Supramolecular organization of the photosynthetic apparatus of \textit{Rhodobacter sphaeroides}

Colette Jungas, Jean-Luc Ranck\textsuperscript{1}, Jean-Louis Rigaud\textsuperscript{1}, Pierre Joliot\textsuperscript{2} and André Verméglio\textsuperscript{3}

CEA/Cadarache-DSV-DEV Laboratory de Bioenergetique Cellulaire, 13108 St Paul-lez-Durance Cedex, \textsuperscript{1}Institut Curie, Section de Recherche, UMR 168 et LCR-CEA 8, 11 rue Pierre-et-Marie Curie, 75231 Paris Cedex 05, \textsuperscript{2}Institut de Biologie Physico-Chimique, CNRS UPR 9072, 13 rue Pierre-et-Marie Curie, 75005 Paris, France

\textsuperscript{3}Corresponding author
e-mail: avermeglio@cea.fr

Native tubular membranes were purified from the purple non-sulfur bacterium \textit{Rhodobacter sphaeroides}. These tubular structures contain all the membrane components of the photosynthetic apparatus, in the relative ratio of one cytochrome \textit{bc} \textsubscript{1} complex to two reaction centers, and ~24 bacteriochlorophyll molecules per reaction center. Electron micrographs of negative-stained membranes diffract up to 25 Å and allow the calculation of a projection map at 20 Å. The unit cell (a = 198 Å, b = 120 Å and γ = 103°) contains an elongated S-shaped supercomplex presenting a pseudo-2-fold symmetry. Comparison with density maps of isolated reaction center and light-harvesting complexes allowed interpretation of the projection map. Each supercomplex is composed of light-harvesting 1 complexes that take the form of two C-shaped structures of ~112 Å in external diameter, facing each other on the open side and enclosing the two reaction centers. The remaining positive density is tentatively attributed to one cytochrome \textit{bc} \textsubscript{1} complex. These features shed new light on the association of the reaction center and the light-harvesting complexes. In particular, the organization of the light-harvesting complexes in C-shaped structures ensures an efficient exchange of ubihydroquinone/ubiquinone between the reaction center and the cytochrome \textit{bc} \textsubscript{1} complex.

\textit{Keywords}: cytochrome \textit{bc} \textsubscript{1} complex/LH1/photosynthetic reaction center/Rhodobacter sphaeroides/supercomplexes

Introduction

In bacterial photosynthesis, absorption of light initiates a cyclic electron transfer coupled to proton translocation across the cytoplasmic membrane. Three membrane protein complexes are necessary to collect and convert light energy into chemical energy: the light-harvesting complexes (LHC), the reaction center (RC) and the cytochrome (cyt) \textit{bc} \textsubscript{1} complex. To ensure an efficient collection of light energy, the purple photosynthetic bacteria usually contain two types of LHC; LH1 and LH2. The amounts of LH2 are modulated by several factors, such as light intensity and oxygen tension, while LH1 are synthesized in stoichiometric amounts with the RC (Drews and Golecki, 1995). After light absorption by the LHC, the excitation energy is transferred to the RC where a charge separation occurs between donor and acceptor molecules. The photo-oxidized primary electron donor, a dimer of bacteriochlorophyll (Bchl) molecules, is reduced by the high potential carriers of the \textit{bc} \textsubscript{1} complex (Meyer and Donohue, 1995) via soluble carriers such as cyt \textit{c} \textsubscript{2}. To complete the cyclic electron transfer, this complex is reduced in turn by the photoreduced acceptor. The mechanisms of excitonic energy migration and photoinduced electron transfer have been studied in detail using biophysical approaches, and analyzed at the molecular level following the resolution of the atomic structure of the RC, LH2 and \textit{bc} \textsubscript{1} complexes (Deisenhofer et al., 1985; Allen et al., 1986; McDermott et al., 1995; Xia et al., 1997; Zhang et al., 1998). However, despite the wealth of information available on the individual proteins, their supramolecular organization in the membrane remains unclear.

