Biochimica et Biophysica Acta 1837 (2014) 1665–1673

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbabio

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

Sandrine E. D'Haene [a,*], Lucy I. Crouch [b,1], Michael R. Jones [b,*], Raoul N. Frese [a,*]

[a] Biophysics of photosynthesis/Physics of Energy, Department of Physics and Astronomy, Faculty of Sciences, VU University Amsterdam, De Boelelaan 1081, Amsterdam 1081 HV, The Netherlands
[b] School of Biochemistry, Medical Sciences Building, University of Bristol, University Walk, Bristol BS8 1TD, United Kingdom

ARTICLE INFO

Article history:
Received 26 March 2014
Received in revised form 1 July 2014
Accepted 3 July 2014
Available online 10 July 2014

Keywords:
Photosynthetic membranes
Supramolecular organization
Linear dichroism spectroscopy
Carotenoid
Photoprotection
Rhodobacter sphaeroides

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*

* Corresponding authors.
E-mail addresses: s.e.d.haene@vu.nl (S.E. D'Haene), m.r.jones@bristol.ac.uk (M.R. Jones), r.n.frese@vu.nl (R.N. Frese).
[1] Present address: Institute for Cell and Molecular Biosciences, Medical School, University of Newcastle, Newcastle upon Tyne, NE2 4HH, United Kingdom.

http://dx.doi.org/10.1016/j.bbabio.2014.07.003
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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,

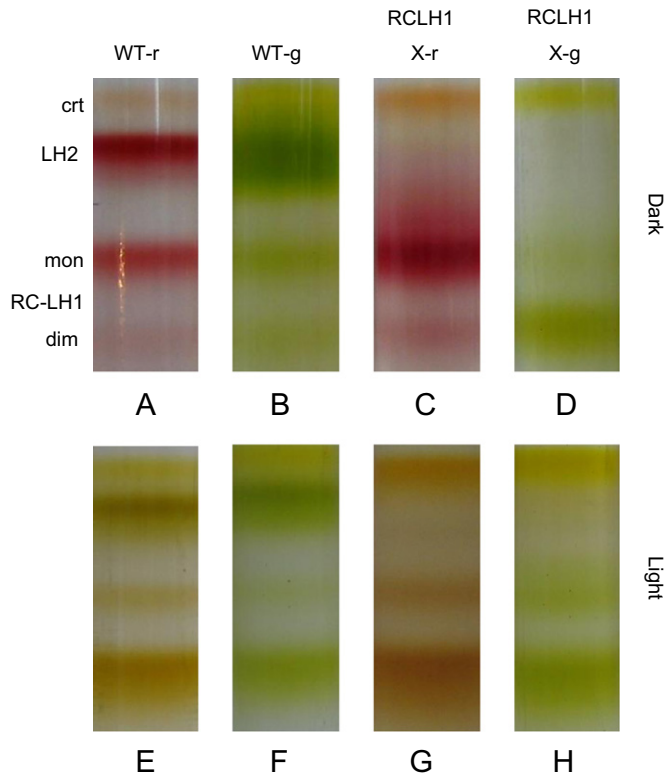

Fig. 1. Sucrose gradient fractionation of complexes solubilized from intracytoplasmic membranes from four strains (columns) grown under dark or light conditions (rows). Colored bands are attributable to (top to bottom) free carotenoid (crt), LH2 (A, B, E and F only), RC–LH1 monomers (mon) and RC–LH1 dimers (dim).

3.3% C) gel containing 50% glycerol (v/v), 10 mM Tris–HCl (pH 7.5), 0.07% N,N,N′,N′-tetramethylethylenediamine (TEMED) and 0.02% ammonium persulfate. After polymerization on ice for 30 min in a home-built gel press with a width of 13 mm × 13 mm, the formed gel is pressed in two perpendicular directions to be transferred into a cuvette with a path length of 10 mm. The expansion of the gel allows the sample to slightly orient along its longest axis. A schematic representation of the sample orientation by gel squeezing and the sample position relative to the light beam is given in S.I. Fig. 3. Samples are cooled in the dark in an Oxford cryostat (DN1704).

The signals of the transmitted light (I) and the difference of parallel and perpendicular (to the direction of expansion of the gel) polarized light (ΔI) were recorded simultaneously with two lock-in amplifiers to obtain the LD spectra (LD = ΔI / I) with a resolution of 1.5 nm at 77 K as previously described [20]. Light minus dark spectra were recorded using a third lock-in amplifier at 3 ms integration time with a continuous laser (Coherent CR-599 CW Dye-laser, DCM dye, Coherent, St. Louis MO) with a bandwidth of about 1 nm, pumped by an Argon laser (Coherent Innova 310) modulated at 20-Hz with excitation at 670 nm and 15 mW cm^{-2} intensity.

3. Results

3.1. Profiles of photosynthetic proteins

The structure of the *Rba. sphaeroides* RC–LH1 dimer and the organization of RCs and antenna complexes in the membrane have mainly been studied in wild type strains [10,17,19,31], or in strains lacking the LH2 antenna complex [1,5,6,8,11,20,32], employing either EM, AFM or LD spectroscopy. LH2-deficient strains are of particular value when studying the structure and membrane-bending properties of the RC–LH1 complex, and can be obtained by deleting the *puc* and *puf* operons encoding the LH2 and RC–LH1 complexes, and expressing the *puf* operon in the resulting strain on a low copy number plasmid [33]. This arrangement allows for additional manipulation of the structure of the RC–LH1 complex, such as point mutations in the various polypeptides or removal of individual components such as PufX. The present work examined the photosystem assembled in *Rba. sphaeroides* wild type strain NCIB8253 (named "WT-r" for convenience in the following), a derivative with a spontaneous mutation of *crtD* expressing green carotenoids (named "WT-g"), and two strains in which a plasmid-encoded *pufBALMX* operon was used to complement a double *puc*/*puf* deletion mutant expressing either native red/brown carotenoids (strain RCLH1X-r) or green carotenoids (strain RCLH1X-g).

To examine the profile of pigment–proteins assembled in these four strains under the two sets of growth conditions, intracytoplasmic membranes prepared from intact cells were mixed in a 3:2 ratio with a 10% solution of n-dodecyl-β-D-maltoside (β-DDM), and the resulting solubilized pigment proteins were fractionated on a five-step sucrose density gradient. As discussed previously [22,23], the types of pigment–protein resolved by this procedure were highly reproducible, and typical gradients are shown in Fig. 1. Gradients loaded with proteins from strain WT-r (Fig. 1A, E) exhibited four colored bands attributable to (top to bottom) free carotenoid, the major LH2 antenna, RC–LH1 monomers and RC–LH1 dimers. Gradients loaded with proteins from strain RCLH1X-r lacked the major colored band attributed to LH2 (Fig. 1C, G). Irrespective of the presence of LH2, most of the RC–LH1 complexes solubilized from cells of these two strains grown under anaerobic/photosynthetic conditions were of the dimeric form (Fig. 1E, G). In striking contrast to this, when the same strains were grown under dark/semiaerobic conditions the predominant form of RC–LH1 complex fractionated on sucrose gradients was monomeric (Fig. 1A, C). The other, very obvious difference was a change in color of the pigmented bands from the yellow-brown obtained with spheroidene (Fig. 1E, G) to the bright red obtained with spheroidenone (Fig. 1A, C).

