by singlet oxygen either by direct quenching or by accepting excited state energy from triplet BChl, the carotenoid triplet having insufficient energy to sensitize singlet oxygen [15].

A specific mechanism for photoprotection by carotenoids in the RC–LH1 complex from *Rba. sphaeroides* was recently elucidated by Šlouf and co-workers [16]. Experiments carried out on photosynthetic membranes from cells grown under semiaerobic conditions, where the principal carotenoid is spheroidenone, established that the lowest energy triplet state of this carotenoid acts as an effective quencher of BChl triplet states. Such quenching would be expected to prevent sensitization of singlet oxygen. This mechanism for BChl triplet quenching was not observed in RC–LH1 complexes isolated from cells grown in the absence of oxygen, where the principal carotenoid is spheroidene (spheroidenone being the keto derivative of spheroidene, the conversion being catalyzed under aerobic conditions by CrtA–spheroidene monooxygenase). As a result the formation of spheroidenone on exposure of anaerobic growing cells to oxygen appears to switch on a specific photoprotective mechanism to prevent photodamage. In addition to introduction of the additional keto oxygen of spheroidenone, it was suggested that spheroidenone is twisted into an S-trans conformation as part of the photoprotective response [16]. This change in structure would increase the conjugation length of the carotenoid, lowering the energy of its triplet excited state such that it can act as an acceptor for energy from BChls that have undergone intersystem crossing from the singlet to triplet configuration.

An intriguing feature of the RC–LH1 complexes that are assembled in different strains of *Rba. sphaeroides* is that the relative amounts of monomers and dimers that can be isolated from photosynthetic membranes seem to depend on the type of carotenoid present, the isolation procedure being to solubilize complexes from the membrane using the mild detergent n-dodecyl-β-D-maltoside and size fractionation on a sucrose density gradient. Relatively few RC–LH1 dimers can be isolated from cells with native carotenoids grown in the presence of oxygen [7,17,18], where the principal carotenoid is spheroidenone. In contrast the relative amount of dimer is much greater if complexes are isolated from cells grown in the absence of oxygen, where the principal carotenoid is spheroidene, or from cells of mutant strains that express the green carotenoid neurosporene rather than spheroidene/spheroidenone due to a spontaneous mutation of the *crtD* gene (hydroxyneurosporene desaturase). Such green cells have been employed in the majority of studies of the organization of RC–LH1 complexes in photosynthetic membranes [20], the structure of the RC–LH1 dimer [1,5,6,8,9,11,21] and the structural role of PufX [22,23]. A possible explanation for this variability could be that the principal form of the RC–LH1 complex in cells with native carotenoids grown in the presence of oxygen is the monomer, and so the architecture of the photosynthetic membrane in such cells would be different from that typically depicted in molecular models of *Rba. sphaeroides* membranes that are based largely on the premise that RC–LH1 complexes are arranged as arrays of dimers [24–28]. However this explanation would be at odds with AFM data collected from *Rba. sphaeroides* cells with native carotenoids grown in the presence of oxygen that show complexes mainly arranged as dimers [18,29]. An alternative explanation could be that the dimer architecture is ubiquitous in membranes, but that spheroidenone-containing dimers are structurally different in a manner that makes them unusually prone to monomerization on removal from the membrane, relative to dimers containing either spheroidene or neurosporene.

To investigate this issue, the present work examines the organization of RC–LH1 complexes in cells grown under different conditions, in intact membranes from those cells, and in detergent solution, employing a combination of sucrose gradient fractionation and linear dichroism spectroscopy (LD) [30]. The latter has been used previously to distinguish between ordered arrays of dimeric RC–LH1 complexes and disordered assemblies of PufX-deficient monomeric RC–LH1 complexes in intact membranes [20,31,32]. It is found that, irrespective of the carotenoid present, membranes display the spectroscopic fingerprint associated with ordered RC–LH1 complexes. This includes a membrane containing spheroidenone from which only very low levels of dimeric RC–LH1 complexes could be isolated. It is concluded that it is likely that RC–LH1 complexes form ordered assemblies in these spheroidenone-rich membranes, but they differ in structure from the dimeric RC–LH1 complexes assembled in cells containing spheroidene or neurosporene. The findings are discussed with respect to the possible physiological significance of a change to the structure of the dimeric RC–LH1 complex in cells that are exposed to oxygen.

2. Materials and methods

2.1. Bacterial strains and growth

The wild-type strain used was NCIB8253, which for convenience is referred to as "WT-r" in the main text, the suffix "-r" denoting the presence of native red/brown carotenoids. Strain WT-g was a spontaneous derivative of WT-r expressing green carotenoids; this strain was stable, showing very low levels of reversion to the native carotenoid type. Strains RCLH1X-r and RCLH1X-g were constructed by complementation of deletion strains DD13 (red/brown carotenoids) and DD13/G1 (green carotenoids), respectively [33], with a plasmid-borne copy of the *pufBALMX* operon which encodes the β- and α-polypeptides of the LH1 antenna, the L- and M-polypeptides of the RC and PufX. The strains were grown under either dark/semiaerobic or light/anaerobic conditions, as described previously [22,23].

2.2. Pigment–protein profiles by sucrose density gradient sedimentation

Intracytoplasmic membranes for detergent solubilization were prepared using a French pressure cell, as described previously [34]. Membrane pellets were suspended in 20 mM HEPES (pH 8) to a final concentration of 60 absorbance units cm^{-1} at 850 nm for LH2-containing strains or 875 nm for LH2-deficient strains, and the suspension mixed in a 3:2 ratio with 10% (w/v) β-DDM [11]. After incubation on ice for 30 min in the dark, membrane debris was removed by centrifugation at 78,100 *g* for 1 h at 4 °C in a TLA100 rotor.

Sucrose density gradients were constructed in transparent ultracentrifuge tubes by carefully layering five steps of 20%, 21.25%, 22.5%, 23.75% and 25% (w/w) sucrose in 20 mM HEPES (pH 8)/0.04% β-DDM. Solubilized membrane proteins (150 μl of sample with an absorbance of 25 at 850 or 875 nm) were loaded on to each gradient, and these were centrifuged in a Sorvall TH-641 swing-out bucket rotor at 180,000 g for 20 h at 4 °C. For each strain/growth condition, multiple gradients were run using several cultures, membrane preparations and solubilizations — representative examples are shown in Fig. 1.

2.3. Linear dichroism and absorption

To preserve large membrane fragments for analysis by LD spectroscopy, harvested bacterial cells were lysed in a French pressure cell at a low breaking pressure of 3000 psi (2×10^7 Pa), as described previously by Siebert and co-workers [8]. After a clearing spin, membranes were fractionated on a 15/40/50% (w/w) three step sucrose density gradient in 20 mM HEPES (pH 8.0), and colored bands at the 15/40 and 40/50 interfaces harvested. Data shown in the main text is from the lower membrane band, but the membranes from the two bands gave very similar results.

Harvested cells and isolated photosynthetic membranes were kept cold on ice before gel preparation to perform LD measurements on intact cells and membranes. ICM and cells at a final OD of about 0.5 absorbance units cm^{-1} at 850 or 875 nm were immobilized by polymerization in a 12% acrylamide/bis-acrylamide (40% solution 29:1 ratio,