A first issue is the spatial organization between LH1 complexes and RCs. Native membranes and LH1–RC complexes isolated from \textit{Rhodopseudomonas (Rps.) viridis} (Miller, 1982; Engelhardt et al., 1986; Ikeda-Yamasaki et al., 1998), \textit{Ectothiorhodospira halochloris} (Stark et al., 1984; Engelhardt et al., 1986), \textit{Rps.molischianum} (Boonstra et al., 1994) and \textit{Rhodospirillum (Rs.) rubrum} (Walz and Ghosh, 1997; Stahlberg et al., 1998) have been analyzed by various electron microscopic techniques. All these studies reveal a single RC inside a closed ring of LH1 complexes. Organization of LH1 complexes in a closed ring was also observed by a cryo-electron microscopy study of two-dimensional (2D) crystals of \textit{Rs.rubrum} purified complexes (Karrasch et al., 1995). The 8.5 Å projection map, obtained in this latter study, showed 16 subunits arranged in a ring-like structure (116 Å overall diameter, 68 Å inner diameter) that could only accommodate one RC. The closed LH1 structure raised the question of how the quinone/quinol transfer took place between the RC acceptor site and the \textit{bc} \textsubscript{1} complex during cyclic electron transfer. Several hypotheses have been proposed, such as slight movements of the α-helices allowing diffusion of the quinone molecules through the ring (Karrasch et al., 1995). It has also been suggested that the presence in the ring of additional proteins such as PuF, an essential polypeptide for photosynthetic growth of \textit{Rhodobacter (Rh.) sphaeroides} and \textit{Rh.capsulatus} (Farchaus et al., 1990; Lilburn et al., 1992), or the Ω polypeptide in the case of \textit{Rs.rubrum} (Ghosh et al., 1994; Stahlberg et al., 1998), may catalyze quinone transfer by forming specific channels through the ring of LH1 complexes (McGlynn et al., 1996).

A second issue is the organization of the RC and \textit{bc} \textsubscript{1} complexes in the photosynthetic unit. A series of experiments performed with intact cells of \textit{Rb.sphaeroides}
prompted the proposal that the elements of the photosynthetic electron transfer chain might be organized in supercomplexes (Joliot et al., 1989), each one comprising two RCs, one cyt c2 and one bc1 complex. This proposal originated from the observation that the apparent equilibrium constant between the different reactants, measured during photooxidation of the donor chain under continuous illumination, was much lower than that deduced from their redox mid-point potentials measured at equilibrium (Joliot et al., 1989; Lavergne et al., 1989). The supercomplex hypothesis has been challenged by Fernández-Velasco and Crofts (1991), and an alternative model was recently proposed to explain the low value of the observed equilibrium constant (Crofts et al., 1998). The basic assumption in this alternative model is a heterogeneity in the ratio of electron transfer components in individual chromatophores acting as separate compartments. However, this model fails to explain the following important observations made on intact cells of Rb.sphaeroides: (i) the addition of subsaturating concentrations of myxothiazol, a specific inhibitor of the bc1 complex, decreases the number of active bc1 complexes but does not affect the rate of cyclic electron transfer for the uninhibited complexes (Verméglio et al., 1993; Joliot et al., 1996); (ii) the complete photoinduced cyclic electron flow functions even at −20°C in a frozen medium (Joliot et al., 1997); (iii) evidence, by freeze-fracture electron microscopy, of a dimeric arrangement of the photosynthetic membrane proteins (Golecki et al., 1991; Sabaty et al., 1994) in the tubular membranes of Rb.sphaeroides cells deleted in LH2 complexes (Hunter et al., 1988; Kiley et al., 1988) or grown in the presence of nitrate (Sabaty et al., 1994); and (iv) the very recent biochemical evidence that dimeric LH1–RC complexes can be isolated by gentle solubilization of photosynthetic membranes of Rb.sphaeroides (Francia et al., 1998).

Here, we report on the biochemical and structural characterization of these tubular membranes. A projection map at 20 Å resolution was obtained by electron microscopy of negatively stained samples. The electron density distribution agrees with the supramolecular arrangement of the photosynthetic apparatus determined by functional approaches, in particular the dimeric association of the photosynthetic RCs (Joliot et al., 1989). An important additional finding is the organization of the LH1 complexes in C-shaped structures, which sheds new light on the coupling of electron transfer between RC and bc1 complex by the quinone molecules.