A further demonstration that there was a connection between carotenoid type and the variation in relative amounts of monomeric and dimeric RC–LH1 complex came from a comparison with the strains expressing green carotenoids (Fig. 1B, D, F and H). Coloration of pigment–proteins from these green strains did not vary between cells grown under dark and light conditions. For the LH2-deficient green strain, dimeric RC–LH1 complexes were the major form solubilized from cells grown in the dark (Fig. 1D). For the LH2-containing green strain (Fig. 1B) approximately equal amounts of monomer and dimer were solubilized from dark-grown cells. Mostly dimers were obtained from either strain grown in the light (Fig. 1F and H), matching the result obtained with the equivalent strains with native brown carotenoids grown in the light (Fig. 1E and G).

An additional point to note was a smear of red pigmentation above the band corresponding to monomeric RC–LH1 complexes in gradients fractionating proteins from the RCLH1X-r strain grown in the dark (Fig. 1C). Absorption spectroscopy of intact gradients using a spectrophotometer fitted with a pair of optical fibers revealed this material to be LH1 antenna protein that was free of RCs (data not shown). Thus it would appear that in this red, LH2-deficient strain there was some over-expression of LH1. This LH1 fraction was not seen in material obtained from this strain when grown in the light (Fig. 1G), in the green LH2-deficient strain (Fig. 1D, H), or in any of the LH2-containing strains (Fig. 1A, B, E and F).

3.2. Organization of complexes probed by LD spectroscopy

One way to probe how the composition of the RC–LH1 complex affects its organization in intact membranes is through linear dichroism spectroscopy (LD) [35]. The sample prepared in a polyacrylamide gel is oriented by expansion of the gel through compression along two perpendicular axes [36]. In previous work it has been shown that in native membrane samples prepared in this way the optical transitions, arising

from the monomeric BChls, BPhes and primary electron donor BChl pair of the RC, possess a uniform alignment relative to the long axis of the oriented membrane [20,31,32,37]. For strains with native RC–LH1 complexes the interpretation is that dimeric RC–LH1 complexes assemble into long-range, ordered linear arrays that are oriented preferentially along the longest membrane axis, even in the presence of LH2. However, LD spectra indicating a lower degree of ordering have also been obtained for membranes containing PufX-deficient RC–LH1 complexes that are known to be monomeric [20,31,32], showing that RC–LH1 dimers are not a prerequisite for detecting a uniform orientation of the RC within membranes. As a result the technique does not reveal whether the RC–LH1 complex is assembled as dimers or monomers, but rather whether RC–LH1 complexes present in the membrane are packed in a regularly ordered manner. The relative amount of order can be estimated by decomposing the LD spectra into ordered and unordered components [32].

In the present work, LD spectroscopy was used to compare the overall spatial arrangement of RC–LH1 complexes in isolated intracytoplasmic membranes. We also probed the orientation of RCs within membranes in intact cells to obtain information on the general orientation of the chromatophores. Fig. 2A shows 77 K absorption spectra of membranes (solid lines) and cells (dashed lines) from strains RCLH1X-r and RCLH1X-g grown in the dark, normalized to the same absorption at the maximum of the dominant LH1 Q_y band. These strains were selected because they showed the greatest contrast between yields of dimer under dark growth conditions — very low for strain RCLH1X-r (Fig. 1C) and very high for strain RCLH1-g (Fig. 1D). The absorption spectra for the RCLH1X-r and RCLH1X-g strains were similar, the main difference being the precise maximum of the LH1 band, which varied between 885 nm and 887 nm for red and green membranes, respectively. In addition to absorption from LH1, these spectra have small bands at 804 nm and 756 nm arising from the two monomeric BChls and two BPhes of the RC, respectively.

LD spectra recorded for these membranes or cells are shown in Fig. 2B, the inset showing the region between 720 and 840 nm on an expanded scale; these spectra have been normalized to the same LH1 absorption at 77 K. In the case of oriented membranes (solid lines), both spectra showed a negative LD at 804 nm characteristic of order in the arrangement of the RC accessory BChls relative to the axis of orientation of the chromatophores, consistent with the previous work [20]. The intensity of this negative LD band and that of the dominant positive LH1 LD at ~890 nm were higher for RCLH1X-g membranes (green-solid) than for RCLH1X-r membranes (red-solid).

LD spectra were also recorded for oriented intact cells of strains RCLH1X-g and RCLH1X-r (Fig. 2B, dashed lines). In this case there was a marked difference in the direction of the LD signal at 804 nm, that for RCLH1X-g cells being negative and indicative of order in the orientation of the RC accessory BChls with respect to the axis of orientation of the cells, but that for RCLH1X-r cells having a positive direction indicative of a lack of order of the RC components relative to the orientation axis [20]. Given the similarity in the LD spectra of membranes isolated from these strains, the difference in the LD spectra of the corresponding cells indicated a difference in the internal arrangement of photosynthetic membranes within these two types of cell, as discussed below.

Dark-minus-light absorption difference spectra and dark-minus-light difference LD spectra (ΔLD) were also recorded for these cells and membranes to monitor the degree of order exhibited by the RC though absorbance bands that are normally hidden by those of the LH complexes (Fig. 3). ΔLD probes the polarization dependency of light-induced spectral changes that occur in response to charge separation within the RC [20]. The absorption difference spectra were similar in line shape for all four samples (Fig. 3A) since charge separation within the RC is not affected by growth conditions or carotenoid composition. In the case of membranes, the ΔLD spectra had a similar differential feature centered at 800 nm (positive at 795 nm/negative at 808 nm) and negative feature at ~890 nm (Fig. 3B), both of which indicated order in the arrangement of the RC component relative to the axis of orientation of the sample by gel squeezing. The ΔLD spectrum of RCLH1X-g cells also had this spectral fingerprint, but that of RCLH1X-r cells did not, with a reverse blue-negative/red-positive feature at 800 nm and

