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The reaction centre of the photounit of *Rhodospirillum rubrum* is anchored to the light-harvesting complex with four-fold rotational disorder

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Abstract

The minimal photounit of the photosynthetic membranes of the purple non-sulphur bacterium *Rhodospirillum rubrum*, comprising the reaction centre and the light-harvesting complex has been purified and crystallised in two dimensions in the presence of added phospholipids, and subsequently visualised by electron microscopy after negatively-staining. The position of the reaction centres within the light-harvesting ring has been determined at low resolution by the application of a new analysis for rotationally disordered identical units (here the reaction centres) within a two-dimensional crystalline lattice comprised of perfectly aligned unit cells (here the light-harvesting complexes). The reaction centre was found to preferentially occupy one of four orientations within the light-harvesting complex. The light-harvesting complex appears to be distorted to C_4 symmetry, thus assuming a squarish shape when visualised by negative staining. A tentative structural model of the reaction centre-light-harvesting complex photounit which fits the experimental data is proposed.

Abbreviations: 2D – two-dimensional; BPh – bacteriopheophytin; βOG – β-octyl-D-glucoside; DHPC – 1,2-diheptanoyl-*sn*-phosphatidylcholine; DOPC – 1,2-dioleoyl-*sn*-phosphatidylcholine; LHI – light-harvesting complex I; RC – reaction centre; RC-LHI – reaction centre associated with the light-harvesting complex I; TEM – transmission electron microscopy

Introduction

The elucidation of the mechanisms of light energy capture and utilisation are of great interest at the present time. All photosynthetic organisms possess specific protein structures, the light-harvesting (LH) complexes for efficiently collecting light-energy and transferring it to the reaction centres for further utilisation. In photosynthetic purple non-sulphur and sulphur bacteria, at least 2 LH complexes are observed, characterised by their near-IR absorption maxima at 880 nm (B880 or LHI), and 800–850 nm (B850 or LHII). In both cases the complexes are composed of non-identical polypeptides, α and β, which bind the two major photosynthetic pigments, bacteriochlorophyll and carotenoids.

For the LHII complexes from the purple bacteria *Rhodopseudomonas acidophila* and *Rhodospirillum molischianum*, respectively, two structures have been solved in three dimensions at atomic resolution and have been shown to be built from circularly interacting dimers of the basic $\alpha\beta$ unit, with ring sizes of 9 and 8 $\alpha\beta$ dimers respectively (McDermott et al. 1995; Koepke et al. 1996). For the LHI complex from *Rho-*

dospirillum rubrum, an 0.85 nm projection map has been obtained by cryoelectron microscopy which revealed once again a circular arrangement of dimeric transmembrane subunits, presumably $\alpha\beta$ dimers, but this time with 16 $\alpha\beta$ dimers as the oligomeric unit (Karrasch et al. 1995). Although the ring-like arrangement of LHI oligomers agreed with early studies using negatively-stained 2D LHI crystals (Meckenstock et al. 1992; Ghosh et al. 1993), 12 $\alpha\beta$ subunits were determined to be present in these preparations. The lower determination of the number of $\alpha\beta$ oligomers in these latter studies is now thought to lie with the lower resolution obtained, leading to the dominance of pseudo-6-fold symmetry in the diffraction patterns. Recently, 2D crystals have also been obtained from a reaction centre-light-harvesting photounit (RC-LHI) from a carotenoid-less mutant of *R. rubrum* which showed the same geometrical arrangement of ring subunits, this time surrounding a central diffuse projection, assigned to the reaction centre (Walz et al. 1997). Although only a single reaction centre was assigned per LHI ring, this latter study was unable to resolve the reaction centre unambiguously. In contrast to the structural data, kinetic studies have sometimes indicated that two reaction centres per photounit may be interacting (Joliot et al. 1990). A further open question, not yet resolved, concerns the mechanism of transfer from a membrane-bound quinone molecule across an apparently closed LHI ring.

In this study, we have employed a new rotational analysis of 2D crystals of the RC-LHI photounit from *R. rubrum* and have determined with reasonable certainty the stoichiometry of the reaction centre to the LHI ring. In addition, we have determined that the LHI ring of the carotenoid-less mutant appears to be squarish rather than round, which may indicate the presence of a protein component in addition to the α and β polypeptides. It is tempting to assume that this component may play a role in quinone transfer. A protein candidate for this third component, the Ω polypeptide, detected in the random carotenoid-less mutant *R. rubrum* G9+ (Ghosh et al. 1994) is shown here to be also present in the Tn5-induced carotenoid-less mutant ST2 (Wiggli et al. 1996).