**Results**

**Purification and biochemical characterization of the tubular membranes**

The cytoplasmic membrane of Rb.sphaeroides forma sp. denitrificans grown photosynthetically in the presence of 200 mM nitrate or under dark semi-aerobic conditions
presents a large number of tubular membranes (Sabaty et al., 1994). The presence of these tubes is correlated with the decreased synthesis of LH2 complexes. Similar tubular membranes were found in various mutants of *Rh. sphaeroides* in which this complex is deleted (Hunter et al., 1988; Kiley et al., 1988). Figure 1A shows an electron micrograph of negatively stained spheroplasts of *Rh. sphaeroides* strain RCLH10. These tubes are between 0.5 and 2 μm long and ~116 nm in diameter as shown by the freeze-drying electron micrograph depicted in Figure 1B (see also Golec et al., 1991). Interestingly, these freeze-drying pictures present well-organized rows of dimeric particles, in agreement with freeze-fracture experiments (Golec et al., 1991; Sabaty et al., 1994). We purified these tubes from cells of *Rh. sphaeroides* forma sp. *denitrificans*, and from mutant RCLH10 deleted in LH2 complexes, as described in the Materials and methods. The purity of the preparations was assayed by electron microscopy of negatively stained samples. The best samples contained <10% contamination in the form of cytoplasmic membrane fragments and chromatophores. The upper part of Figure 2 shows the absorption spectrum of purified tubular membranes recorded at 77 K. In the near-infrared spectral region, the main absorption band is centered at 880 nm and corresponds to the Qy transition of the LH1 complexes. In addition, typical bands of Bchl and bacteriopheophytin (Bpheo) molecules of the RC are centered at 804 and 760 nm, respectively. The blue region is dominated by the Qx transitions of the LH1 complexes at 590 nm and by the carotenoid bands centered at 445, 472 and 505 nm. The presence of bc1 complex is revealed by the characteristic α-bands of cyt c1, cyt b1 and cyt b1, centered at 548, 556 and 561 nm, respectively. From five different preparations of purified tubular membranes, a relative ratio of 0.9 ± 0.05 bc1 complex per 2 RCs was determined. This ratio did not significantly change during the purification procedure, and is similar to that measured for chromatophores of wild-type *Rh. sphaeroides* (van der Berg et al., 1979). The presence of RCs, bc1 complexes and LH1 complexes was further confirmed by gel electrophoresis (Figure 2B). This gel presents characteristic polypeptide bands of the RC at 28, 26 and 24 kDa. The bands of the cyt b, cyt c1, Rieske protein and subunit IV were observed at 43, 33, 20 and 14 kDa, respectively. These assignments were confirmed by Western blots (not shown). LH1 subunits were detected at 11 and 8 kDa. Part of the LH1 migrates as oligomers of high molecular weight as already observed by Hunter et al. (1988). We also measured the relative ratio of Bch1 to RC, which we found to be 24–25 Bch1 molecules per RC in agreement with previous work on membrane or isolated complexes (van Grondelle et al., 1994; McGlynn et al., 1996; Francia et al., 1998).

**Electron microscopy of the tubular membrane**

The high degree of organization observed by freeze-etching (Golec et al., 1991; Sabaty et al., 1994) and freeze-drying (Figure 1B) suggests that the tubular membrane purified from *Rh. sphaeroides* is a natural 2D crystal of membrane proteins that can be analyzed by negative staining and electron microscopy. Figure 3A shows low-dose electron micrographs of negatively stained tubular membranes. Optical diffraction analysis of such images clearly shows two distinct patterns, both with sharp strong spots. The Fourier transform and the filtered image of one tubular crystal are depicted in Figure 3B and C, respectively. These two diffraction patterns correspond to two layered 2D crystals diffracting independently and originating from the top and bottom parts of the tube flattened on the electron microscope grid. Only the pattern with the strongest non-overlapping spots, which corresponds to the top layer, was subsequently used for image processing. The indexation clearly indicated a monoclinic unit cell with parameters: a = 198 Å, b = 120 Å and γ = 103°, and the resolution extended up to 25 Å. Eight images were selected, by optical diffraction, digitized and processed using the procedure of Crowther et al. (1996). Five images were kept for the final merging step. Reflections with quality index for image phases (IQ) values of 5 are visible out to 20 Å resolution (Figure 4A).
Fig. 3. (A) Low-dose electron micrographs of native tubular membranes from *Rb.sphaeroides*. Samples were negatively stained with 1% uranyl acetate. Bar = 100 nm for all the tubes. (B) Fourier transform of one tube from digitized scan. The arrow indicates the resolution at 25 Å. (C) Filtered image from masked Fourier transform of electron micrograph. Bar = 200 Å.