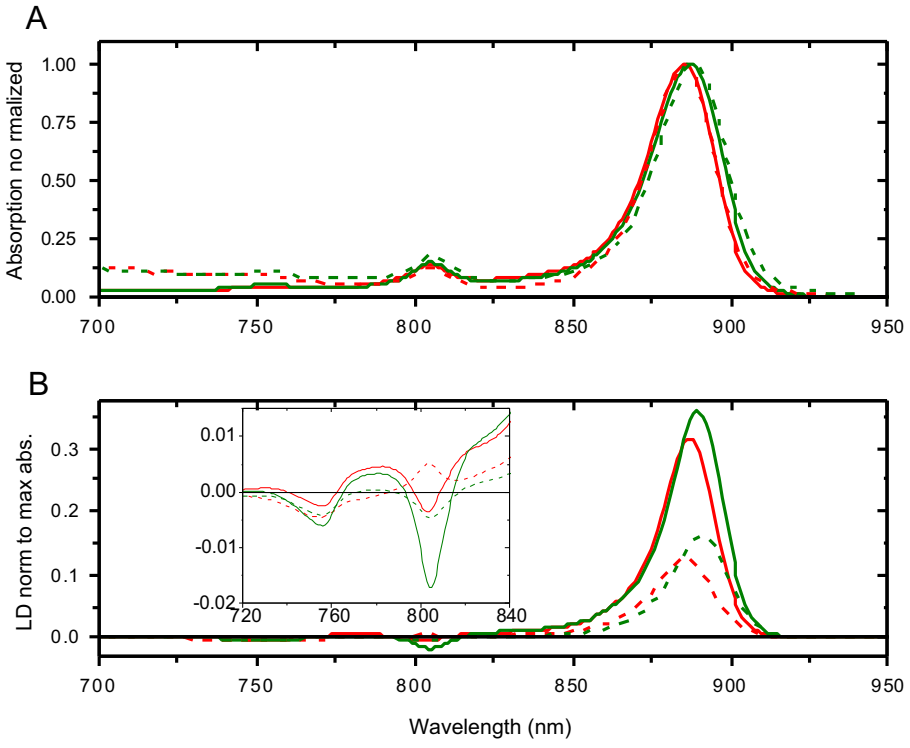

Fig. 2. Normalized 77 K absorption (A) and LD (B) spectra of membranes (solid) and cells (dashed) from strains RCLH1X-r (red) and RCLH1X-g (green) grown in the dark. *Inset*: Magnification of the LD spectra in the monomeric BChl and BPhe region.

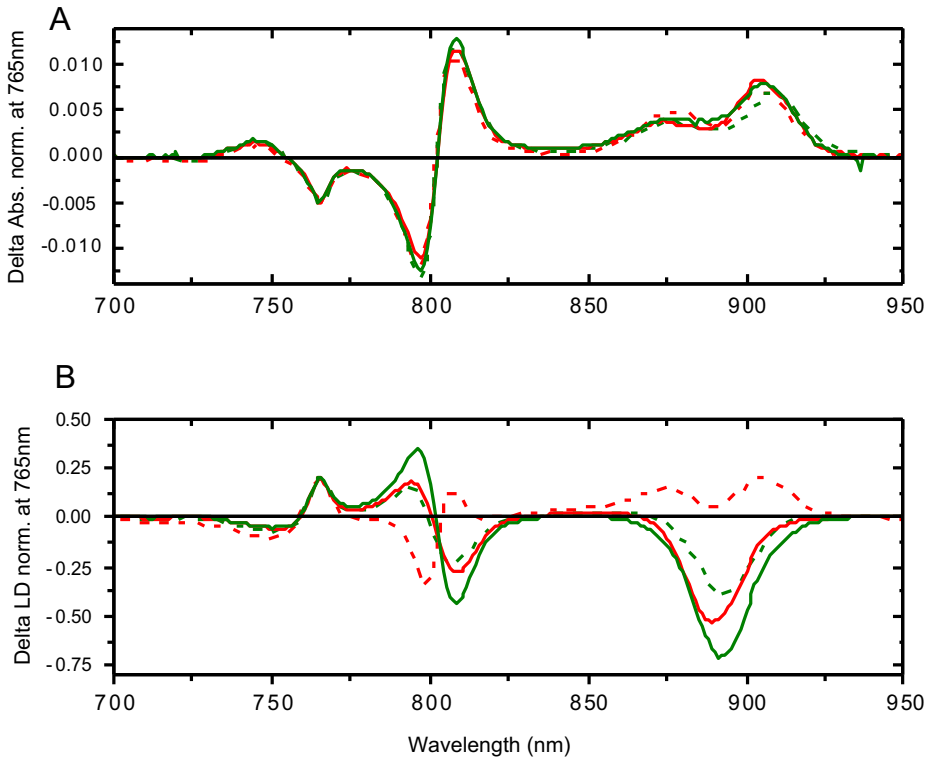

Fig. 3. Normalized 77 K light minus dark (delta) absorption (A) and delta LD (B) spectra of membranes (solid) and cells (dashed) from strains RCLH1X-r (red) and RCLH1X-g (green) grown in the dark.

positive LD in the long-wavelength region. Thus the ΔLD spectra reinforced the conclusions deduced from the LD spectra that RCs in membranes in RCLH1X-g cells show macroscopic order but those in RCLH1X-r cells do not. However, order in the arrangement of RCs relative to the long axis of the membranes was revealed in both cases when membranes were removed from cells.

A second case where there was a marked difference in the RC–LH1 complexes that could be fractionated on sucrose gradients was between the WT-r strain grown under dark or light conditions, where mostly monomers or dimers, respectively, were resolved (Fig. 1A and E). Information on the alignment of RCs in oriented samples of cells and membranes from this strain could not be obtained from LD spectra due the spectral overlap between the RC and LH2 absorption bands at 800 nm, but could be obtained from ΔLD spectra which selectively probe absorption changes due to charge separation within the RC [31]. Both sets of membranes gave ΔLD spectra (not shown) that were similar to spectra reported previously for completely ordered RCs [31,38]. In contrast, both types of cell had a signal in this region with the reverse orientation indicative of a lack of order (as for RCLH1X-r cells in Fig. 3B, red-dashed line). Again, when taken together with the order exhibited by isolated membranes, this indicated a lack of specific order in the arrangement of membranes within cells similar to the case for strain RCLH1X-r but contrasting with strain RCLH1X-g grown in the dark.

4. Discussion

4.1. Organization of RC–LH1 complexes within intact cells

Extensive microscopic and spectroscopic investigations have led to detailed structural models of the photosynthetic membranes from *Rba. sphaeroides*, where the overall spatial organization and orientation of the photosynthetic complexes and their pigments have been described [6,20,24–27,31,39]. In previous work the use of LD spectroscopy gave the possibility to distinguish between PufX-containing RC–LH1 complexes that show long-range order in the arrangement of the RC relative to the long membrane axis, and PufX-deficient monomeric RC–LH1 complexes that lack such order [20]. When put together with structural information from EM and AFM, this LD and ΔLD data has been interpreted in terms of dimeric, PufX-containing RC–LH1 complexes forming ordered linear arrays within the photosynthetic membrane. The fixed orientation of the RC BChls within the LH1 ring (shown in yellow in Fig. 4A) gives rise to characteristic LD signals when dimers associate in an ordered fashion in membranes [20,31]. In the presence of LH2 (Fig. 4B), it is proposed that, because of the arrangement of RC–LH1 dimers in linear rows, isolated chromatophores display a similar characteristic LD and ΔLD spectra indicating order [31]. In the absence of LH2 these RC–LH1 rows aggregate in arrays (shown for two adjacent linear arrays in Fig. 4C). The inherent curvature of the dimer along its long axis results in the formation of tubular membrane structures that can be purified as a heavy membrane fraction and have been analyzed by EM [5,6,8,20,40–42].