Materials and methods

Chemicals

All chemicals were of the highest purity and in general were obtained from Fluka Chemie AG (Buchs, Switzerland). Exceptions were: 1,2-diheptanoyl-*sn*-phosphatidylcholine (DHPC) and 1,2-dioleoyl-*sn*-phosphatidylcholine (DOPC) (Avanti Polar Lipids, Alabaster, Alabama, USA) and β-octyl-D-glucoside (βOG) (BACHEM, Bubendorf, Switzerland).

Preparation of purified RC-LHI complexes

The carotenoid-less Tn5 mutant *R. rubrum* ST2 (Wiggli et al. 1996) was grown in 20 l bottles in dim light and under anaerobic heterotrophic conditions using Sistrom medium A (Sistrom 1977) as described (Saegesser et al. 1992) and harvested in the late exponential phase. Chromatophore membranes were prepared as described (Wiggli et al. 1996). The purification of the RC-LHI complexes and the preparation of single detergent-free RC-LHI complexes was performed as described (Stahlberg et al. 1997).

2D crystallisation

2D membrane crystals were grown with the dialysis method (Jap et al. 1992) as described (Stahlberg et al. 1997). In short: 200 μg of 1,2-diheptyl-*sn*-phosphatidylcholine (DHPC)-solubilised and purified RC-LHI photounits plus the phospholipid 1,2-dioleoyl-*sn*-phosphatidylcholine (DOPC) at a molar ratio of about 1:1 (DOPC:LHI) were adjusted to 100 μl volume with 10 mM TrisHCl pH 8.0, containing 200 mM NaCl and 2 mM $MgCl_2$. Dialysis was performed for 6 days at 8 °C in microdialysis setups against 2.4 ml of 50 mM NH_4HCO_3 pH 7.9 containing 200 mM NaCl and 10 mM $MgCl_2$ (Buffer A) and containing 0.8% (w/v) βOG. A second dialysis of 7 days followed against 2.4 ml Buffer A at 8 °C.

SMART® (Pharmacia) chromatography of isolated LHI complexes

SMART® (Pharmacia) chromatography of isolated LHI complexes was performed using the same conditions as described for FPLC® (Pharmacia) chromatography in Ghosh et al. (1994). Selective fractions from the SMART® elution profiles were analysed for the amino acid composition as described in Ghosh et al. (1994).

Transmission electron microscopy

Negatively stained grids for transmission electron microscopy (TEM) were prepared within 4 h after sample collection as described (Stahlberg et al. 1997). TEM was carried out with a Philips CM12, operated at 80 kV. Images were recorded under low-dose conditions at nominal magnifications of 45 000× on Kodak SO-169 plates, which were developed in D19 standard developer for 10 min. The electron dose for acquiring an image was between 5 and 10 electrons/Å^2.

Image treatment

Micrographs were examined with an optical diffractometer (Aebi et al. 1973) and suitable areas were digitised using an Eikonix 850 CCD imaging camera. The pixel size corresponded to 0.36 nm in the specimen plane. Images of 2D crystals were treated using the MRC-programs developed by R. Henderson et al. (1986, 1990).

Within the images of negatively stained 2D crystal the differing orientations of the RCs in each LHI ring were corrected with the program SPIDER (Frank et al. 1985; Radermacher 1988) by rotational alignment as described (Stahlberg et al. 1997).

Results and discussion

We have recently developed a new rotational analysis (Stahlberg et al. 1997) for 2D crystals containing identical rotationally disordered units (here the reaction centre) placed within a perfect crystal lattice (here the LHI complex). This analysis utilises the program SPIDER, which was originally developed for single particle analysis (Frank et al. 1985; Radermacher 1988). Figure 1a shows the result of a typical rotational analysis performed for 2D crystals of the RC-LHI complex from *R. rubrum* and indicates that the reaction centre, which is present in the centre of the LHI ring (Figure 1b), can be localised and appears to assume only 4 orientations in the crystal lattice. Full details of this rotational treatment have been presented elsewhere (Stahlberg et al. 1997). The superposition of 3900 unit cells allowed a projection of the reaction centre to be visualised at low resolution (1.6 nm) and a montage of this analysis is shown in Figure 1b. Conveniently, neighbouring LHI rings are packed with the cytoplasmic and periplasmic faces shown alternately, thus allowing both faces of the reaction centre to be obtained. As the RC from *R. rubrum* contains only a tightly-bound H-subunit protruding into the cytoplasmic compartment and no tightly-bound cytochrome, we interpret the stain-excluding regions (shown as bright regions of electron density) and the stain-accumulating regions (shown as darker regions of electron density) to correspond to the cytoplasmic and periplasmic faces, respectively. Computer-aided processing of the 2D crystal-images of the total lattice using the MRC program suite (Henderson et al. 1986, 1990) revealed the projection map shown as an insert in Figure 1b. This map is essentially identical to images obtained from naturally occurring 2D RC-LHI crystals from other organisms (Engelhardt et al. 1986; Miller, 1982; Stark et al. 1984), differing slightly in the dimensions of the unit cell. Finally, the projection of the LHI complex appears to be squarish, in contrast to the circular projection recently obtained by Karrasch et al. (1995) from 2D crystals of the purified wild-type LHI complex from *R. rubrum* (Figure 1c). This unexpected geometry has also been observed