A projection map was calculated (Figure 4B). The unit cell (198×120 Å) contains an elongated S-shaped supercomplex composed of two C-shaped structures of external diameter 112 Å, their open sides facing each other and enclosing a large protein mass. In addition to the density localized in the center of each ring, a positive density is detected between the two C-shaped structures. This S-shaped supercomplex corresponds closely to the dimeric appearance of the rows observed in freeze-dried (Figure 1B) and freeze-fractured samples (Golecki et al., 1991; Sabaty et al., 1994). However, at the higher resolution achieved in this study, the S-shaped arrangement displays a clear asymmetry for each individual picture (Figure 3C).

**Discussion**

In this work, we purified and analyzed in detail the tubular membranes of *Rb.sphaeroides* induced by the absence of LH2 complexes. These membranes contain all the proteins necessary for photosynthetic activity: RC, LH1 and bc₁ complexes. We found a bc₁ complex to RC ratio of 0.5, and 24–25 Bchl molecules per RC. The organization of the photosynthetic unit was analyzed by electron microscopy, taking advantage of the 2D crystal organization of the native membranes. The excellent quality of these tubular membranes enabled us to record low dose images of negative stained specimens and to resolve, for the first time, the supramolecular organization of the complete photosynthetic apparatus in a native membrane. The 20 Å projection map revealed that the membrane proteins are arranged as two connected C-shaped structures enclosing a large protein mass. Taking into account the composition and stoichiometry of the membrane complexes in the tubular membranes, the following interpretation of the projection map shown in
Fig. 4. (A) Diffraction properties of negatively stained native tubular membranes from *Rb. sphaeroides*. Plot showing the Fourier transform of five negatively stained images after processing and merging. The size of the circles indicates the IQ value. The two dotted rings are at radii corresponding to 1/40 and 1/20 Å⁻¹. (B) Projection map at 20 Å resolution after processing and averaging of negatively stained native tubular flat membrane from *Rb. sphaeroides*. The unit cell (a = 198 Å, b = 120 Å and γ = 103°) is outlined in black. Positive density representing the protein is shown as solid lines and negative density as dotted lines.

Figure 4 is proposed. Each C-shaped structure in the projection map corresponds to LH1 complexes. This attribution agrees with the 8.5 Å projection map of the LH1 complex of *Rs. rubrum* (Karrasch et al., 1995) which revealed a circular arrangement of 16 transmembrane αβ subunits in a 116 Å diameter ring with a 68 Å hole in the center, similar in size to that observed in our projection map. An important observation is the C-shape of the LH1 complex in the tubular membrane, as opposed to the closed ring observed for the reconstituted LH1 complexes of *Rs. rubrum*. Given that the 16 αβ subunits present in the closed ring of *Rs. rubrum* LH1 correspond to 32 molecules of Bchl, and that we measured ~24 Bchl molecules per RC in the tubular membrane, we deduce that the C-shaped structure should contain ~12 αβ subunits. The superimposition of 3/4 of the projected structure of the closed ring of LH1 complexes of *Rs. rubrum* with our projection map, shown in Figure 5, supports this stoichiometry. The protein density observed within each LH1 C-shaped structure can be attributed to one RC, in agreement with previous observations in other species (Miller, 1982; Stark et al., 1984; Engelhardt et al., 1986; Boonstra et al., 1994; Ikeda-Yamasaki et al., 1998). This protein density is well resolved, suggesting that the RC can occupy a single well-defined position with respect to the LH1. The remaining density, localized between the two C-shaped structures, can tentatively be attributed to the *bc₁* complex. This accounts for both the presence of the *bc₁* complex in the ratio of 1:2 RCs, and for the asymmetry of the unit cell. However, at the present stage it is difficult to localize the *bc₁* complex precisely in this negative-stain projection map. Because the stain does not penetrate the membrane, the density contribution in the projection map arises solely from the cytoplasmic surface of the flattened tube. The contribution of the *bc₁* complex in the projection map is thus expected to be very weak because most of the extramembranous parts of this complex (Rieske protein and cyt c₁) are located in the internal membrane of the tube and do not contribute significantly to the projection map.