Here we applied LD and ΔLD spectroscopy to cells to assess the general orientation of the chromatophores *in vivo*. Data obtained for the RCLH1X-r and RCLH1X-g strains grown in the dark are shown in Fig. 2B (LD) and Fig. 3B (ΔLD). For completeness, spectra (not shown) were also recorded for cells of the RCLH1X-r and RCLH1X-g strains grown in the light, and for a WT-r and WT-g strains grown under both conditions (*i.e.* the eight variations shown in Fig. 1). In all but one case the LD and/or ΔLD spectra obtained indicated a lack of a preferential alignment of the RC population relative to the axis of orientation of the cells, despite the fact that the membranes isolated from these cells did exhibit order when oriented in a gel. The obvious explanation is that photosynthetic membranes within these cells are randomly oriented with respect to the length of the cell, such that macroscopic ordering of the photosynthetic complexes of these cells could not be detected. The presence of detached chromatophores and the arrangement of attached ICM into a reticulum formed by linked photosynthetic membranes [43], recently imaged by Scheuring and co-workers [44], clarify this result. The

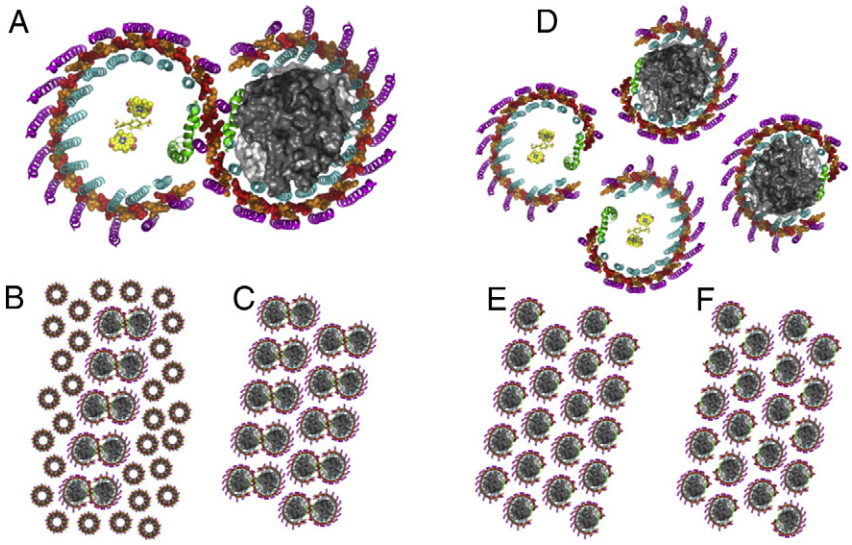

Fig. 4. Models of the dimeric RC–LH1 complex and the organization of RC–LH1 complexes in different *Rba. sphaeroides* membranes represented with the alignment axis, membrane length, in the vertical direction. (A) Model of an RC–LH1 dimer constructed as described in detail previously [22,23]; on the right the RC is shown as a solid object with the largely extra-membrane H-polypeptide highlighted in dark grey, while on the left only the macrocycles of the accessory BChls (spheres) and primary electron donor BChls (sticks, edge-on) are shown (carbons in yellow). For both monomers the 28 LH1 BChls are shown as spheres colored alternating red and orange, the 14 LH1 α- and β-polypeptides as cyan or magenta ribbons, respectively, and the single PufX as a green ribbon. (B) Linear array of RC–LH1 dimers in membranes also containing LH2 (smaller circles); (C) 2-D array of RC–LH1 dimers in membranes lacking LH2; (D) monomeric, elliptical RC–LH1 complexes; (E) Array of monomeric, elliptical RC–LH1 complexes, each with a uniform orientation; (F) more heterogeneous array of monomeric RC–LH1 complexes, each in one of two possible orientations.

one exception to this trend was the LH2-deficient strain RCLH1X-g when grown under dark, semi-aerobic conditions. In this case both the LD and ΔLD spectra gave a clear indication of order in the arrangement of the RC population, and we attribute this to the likely presence of extensive tubular RC–LH1 membranes in this LH2-deficient strain that extend along the length of the cells. As a result the orientation of the membranes in the cell is similar to the orientation of membranes after isolation and alignment in the gel by squeezing.

In contrast to dark-grown cells, when strain RCLH1X-g was grown in the light the intact cells did not produce a LD or ΔLD spectrum indicative of order, and we attribute this to a three to four-fold lower level of expression of RC–LH1 complexes under photosynthetic conditions as judged from the absorption spectra of intact cells (data not shown). Apparently during photosynthetic growth there is stronger regulation of the expression levels of the RC–LH1 complex, such that tubular membranes are either fewer in number or, more probably, less extensive and therefore less well aligned with the long axis of the cells.

Although, as assessed through absorption spectra of growing cultures (data not shown), levels of the RC–LH1 complex were even higher in whole cells of strain RCLH1X-r grown in the dark than in cells of strain RCLHX-g, the LD and ΔLD spectra for these red cells did not contain the spectral fingerprint indicating order. This was despite the fact that, as with the equivalent green strain, isolated oriented membranes did contain ordered RCs. Here a significant factor may have been the presence of a population of LH1 complexes that were not associated with RCs, indicated by a smear of pigmentation on the sucrose density gradient settling above the band corresponding to RC–LH1 monomers (Fig. 1C). When present in the membrane with monomeric or dimeric RC–LH1 complexes, significant levels of RC-free LH1 rings could disrupt the aggregation of the RC–LH1 complexes to form ordered and extensive tubular membrane structures. The RCLH1X-g strain did not assemble significant levels of RC-free LH1 complexes that could cause such disruption (Fig. 1D).

Tubular membranes have been observed earlier in cell sections and isolated fractions by electron microscopy [5,6,8,40–42]. Our results confirm the presence of this type of membranes in the RCLH1X-g strain *in vivo*, without manipulation that could produce artifacts. By comparison with ΔLD spectrum of Frese and co-workers that was constructed from membranes containing 50% RC–LH1–PufX and 50% PufX-deficient RC–LH1 [32] our data indicate a large percentage (more than 50%) of oriented RCs relative to the long axis of the cell. Such an extensive long-range ordering detected in cells would indicate an overall high degree of organization into densely packed arrays. It can be interpreted as a general formation of elongated membranes instead of the usual spherical shape. It implies that elongated membranes are present in a larger amount in the cell than can be observed when isolated [8].