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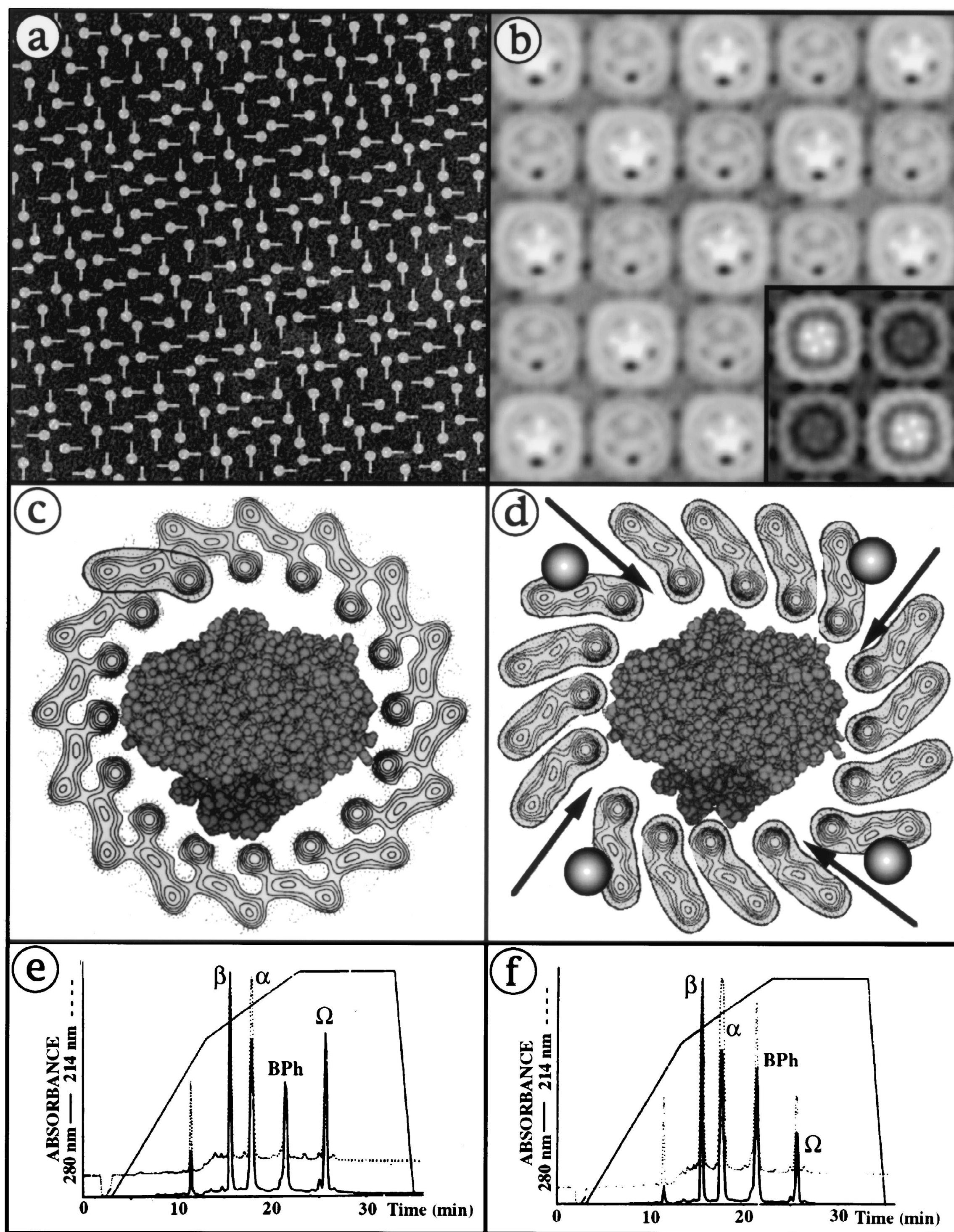
Figure 1. (a) The angle distribution of the RCs found in a 2D crystal of RC-LHI complexes is shown. Four allowed orientations separated by 90° are indicated by arrows; (b) A collage of the image treatment results obtained by rotational analysis of the reaction centre units is shown. The images have been corrected for the four allowed orientations of the cytoplasmic and periplasmic sides of the RC-LHI units. The inset is the RC-LHI structure as reconstructed with the so-called MRC program suite without rotational correction for the RC orientations. Lattice dimensions are a=b=16.65 nm ± 0.1 nm, which corresponds to a mean diameter of a photounit of 11.77 nm ± 0.07 nm. For details see (Stahlberg et al. 1997); (c) A model of the RC-LHI complex, based upon the atomic coordinates of the RC of *Rhodobacter sphaeroides* (Allen et al. 1987) and the projection map of the LHI complexes from the wild-type of *R. rubrum,* obtained by cryoelectron microscopy (Karrasch et al. 1995); (d) A speculative model of the RC-LHI complex combining the model in (c) with the experimentally obtained projections obtained here as shown in (b). In this model four putative additional transmembrane polypeptides (shown in projection as balls), possibly the polypeptide Ω, have been placed at the edge of the ring to generate four 'corners'; (e) Elution profile obtained by SMART® (Pharmacia) chromatography of highly purified LHI complexes from the Tn5 carotenoid-less mutant *R. rubrum* ST2 using a PepRPC column. The conditions for the chromatography run were identical to those described previously for the analogous PepRPC column used for FPLC® (Pharmacia) chromatography of the same complexes (Ghosh et al. 1994). The positions of the components α, β, Ω and bacteriopheophytin (BPh) (derived from the acidification of bacteriochlorophyll a by the running solvent) are indicated. The indicated elution gradient ranged from pure buffer A (0.2% trifluoraceticacid (TFA) in water) to pure buffer B (0.2% TFA in acetonitrile/isopropanol 1:1 (v/v)); (f) Elution profile obtained from purified LHI complexes from the random nitrosoguanidine-induced mutant *R. rubrum* G9+.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by electron microscopy of single RC-LHI particles (Stahlberg et al. 1997). An interpretation of the C_4 symmetry might be that an additional protein component, possibly a single transmembrane α-helix which can bind negative stain, is present at the 'corners' of the LHI ring. Our interpretation of the present structural data is shown in Figure 1d.