Fig. 5. Proposed model of the photosynthetic unit of *Rb. sphaeroides* viewed from above the membrane. The projection structure of the RC of *Rb. sphaeroides* (Allen et al., 1987; Ermel et al., 1994) and the projection map of 3/4 of LH1 of *Rs. rubrum* (Karrasch et al., 1995) are shown in red and green, respectively.

The supramolecular organization of the photosynthetic components observed in this electron microscopic study
agrees with the suggestion, based on diverse functional evidence, that the photosynthetic electron chain of *Rb.sphaeroides* is organized in supercomplexes that include two RCs and one bc₁ complex as membrane partners (Joliot et al., 1989, 1996, 1997; Verméglio et al., 1993). The observation that only one cyt c₂ can bind to two RCs even when an excess of cyt c₂ is present in the periplasmic space was an important argument in favor of a dimeric association of the RCs in the supercomplex of *Rb.sphaeroides* (Verméglio et al., 1993). Similarly, analysis of flash-induced absorbance changes on intact cells of *Rs.rubrum* has shown that the anticooperative interactions between the two cyt c₂-binding sites prevent the simultaneous binding of two cyt c₂ molecules on the same dimer of RCs (Joliot et al., 1990). In the case of *Rb.sphaeroides*, this behavior can be tentatively explained by postulating that the RCs in the supercomplex are positioned so that their M-subunits, and hence the interaction site between the cyt c₂ and the RC (Tiede et al., 1993; Adir et al., 1996) are close together. This positioning of the RCs would also allow an efficient exchange of quinone molecules between the RC and the bc₁ complex (see below). A tentative match between our density map and a projection map derived from the coordinates of the *Rb.sphaeroides* RC determined by X-ray crystallography (Allen et al., 1986; Ermler et al., 1994) where the M-subunit and hence the secondary electron acceptor (Q₉) sites of both RCs are facing the open side of the two LH1 rings in the pseudo-dimeric association, is depicted in Figure 5.

It must be emphasized that the functional evidence for an organization in supercomplexes of the photosynthetic electron carriers was not dependent on the LH2:LC1 ratio (Verméglio et al., 1993; Sabaty et al., 1994). This suggests that the supercomplex organization observed in the tubular membrane is also present in the invaginated membrane. The presence of LH2 complexes in non-stoichiometric amounts prevents a crystalline arrangement of the photosynthetic components and leads to the formation of the chromatophores. On the other hand, in the absence of LH2 the formation of the tubular membrane is induced by the crystallization of the membrane proteins present in a stoichiometric ratios. Finally, it is noteworthy that the dimeric association of the RCs and LH1 complexes reported here is also consistent with the recent observation that dimeric cores composed of two RCs, two PuF polypeptides and ~27 LH1 subunits can be isolated from wild-type *Rb.sphaeroides* membranes after gentle solubilization (Francia et al., 1998). From the functional results obtained with *Rs.rubrum* intact cells (Joliot et al., 1990), we postulate that a similar dimeric association of RCs is also present in this species, although further supporting structural and biochemical evidence is required. Finally, it is also interesting to note that PSI1 RCs, which are highly homologous to the purple bacteria RCs, have been suggested to present a dimeric association in vivo where the D1/D2 heterodimers are in close contact and surrounded by LHC (Rögnér et al., 1996; Hankamer et al., 1997).