4.2. Organization of RC–LH1 complexes within isolated membranes

In contrast to the general assumption that RC–LH1 is present in a mostly dimeric form in membranes, when spheroidenone was present as the principal carotenoid then the RC–LH1 complexes extracted from intact membranes were largely monomeric (Fig. 1 A and C). There are three possible interpretations of this observation. The first is that the principal form of the RC–LH1 complex assembled in all membranes is dimeric, but the use of β-DDM to solubilize proteins from the membrane produces varying amounts of monomers during the solubilization procedure. As discussed in previous publications [22,23] this possibility can be discounted, as the profile seen for any given combination of genotype and growth condition was highly reproducible across multiple cultures, membrane preparations and solubilizations, with no particular sensitivity to precise solubilization conditions such as the exact concentration of β-DDM, time or temperature.

To reinforce that point, Supplementary Fig. 1 shows the highly similar profiles obtained from sucrose gradients loaded with material from six repeat cultures/solubilizations for strain WT-r grown in the dark, where the RC–LH1 complex is reproducibly mostly monomeric, and strain WT-g grown in the light where the RC–LH1 complex is reproducibly mostly dimeric. The sucrose gradients in Supplementary Fig. 2 show that, for strain WT-r grown in the dark, the relative amounts of monomer and dimer did not change significantly if the concentration of β-DDM used for solubilization was varied from the standard 4% to between 2 and 7% (concentrations above 7% caused general degradation of RC–LH1 and LH2 complexes, and concentrations below 2% led to incomplete solubilization of membranes). Similarly, no variation in relative dimer yield was seen if the solubilization using 4% β-DDM was carried out at room temperature or for double standard time of 30 min.

Another possible explanation of our results is that the RC–LH1 complexes are always mainly dimeric when assembled in intracytoplasmic membranes, but differences in the detailed structure of the dimeric complex, that depend on strain type and growth conditions, result in different, but highly reproducible levels of monomerization of dimers after solubilization with β-DDM. If this hypothesis is correct then the results obtained from sucrose gradients reveal how variations in the type of carotenoid present may modulate the strength of the association between RC–LH1 monomers. In this case, when spheroidenone is present, in contrast with spheroidene or neurosporene, the structure of the dimer is somewhat modified. The interaction between RC–LH1 monomers becomes weaker and dissociates under even the mildest solubilization conditions we have used. We should note here that β-DDM is the most appropriate detergent for the isolation of integral complexes because of its ability to keep protein–protein interactions intact. In higher plants, for example, integral and large photosynthetic supercomplexes can be obtained from the solubilization of thylakoid membranes with β-DDM [45–47]. However, it is perhaps surprising that the extent of monomerization of a given complex is not sensitive to parameters such as detergent concentration.

The last possible interpretation is that there are variations in the relative amounts of monomeric and dimeric RC–LH1 complex that are assembled in intracytoplasmic membranes in different strains and under different growth conditions, and the profiles observed on sucrose density gradients faithfully represent these differences. Given the reproducibility described above this would seem to be the most likely interpretation, and it implies that β-DDM does not cause significant monomerization of dimers during the solubilization procedure, irrespective of the starting material.

The likely presence of monomers in the intact membrane raises questions about their effect on the organization of RC–LH1 complexes in those membranes, and these can be addressed through the use of LD spectroscopy. Previous work has shown the importance of the PufX for the dimeric form of RC–LH1. LD spectra for PufX deficient strains [20] or strains with a mutated PufX [38] indicated a lack of long-range order in the arrangement of the RC–LH1 complexes in intact membranes, leading to the conclusion that the mutations cause a modification in the organization of RC–LH1 complexes from ordered dimers to unordered monomers. In contrast to these previous studies we found that, irrespective of growth conditions or carotenoid composition or the presence/absence of LH2, all preparations of intact intracytoplasmic membranes yielded LD and/or ΔLD spectra indicating an ordered arrangement of RCs when the native PufX was present. In the case of LH2-deficient membranes that contain the native PufX this LD spectral signature of ordering has been associated with the presence of arrays of RC–LH1 dimers [20]. This tallies with the high yields of dimers that could be isolated from strains with native carotenoids grown under photosynthetic conditions (Fig. 1E, G), or either of the strains containing green carotenoids (Figs. 1B, D, F, H), but not with the high yields of monomers obtained from strains WT-r or RCLH1X-r grown in the dark (Figs. 1A and C, respectively). The data in Fig. 1 suggest that RC–LH1 complexes have a different structure in the latter strains when grown under aerobic conditions, with the crucial factor likely to be the type of carotenoid (spheroidenone) present in the complex, as there are no indications that any other components vary according to growth conditions (and it should be noted that results obtained with green strains were essentially independent of growth conditions). In contrast to the PufX-mutant strains previously described [20,38] long range order is observed with the spheroidenone-containing complexes. This comparison leads towards the conclusion that a change in carotenoid composition does not have such a strong effect on the membrane organization as a modification of the structure of PufX, or its absence.

Taken together with the profiles obtained using sucrose gradients, the simplest interpretation of our data on spheroidenone-containing membranes is that they contain mostly monomeric RC–LH1 complexes, but these are packed together in the crowded membrane in a largely ordered fashion that produces similar LD and ΔLD spectra to membranes from strains with spheroidene or neurosporene as the principal carotenoid, where the dimeric form is predominant. This requires the assumption that the inherent elliptical shape of an individual monomeric RC–LH1 complex (Fig. 4D) can induce order in the arrangement of multiple such complexes when packed together in the membrane. In fact this is in line with previous work on a PufX-deficient strain that contains LH2, where the flexibility of the LH1 ring, together with the inherent asymmetry of the RC, results in an elliptical shape for the PufX-deficient monomeric RC–LH1 complex with a closed ring of LH1 around each RC. ΔLD spectra of membranes from this strain indicated partial order in the arrangement of its PufX-deficient RC–LH1 complexes which was attributed to close packing of multiple elliptical monomers [5,6,8,11,20,32]. In the present case a similar packing phenomenon could result in largely monomeric and elliptical PufX-containing RC–LH1 complexes being organized into ordered domains in a similar way to the dimeric form (Fig. 4E, F). As the RC cofactors exhibit a C_2 symmetry each elliptical RC–LH1 complex could adopt one of two orientations within an ordered array, and so such arrays could either be highly uniform in nature with a single orientation (Fig. 4E) or more heterogeneous with a mixture of two opposing orientations (Fig. 4F). Furthermore, the presence of some dimeric RC–LH1 complexes could aid in the uniform alignment of RC–LH1 monomers more precisely than LH2 complexes could in the LH2-containing PufX-deficient membranes as stated above.