A candidate for the additional protein component may be the polypeptide Ω (Ghosh et al. 1994). The Ω polypeptide is a highly hydrophobic peptide of molecular weight 4 kDa containing about 28 amino acids and is found in a molar stoichiometric ratio to the α and β LHI polypeptides of approximately 1 Ω/10 $\alpha\beta$ in purified LHI complexes from the carotenoid-less mutant *R. rubrum* G9+ (Ghosh et al. 1994). However, in the latter study the interpretation of the data was complicated by the fact that the carotenoid-less mutant *R. rubrum* G9+, which was obtained using random nitrosoguanidine mutagenesis, may contain polypeptide fragments generated by the creation of stop codons, possibly leading to the production of small polypeptides which copurify with the LHI complex. However, SMART® chromatography profiles of purified LHI complexes from the Tn5-directed mutant *R. rubrum* ST2, which contains only a single chromosomal lesion (Wiggli et al. 1996) also shows the presence of the same polypeptide (Figure 1d) as observed for *R. rubrum* G9+ (Figure 1e) and in the same molar ratio as determined by amino acid analysis (data not shown). We have also observed the Ω polypeptide in SMART® profiles of LHI complexes obtained from the wild-type organism (data not shown). Although we have not yet been successful in microsequencing the Ω polypeptide, due to high hydrophobicity and N-terminal blockage, the SMART® data are nevertheless suggestive that it is not artefactual.

The present work suggests the following conclusions for the RC-LHI complex from *R. rubrum*: (1) The LHI ring from the carotenoid-less mutant contains 16 subunits, as was found for the wild-type complex (Karrasch et al. 1995); (2) the LHI ring surrounds a single reaction centre; (3) the LHI ring from the carotenoid-less complex is closed and appears to have a squarish shape, possibly due to the presence of another protein component in addition to the α and β polypeptides; (4) a possible candidate for this additional component, the hydrophobic polypeptide Ω (Ghosh et al. 1994) has also been shown to be present in highly purified LHI preparations of the Tn5-directed carotenoid-less mutant *R. rubrum*, as well in those obtained from the wild-type carotenoid-containing organism.

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