Besides the structural evidence for a supercomplex organization of the photosynthetic chain of *Rb.sphaeroides*, other important information can be drawn from our electron microscopy study. One striking feature is the organization of the LH1 complexes in C-shaped structures. This finding contrasts with previous proposals based on image processing and analyses of electron microscopy pictures recorded for both highly organized native membranes and purified LH1–RC and LH1 complexes. The picture that emerges from these various studies is that the LH1 complexes form a closed ring surrounding the RC when present. In the work of Karrasch et al. (1995), it should be noted that the LH1 complexes of *Rs.rubrum* were dissociated into αβ dimers prior to crystallization, and therefore the closed ring structure occurring in the reconstituted system gives no direct information on the arrangement in the native membrane. Similarly, the formation of a closed ring in the isolated LH1–RC complexes of other species may be induced during solubilization, as discussed by Francia et al. (1998). When the dimeric arrangement is disrupted by solubilization, a closed ring of LH1 completely encircling the RC may well be more stable than a C-shaped structure. In the case of *Rps.viridis*, the circular appearance of the LH1 ring could be an artifact caused by averaging. Nevertheless, at the present stage, it is difficult to know how far the organization in C-shaped structures of LH1 observed in *Rb.sphaeroides* can be generalized to other species of purple bacteria, in particular to those containing a low bc₁:RC ratio and (or) possessing no PuF-like polypeptide.

Whatever the final answer to this question is, organization in C-shaped structures of the LH1 complexes of *Rb.sphaeroides* in native membrane has important functional and structural implications. This geometry would facilitate diffusion of quinone molecules between RCs and the bc₁ complex, especially if the RCs are localized so that the Q₉ sites, located on the M subunit, face the open side of the LH1 ring. Organization of LH1 complexes in C-shaped structures may also account for certain spectroscopic observations. Inhomogeneous absorption of LH1 complexes has been demonstrated by different approaches. Approximately 10% of the Bchl molecules of the LH1 complexes absorb at a long wavelength (Bchl 896) (Kramer et al., 1984) and present a high value (0.25) of time-independent anisotropy (van Grondelle et al., 1987, 1994; Hunter et al., 1989). This minor fraction of the Bchl molecules was suggested to act as a trap for the excitation energy (van Grondelle et al., 1994). One possible interpretation is that the two Bchl molecules localized at the extremities of the C-shaped structure correspond to this long wavelength form of LH1 and are a privileged site of energy transfer between the LH1 complexes and the RC.

Several lines of evidence favor a model in which the formation of the C-shaped structures of LH1 we observed are related to the presence of the PuF polypeptide. The PuF protein is a small hydrophobic polypeptide found so far only in *Rb.capsulatus* and *Rb.sphaeroides*. The presence of this polypeptide is essential for photosynthetic growth (Farchaus et al., 1990; Lilburn et al., 1992) and facilitates the exchange of quinone molecules between the Q₁ site of the bc₁ complex and the Q₉ site of the RC (Farchaus et al., 1990; Lilburn et al., 1992; Barz et al., 1995a,b). The evidence for its involvement in the formation of C-shaped structures of LH1 can be summarized as follows: (i) deletion of the puF gene induces a significant increase in the ratio of Bchl molecules to RC in both *Rb.capsulatus* and *Rb.sphaeroides* species (Farchaus et al.,

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involved in supercomplex formation. It will also be important to determine, by detailed electron microscopic studies of native membranes of several other species of photosynthetic bacteria, whether the organization in C-shaped structures of the LH1 complexes of Rb. sphaeroides is restricted to this species, or is a more general feature allowing an efficient diffusion of quinone molecules between the RCs and the bc$_1$ complex.

Materials and methods

Growth of Rb. sphaeroides

Rb. sphaeroides forma sp. denitrificans was grown under anaerobic heterotrophic conditions using Hutner medium in the presence of 200 mM potassium nitrate as described previously (Sabaty et al., 1994). Mutant RCLH10 of Rb. sphaeroides deleted in LH2 complexes was grown photosynthetically as described by Hunter et al. (1988). In both cases, cells were harvested in the late exponential phase and washed with 50 mM Tris–HCl pH 8, 1 mM 4-(2-aminoethyl)-benzenesulfonyl fluoride (AEBSF).