Since there is a direct relation between the structure and interaction of the complexes and the vesicle formation, a modification of the carotenoid composition could induce an alteration of the way complexes are embedded in the membrane resulting in a different vesicle shape. The arrangement of RC–LH1 into ordered arrays, if similar for monomers and dimers, should lead to the formation of some chromatophores with a tubular shape. However, according to our LD results on intact cells, it does not seem to be the case. As the amplitude of the LD measured on isolated membranes is slightly lower in the presence of the monomeric RC–LH1 compared to membranes containing dimers, we can conclude that the ordering of the RC–LH1 complexes in spheroidenone-containing membranes is somehow altered and membranes are smaller in length. The presence of LH1 empty ring probably participates in this alteration. The tilt of about 5° between the two LH1 of the dimer observed by AFM might be absent in monomers and induce a different membrane shape [10]. Notably, when PufX is not expressed and results in a complete absence of dimers, chromatophores are larger than in native cells grown under light conditions [29].

At present, we cannot exclude the possibility that a weak interaction between RC–LH1 monomers is present in spheroidenone-containing membranes and leads to a similar organization as obtained in membranes with dimeric spheroidene- or neurosporene-containing complexes. More extensive AFM studies of membranes from such spheroidenone-containing cells could shed more light on this if the resolution was sufficient to distinguish RC–LH1 dimers from closely-associated, ordered RC–LH1 monomers.

4.3. Physiological relevance of changes in membrane organization

Finally, it is relevant to consider the importance of the modification of carotenoid composition and its effect on the RC–LH1 structure at the physiological level. As outlined in the Introduction, Šlouf and coworkers [16] have recently delineated a photoprotective mechanism that would be activated on the conversion of spheroidene to spheroidenone in response to the presence of oxygen. A feature of this spheroidenone-specific mechanism was proposed to be a twisting of the carotenoid into an altered conformation to increase its conjugation length and so tune its energy levels. An accompanying effect of such a change in the conformation of the ~56 carotenoid molecules located in each RC–LH1 dimer could be a structural change at the dimer interface that manifests either as a markedly increased propensity for monomerization on solubilization of intact membranes, or the presence

of ordered monomers rather than ordered dimers in the spheroidenone-rich membrane. The role of carotenoids in the dimerization process can possibly take place where the two LH1 rings interconnect to form an S-shape in dimers. Spheroidenone could induce a structural change altering the necessary discontinuity of the LH1 antenna to assemble into dimers. A possible relevant point to note is the evidence of interactions between carotenoid and the PufX protein that is thought to be positioned close to the dimer interface and play a role in dimerization of the RC–LH1 complex [7,48]. In addition to direct structural effects, a change in carotenoid composition and conformation on switching cells from anaerobic to aerobic growth could affect the properties of the RC–LH1 dimer through an indirect effect on this crucial PufX protein. The role of PufX in the LH1 ring opening for a proper quinone migration has been demonstrated in the dimeric form of the RC–LH1 complex [1]. In the RC–LH1 monomer containing PufX the migration of the quinone pool could be altered by the modification of the structure into a closed ring. The presence of mainly monomeric RC–LH1–PufX complexes in *Rba. sphaeroides*, when cells are grown under semi-anaerobic conditions, offers the possibility to obtain more information about the differences of structure induced by the change of carotenoid composition.

In addition to producing this experimentally observable effect on the amount and/or stability of the RC–LH1 dimer, a significant structural change at the dimer interface could conceivably also serve to partially decouple the antenna pigments in the two halves of the dimer and so further contribute to photoprotection of the system. The triplet excited states that sensitize singlet oxygen tend to be formed at high levels in antenna systems when there is an interruption to the flow of excited state energy into the trap formed by the RC electron transfer chain. Decoupling of the two halves of an RC–LH1 dimer could limit the delivery of excited state energy to a damaged half of the dimer, reducing the probability of further damage while still delivering excitation energy to the functional half. It seems reasonable to postulate that under non-stress conditions the dimeric structure offers advantages in terms of the efficiency with which energy can be delivered from LH1 to an open and functional RC, but such a flexible arrangement could be deleterious under conditions where photodamage is closing RCs and increasing the levels of harmful BChl triplets and singlet oxygen.

A photoprotective response corresponding to a carotenoid conformational change that induces a modification of a protein structure and results in a remodeling of the photosynthetic membrane has also been observed in the main light harvesting complex of plants [49,50], in diatoms [51] and cyanobacteria [52]. Photosynthetic organisms have possibly developed similar mechanisms to deal with deleterious excess of light.

5. Conclusions

We performed an *in vivo* investigation of the organization of purple bacterial photosynthetic membranes by measuring LD on oriented, intact cells. Only one green strain, containing RC–LH1 complexes in a predominantly dimeric configuration and lacking LH2, showed long-range ordering of RCs. This result can best be explained by the packing model where RC–LH1 dimers precisely interlock, forming elongated tubular membranes [20]. Our data indicate a high proportion of elongated membranes with all embedded RC–LH1 complexes in uniform orientation within intact cells from this specific strain.

Sucrose density gradient fractionation of solubilized membranes from different strains and growth conditions showed major and reproducible variations in the relative amounts of the monomeric and dimeric forms of RC–LH1. Despite the main presence of native monomers in some cases, LD spectroscopy performed on all intact membranes preparations indicates that RCs, and by extension RC–LH1 complexes, are organized in a highly ordered fashion. Monomeric RC–LH1 complexes are prevalent when strains with native carotenoids are grown in the presence of oxygen, a growth condition in which spheroidenone is synthesized. As this carotenoid was recently found to have a particular photoprotective role [16], it is tempting to speculate that a switch from dimeric to monomeric architecture for the RC–LH1 complex also forms part of this photoprotective response, a change that could occur with only minor remodeling of the membrane. Although RC–LH1 monomers have been observed before in AFM images, they have been omitted from derived models. Our data highlights the necessity to include RC–LH1 monomers in the models of *Rba. sphaeroides* membranes.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.bbabio.2014.07.003.

Acknowledgements

M.R.J. and L.C. acknowledge financial support by the Biotechnology and Biological Sciences Research Council of the United Kingdom through Doctoral Training Grant BB/D526037/1. R.N.F. acknowledges The Netherlands Organization for Scientific Research.

References

[1] P. Qian, M.Z. Papiz, P.J. Jackson, A.A. Brindley, I.W. Ng, J.D. Olsen, M.J. Dickman, P.A. Bullough, C.N. Hunter, Three-dimensional structure of the *Rhodobacter sphaeroides* RC–LH1–PufX complex: dimerization and quinone channels promoted by PufX, Biochemistry 52 (2013) 7575–7585.
[2] R.J. Cogdell, A. Gall, J. Köhler, The architecture and function of the light-harvesting apparatus of purple bacteria: from single molecules to *in vivo* membranes, Q. Rev. Biophys. 39 (2006) 227–324.
[3] G. McDermott, S.M. Prince, A.A. Freer, A.M. Hawthornthwaite-Lawless, M.Z. Papiz, R. J. Cogdell, N.W. Isaacs, Crystal structure of an integral membrane light-harvesting complex from photosynthetic bacteria, Nature 374 (1995) 517–521.
[4] J. Koepke, X.C. Hu, C. Muenke, K. Schulten, H. Michel, The crystal structure of the light-harvesting complex II (B800-850) from *Rhodospirillum molischianum*, Structure 4 (1996) 581–597.
[5] C. Jungas, J.L. Ranck, J.L. Rigaud, P. Joliot, A. Verméglio, Supramolecular organization of the photosynthetic apparatus of *Rhodobacter sphaeroides*, EMBO J. 18 (1999) 534–542.
[6] P. Qian, P.A. Bullough, C.N. Hunter, Three-dimensional reconstruction of a membrane-bending complex — the RC–LH1–PufX core dimer of *Rhodobacter sphaeroides*, J. Biol. Chem. 283 (2008) 14002–14011.
[7] K. Holden-Dye, L.I. Crouch, M.R. Jones, Structure, function and interactions of the PufX protein, Biochim. Biophys. Acta Bioenerg. 1777 (2008) 613–630.
[8] C.A. Siebert, P. Qian, D. Fotiadis, A. Engel, C.N. Hunter, P.A. Bullough, Molecular architecture of photosynthetic membranes in *Rhodobacter sphaeroides*: the role of PufX, EMBO J. 23 (2004) 690–700.
[9] S. Scheuring, F. Francia, J. Busselez, B.A. Melandri, J.L. Rigaud, D. Lévy, Structural role of PufX in the dimerization of the photosynthetic core complex of *Rhodobacter sphaeroides*, J. Biol. Chem. 279 (2004) 3620–3626.
[10] S. Bahatyrova, R.N. Frese, C.A. Siebert, J.D. Olsen, K.O. van der Werf, R. van Grondelle, R.A. Niederman, P.A. Bullough, C. Otto, C.N. Hunter, The native architecture of a photosynthetic membrane, Nature 430 (2004) 1058–1062.
[11] P. Qian, C.N. Hunter, P.A. Bullough, The 8.5 Å projection structure of the core RC–LH1–PufX dimer of *Rhodobacter sphaeroides*, J. Mol. Biol. 349 (2005) 948–960.
[12] M. Griffiths, W.R. Sistrom, G. Cohen-Bazire, R.Y. Stanier, Function of carotenoids in photosynthesis, Nature 176 (1955) 1211–1214.
[13] W.R. Sistrom, M. Griffiths, R.Y. Stanier, The biology of a photosynthetic bacterium which lacks colored carotenoids, J. Cell Comp. Physiol. 48 (1956) 473–515.
[14] C.F. Borland, D.J. Mcgarvey, T.G. Truscott, R.J. Codgell, E.J. Land, Photophysical studies of bacteriochlorophyll-a and bacteriopheophytin-a — singlet oxygen generation, J. Photochem. Photobiol. B 1 (1987) 93–101.
[15] R.J. Cogdell, T.D. Howard, R. Bittl, E. Schlodder, I. Geisenheimer, W. Lubitz, How carotenoids protect bacterial photosynthesis, Philos. Trans. R. Soc. B 355 (2000) 1345–1349.
[16] V. Šlouf, P. Chábera, J.D. Olsen, E.C. Martin, P. Qian, C.N. Hunter, T. Polívka, Photoprotection in a purple phototrophic bacterium mediated by oxygen-dependent alteration of carotenoid excited-state properties, Proc. Natl. Acad. Sci. U. S. A. 109 (2012) 8570–8575.
[17] I.W. Ng, P.G. Adams, D.J. Mothersole, C. Vasilev, E.C. Martin, H.P. Lang, J.D. Tucker, C. N. Hunter, Carotenoids are essential for normal levels of dimerisation of the RC–LH1–PufX core complex of *Rhodobacter sphaeroides*: characterisation of R-26 as a crtB (phytoene synthase) mutant, Biochim. Biophys. Acta Bioenerg. 1807 (2011) 1056–1063.
[18] E.C. Ratcliffe, R.B. Tunnicliffe, I.W. Ng, P.G. Adams, P. Qian, K. Holden-Dye, M.R. Jones, M.P. Williamson, C.N. Hunter, Experimental evidence that the membrane-spanning helix of PufX adopts a bent conformation that facilitates dimerisation of the *Rhodobacter sphaeroides* RC–LH1 complex through N-terminal interactions, Biochim. Biophys. Acta Bioenerg. 1807 (2011) 95–107.
[19] P.G. Adams, C.N. Hunter, Adaptation of intracytoplasmic membranes to altered light intensity in *Rhodobacter sphaeroides*, Biochim. Biophys. Acta Bioenerg. 1817 (2012) 1616–1627.
[20] R.N. Frese, J.D. Olsen, R. Branvall, W.H. Westerhuis, C.N. Hunter, R. van Grondelle, The long-range supraorganization of the bacterial photosynthetic unit: a key role for PufX, Proc. Natl. Acad. Sci. U. S. A. 97 (2000) 5197–5202.