Purification of the tubular membranes

The photoheterotrophically grown cells were suspended in 50 mM Tris–HCl pH 8, 1 mM AEBSF, 15% (w/v) sucrose, 1 mg/ml lysozyme and 2 mM EDTA. After 30 min incubation at 30°C, a few crystals of DNase I and MgCl$_2$ were added and spheroplasts were disrupted by dilution in 50 mM Tris–HCl pH 8, 1 mM AEBSF and the intact spheroplasts and cells spun down at 4000 g. The tubular membranes were recovered in the supernatants of successive spins at 5000–20 000 g. In a final step, the supernatant was dialyzed against 50 mM Tris–HCl pH 8, 1 mM AEBSF, deposited on a 20–40% sucrose-step gradient and spun at 20 000 g for 10 min (Beckman, model L-8-80M, SW41 rotor). The purified tubular membranes were recovered in the pellet.

Analytical methods

Gel electrophoresis. Polypeptide contents of purified membranes were determined according to Laemmli (1970), except that sodium dodecyl sulfate (SDS) was omitted from the gel and from the lower electrode buffer. Membrane proteins were first reduced by 2% β-mercaptoethanol and then denatured at 50°C for 10 min in the presence of 2% SDS (Ljungdahl et al., 1987). The solubilized samples then underwent polyacrylamide gel electrophoresis with a Mini Protean II apparatus (Bio-Rad). The resolving gel containing 15% acrylamide was stained with Coomassie Blue.

Absorption spectroscopy. Room temperature absorption spectra were recorded on an Hitachi U-2000 spectrophotometer. Low temperature spectra (77 K) were collected using a Varian Cary E5 double-beam scanning spectrophotometer equipped with a flow cryostat (Air Liquide, Grenoble) cooled with liquid nitrogen. The samples were oxidized with potassium ferricyanide (25 mM), and reduced with potassium dithionite (10 mM) or sodium ascorbate (10 mM).

Determination of RC concentration. RC concentrations were calculated from light-induced measurements performed at 603 and 542 nm with a laboratory-built apparatus (Joliot et al., 1989) assuming a Δε$_{603,542}$ = 30/mM/cm (Dutton et al., 1975).

Determination of the bc$_1$ concentration. The bc$_1$ complex concentration was determined by dithionite-reduced minus ascorbate-reduced difference spectra recorded in the 530–580 nm range and assuming a Δε$_{560,574}$ of 28/mM/cm (Dutton et al., 1975).

Determination of the Bchl concentration. The Bchl molecules were extracted from the membranes using the method described by van der Rest and Kingras (1974). A suspension of purified tubes (2 ml, Δε$_{775}$ = 1.5–2) was lyophilized and extracted three times with small volumes of an acetone-methanol mixture (7:2 v/v). The pooled extracts were spun for 5 min at 4000 g. The absorption of the supernatant, adjusted to 10 ml, was measured at 771 nm to determine the concentration of Bchl using the method described by Clayton (1963).

Electron microscopy and image processing

Freeze-drying. After rapid cryofixation in liquid nitrogen, the quenched sample was mounted in a Cryofrac (Reichert-Jung) and kept at −170°C under a vacuum <10$^{-5}$ Torr to avoid contamination. Sublimation was achieved by slowly increasing the sample temperature to ~80°C over 2 days (Chen et al., 1994).
~2 h. To avoid any contamination of the surface and improve the local vacuum, the sample was protected by a cold shroud during etching. The replica surface exposed by etching was obtained by evaporation of platinum-carbon at a low incident angle (20°). To strengthen the replica, a 10–20 nm thick layer of carbon was evaporated onto the surface at an incident angle of 90°. Following the cleaning, the replicas were examined under a Philips CM 120 electron microscope.

Negative staining. Samples negatively stained with 1% (w/w) uranyl acetate were examined under a Philips CM120 electron microscope operating at 120 kV. Low-dose electron micrographs were recorded at a magnification of 45 000 on Kodak SO163 film developed in full-strength Kodak D-19 developer.

Image analysis. Micrographs were evaluated for focus, astigmatism and drift by optical diffraction. The selected area of the best images was digitized on a Leafscan 45S CCD-array microdensitometer (Leaf System, Inc.). The sampling was 0.4 nm on the specimen scale. Initial rectangular areas were padded up to 1024 or 2048 pixel squares. Lattice parameters were first determined using Spectra (Schmid et al., 1993), then further processed using the MRC image analysis package (Crowther et al., 1996). The projection map was calculated after merging data for several tubular membranes.

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