[21] F. Francia, J. Wang, G. Venturoli, B.A. Melandri, W.P. Barz, D. Oesterhelt, The reaction center–LH1 antenna complex of *Rhodobacter sphaeroides* contains one PufX molecule which is involved in dimerization of this complex, Biochemistry 38 (1999) 6834–6845.
[22] L.I. Crouch, K. Holden-Dye, M.R. Jones, Dimerisation of the *Rhodobacter sphaeroides* RC–LH1 photosynthetic complex is not facilitated by a GxxxG motif in the PufX polypeptide, Biochim. Biophys. Acta Bioenerg. 1797 (2010) 1812–1819.
[23] L.I. Crouch, M.R. Jones, Cross-species investigation of the functions of the Rhodobacter PufX polypeptide and the composition of the RC–LH1 core complex, Biochim. Biophys. Acta 1817 (2012) 336–352.
[24] J. Hsin, J. Gumbart, L.G. Trabuco, E. Villa, P. Qian, C.N. Hunter, K. Schulten, Protein-induced membrane curvature investigated through molecular dynamics flexible fitting, Biophys. J. 97 (2009) 321–329.
[25] M.K. Şener, J.D. Olsen, C.N. Hunter, K. Schulten, Atomic-level structural and functional model of a bacterial photosynthetic membrane vesicle, Proc. Natl. Acad. Sci. U. S. A. 104 (2007) 15723–15728.
[26] J. Hsin, J. Strümpfer, M. Şener, P. Qian, C.N. Hunter, K. Schulten, Energy transfer dynamics in an RC–LH1–PufX tubular photosynthetic membrane, New J. Phys. 12 (2010).
[27] M. Şener, J. Strümpfer, J.A. Timney, A. Freiberg, C.N. Hunter, K. Schulten, Photosynthetic vesicle architecture and constraints on efficient energy harvesting, Biophys. J. 99 (2010) 67–75.
[28] T. Geyer, V. Helms, A spatial model of the chromatophore vesicles of *Rhodobacter sphaeroides* and the position of the cytochrome bc1 complex, Biophys. J. 91 (2006) 921–926.
[29] P.G. Adams, D.J. Mothersole, I.W. Ng, J.D. Olsen, C.N. Hunter, Monomeric RC–LH1 core complexes retard LH2 assembly and intracytoplasmic membrane formation in PufX-minus mutants of *Rhodobacter sphaeroides*, Biochim. Biophys. Acta Bioenerg. 1807 (2011) 1044–1055.
[30] G. Garab, Linear and circular dichroism, in: J. Amesz, A. Hoff (Eds.), Biophysical Techniques in Photosynthesis, advances in photosynthesis, vol. 3, Springer Netherlands, 1996, pp. 11–40.
[31] R.N. Frese, C.A. Siebert, R.A. Niederman, C.N. Hunter, C. Otto, R. van Grondelle, The long-range organization of a native photosynthetic membrane, Proc. Natl. Acad. Sci. U. S. A. 101 (2004) 17994–17999.
[32] R.N. Frese, J.C. Pàmies, J.D. Olsen, S. Bahatyrova, C.D. van der Weij-de Wit, T.J. Aartsma, C. Otto, C.N. Hunter, D. Frenkel, R. van Grondelle, Protein shape and crowding drive domain formation and curvature in biological membranes, Biophys. J. 94 (2008) 640–647.
[33] M.R. Jones, G.J.S. Fowler, L.C.D. Gibson, G.G. Grief, J.D. Olsen, W. Crielaard, C.N. Hunter, Mutants of *Rhodobacter sphaeroides* lacking one or more pigment protein complexes and complementation with reaction-center, Lh1, and Lh2 genes, Mol. Microbiol. 6 (1992) 1173–1184.
[34] M.R. Jones, M. Heer-Dawson, T.A. Mattioli, C.N. Hunter, B. Robert, Site-specific mutagenesis of the reaction center from *Rhodobacter sphaeroides* studied by Fourier Transform Raman Spectroscopy: mutations at tyrosine M210 do not affect the electronic structure of the primary donor, FEBS Lett. 339 (1994) 18–24.
[35] G. Garab, H. van Amerongen, Linear dichroism and circular dichroism in photosynthesis research, Photosynth. Res. 101 (2009) 135–146.
[36] I.A. Abdourakhmanov, A.O. Ganago, Y.E. Erokhin, A.A. Solov'ev, V.A. Chugunov, Orientation and linear dichroism of the reaction centers from *Rhodopseudomonas sphaeroides* R-26, Biochim. Biophys. Acta Bioenerg. 546 (1979) 183–186.
[37] J. Breton, Orientation of the chromophores in the reaction center of *Rhodopseudomonas viridis*. Comparison of low-temperature linear dichroism spectra with a model derived from X-ray crystallography, Biochim. Biophys. Acta 810 (1985) 235–245.
[38] K. Sznee, L.I. Crouch, M.R. Jones, J.P. Dekker, R.N. Frese, Variation in supramolecular organisation of the photosynthetic membrane of *Rhodobacter sphaeroides* induced by alteration of PufX, Photosynth. Res. 119 (2014) 243–256.
[39] D.E. Chandler, J. Hsin, C.B. Harrison, J. Gumbart, K. Schulten, Intrinsic curvature properties of photosynthetic proteins in chromatophores, Biophys. J. 95 (2008) 2822–2836.
[40] C.N. Hunter, J.D. Pennoyer, J.N. Sturgis, D. Farrelly, R.A. Niederman, Oligomerization states and associations of light-harvesting pigment protein complexes of *Rhodobacter sphaeroides* as analyzed by lithium dodecyl sulfate-polyacrylamide gel electrophoresis, Biochemistry 27 (1988) 3459–3467.
[41] P.J. Kiley, A. Varga, S. Kaplan, Physiological and structural analysis of light-harvesting mutants of *Rhodobacter sphaeroides*, J. Bacteriol. 170 (1988) 1103–1115.
[42] W.H. Westerhuis, J.N. Sturgis, E.C. Ratcliffe, C.N. Hunter, R.A. Niederman, Isolation, size estimates, and spectral heterogeneity of an oligomeric series of light-harvesting 1 complexes from *Rhodobacter sphaeroides*, Biochemistry 41 (2002) 8698–8707.
[43] J.D. Tucker, C.A. Siebert, M. Escalante, P.G. Adams, J.D. Olsen, C. Otto, D.L. Stokes, C.N. Hunter, Membrane invagination in *Rhodobacter sphaeroides* is initiated at curved regions of the cytoplasmic membrane, then forms both budded and fully detached spherical vesicles, Mol. Microbiol. 76 (2010) 833–847.
[44] S. Scheuring, R. Nevo, L.N. Liu, S. Mangenot, D. Charuvi, T. Boudier, V. Prima, P. Hubert, J.N. Sturgis, Z. Reich, The architecture of *Rhodobacter sphaeroides* chromatophores, Biochim. Biophys. Acta 1837 (2014) 1263–1270.
[45] C. Pagliano, S. Barera, F. Chimirri, G. Saracco, J. Barber, Comparison of the alpha and beta isomeric forms of the detergent n-dodecyl-D-maltoside for solubilizing photosynthetic complexes from pea thylakoid membranes, Biochim. Biophys. Acta Bioenerg. 1817 (2012) 1506–1515.
[46] S. Caffarri, R. Kouřil, S. Kereïche, E.J. Boekema, R. Croce, Functional architecture of higher plant photosystem II supercomplexes, EMBO J. 28 (2009) 3052–3063.
[47] E.J. Boekema, H. van Roon, J.P. Dekker, Specific association of photosystem II and light-harvesting complex II in partially solubilized photosystem II membranes, FEBS Lett. 424 (1998) 95–99.
[48] M. Aklujkar, J.T. Beatty, The PufX protein of *Rhodobacter capsulatus* affects the properties of bacterio chlorophyll a and carotenoid pigments of light-harvesting complex 1, Arch. Biochem. Biophys. 443 (2005) 21–32.
[49] A.V. Ruban, R. Berera, C. Ilioaia, I.H.M. van Stokkum, J.T.M. Kennis, A.A. Pascal, H. van Amerongen, B. Robert, P. Horton, R. van Grondelle, Identification of a mechanism of photoprotective energy dissipation in higher plants, Nature 450 (2007) 575–578.
[50] R. Bassi, S. Caffarri, Lhc proteins and the regulation of photosynthetic light harvesting function by xanthophylls, Photosynth. Res. 64 (2000) 243–256.
[51] J. Lavaud, B. Rousseau, H.J. van Gorkom, A.L. Etienne, Influence of the diadinoxanthin pool size on photoprotection in the marine planktonic diatom *Phaeodactylum tricornutum*, Plant Physiol. 129 (2002) 1398–1406.
[52] A. Wilson, G. Ajlani, J.M. Verbavatz, I. Vass, C.A. Kerfeld, D. Kirilovsky, A soluble carotenoid protein involved in phycobilisome-related energy dissipation in cyanobacteria, Plant Cell 18 (2006) 992–1007.