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The light-harvesting core-complex and the B820-subunit from *Rhodopseudomonas marina*. Part II. Electron microscopic characterisation

Rainer U. Meckenstock[a], Katarina Krusche[b], René A. Brunisholz[a] and Herbert Zuber[a]

[a]*Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, CH-8093 Zürich, Switzerland* and [b]*Institut für Zellbiologie, ETH-Hönggerberg, CH-8093 Zürich, Switzerland*

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Electron micrographs of photosynthetic membranes of the BChla-containing bacterium *Rp. marina* showed a quasi-crystalline structure. The photoreceptor units are arranged in a hexagonal lattice with a reaction center to reaction center distance of 102±3 Å. Purified B880-complex was concentrated up to an OD_{880} of 60 which induced the formation of large protein vesicles. The protein complexes within these vesicles were highly ordered and showed a hexagonal lattice with the same center to center distance of 102±3 Å as was observed in the native membranes. Image processing of the micrographs revealed a ring-like structure of the B880-complex at 26 Å resolution and suggests that the B880-complex consists of 5 or 6 subunits. For the first time it can be shown that an isolated core-complex is in a stable, ring-like structure even without the reaction center which is supposed to be located in the middle of the B880-ring. The data indicate that the isolated B880-complex exhibits the same structure as in the native membrane.

Rhodopseudomonas viridis; Quasi crystalline membrane; Ring-like structure; 2D crystallisation

1. INTRODUCTION

Besides the biochemical and the biophysical attempts (see Part I), electron microscopy has been the most successful method to receive information about the structure of the antenna systems of non-sulfur purple bacteria. It has been shown in the case of a number of BChlb-containing *Rhodospirillaceae* that the photosynthetic reaction center (RC) plus the core-complex, called a photoreceptor unit, form large quasi crystalline areas with hexagonal arrangement [1,2]. Electron micrographs of metal shadowed photosynthetic membranes of *Rp. viridis* showed that the core-complex surrounds the RC in a ring like structure and seems to consist of six subunits [3,4]. The RC-to-RC distance was around 130 Å.

Even though the photosynthetic membranes of the BChlb-containing bacteria show the quasi-crystalline structure of the photoreceptor units, among the BChla-containing bacteria it has only been reported from *Rhodospirillum rubrum* to have the reaction centers arranged in a hexagonal cluster of 10 to 20 units which were not highly ordered [5].

Here we report for the first time that BChla-containing bacteria can exhibit the same quasi-crystalline structure of the photosynthetic membrane as BChlb-containing bacteria do.

Correspondence address: H. Zuber, Institut für Molekularbiologie und Biophysik, ETH-Hönggerberg, CH-8093 Zürich, Switzerland.

Abbreviations: RC, reaction center; B880: Core-antenna complex with an absorption maximum at 880 nm; BChl, bacteriochlorophyll; *Rp.*, *Rhodopseudomonas*; OD, optical density.

2. MATERIALS AND METHODS

2.1. *Preparation of the specimens*

Rhodopseudomonas marina DSM strain 2698 was purchased from the German collection of micro-organisms (Göttingen) [6]. Growth of the cells and purification of the B880-complex and of the photosynthetic membranes was performed as described in Part I.

The purified B880-complex was stored in 5 mM $KHPO_4$, 0.125% LDAO at 4°C and in solution showing an optical density at 880 nm of 60.

Photosynthetic membranes and the B880-complexes were visualised with transmission electron microscopic (TEM) methods. In order to obtain surface information, crude photosynthetic membranes were freeze-dried and heavy metal shadowed. To achieve best preserved structures, the excess of solution remaining on the grid after the washing procedure, was withdrawn by touching the grid against a piece of damp filter paper until leaving just a small film. The samples were then immediately frozen by dipping them into liquid nitrogen. Freeze-drying took place in a Balzer 400 apparatus equipped with a counter-flow loading device, at a temperature of −80°C at 10^{-7} mbar during 3 h. The specimens were unidirectionally shadowed with tantalum/tungsten at an angle of 30° and covered with a thin carbon layer for protection against deterioration. The amount of tantalum/tungsten being evaporated on the specimen was controlled by a quartz monitor.

The B880-vesicles were negatively stained with 1% uranyl acetate.

2.2. *Electron microscopy*

The specimens were examined with a Philips CM12 transmission electron microscope operating at 100 keV. The images were taken at a nominal magnification of 45,000 with a defocus of 450–550 nm and were recorded on Agfa-Gevaert Scientia films.

2.3. *Image processing*

Selected areas of the micrographs were digitised with a 8-bit resolu-

tion on a rotating drum scanner (Optronics Inc. Photoscan system P-1000 HS). The pinel size corresponding to the specimen scale was 4.1 Å. As the specimen provided a quasi-crystalline structure correlation averaging could be performed in order to enhance the signal to noise ratio of the images [7].

The lattice constant was evaluated in both cases (native membranes and B880-vesicles) from the spacing of the diffraction spots visible in the power spectra of the Fourier transform of the image.

3. RESULTS

3.1. *The photosynthetic membranes*

Micrographs of the unidirectional shadowed photosynthetic membranes of *Rp. marina* show large membrane vesicles with a size between 1 and 2 μm (Fig. 1). On the surface there are particles visible which are arranged in a quasi-crystalline structure. These are probably the photosynthetic reaction centers (RC) which extend the membrane. Within the quasi-crystalline structure 500–1000 molecules are arranged in highly ordered clusters. The RC-to-RC distance is 102±3 Å.

The space between the reaction centers which should be occupied by the B880-antenna system shows no surface structures indicating that the intrinsic antenna-protein complex does not extend the membrane surface.

3.2. *Aggregation of the B880-complex*

Upon concentrating of the purified B880-complex the proteins aggregated to large vesicles with up to 0.6 μm in diameter (Fig. 2a). Furthermore there are monolayer sheets of B880-complexes. These sheets, however, were obviously busted vesicles, as they did not appear in frozen hydrated preparations of the same sample (data not shown).

Fourier transformation of micrographs of the flattened vesicles revealed a good hexagonal crystal structure of the B880-hence with a resolution of 26 Å. Image processing of the best ordered areas showed that the crystals consist of tightly packed rings each of which represents a single B880-molecule (Fig. 2b). The outer diameter of one ring is 102±3 Å, which is identical to the RC-to-RC distance of 102 Å in the native photosynthetic membranes.

The hole in the middle of the ring, which in vivo should be occupied by the reaction center, has a diameter of approximately 60 Å. Although the signal to noise ratio of the processed image is low, it is obvious that the B880-ring is composed of either 5 or 6 subunits.

4. DISCUSSION

In the presented micrographs (Fig. 1) it is shown for the first time that BChla-containing bacteria can build quasi-crystalline structures of their photosynthetic membranes. This property was until now only reported from BChlb-containing bacteria [2]. The main difference between the core antennae of BChlb- and BChla-containing bacteria is, besides the different BChl type, the presence of an additional γ-polypeptide in the BChlb-containing antenna which carries no BChl [8]. As the number of polypeptides per reaction center is 36 in BChlb-containing bacteria [3] compared to 24 in BChla-containing bacteria (see Part 1) the size of the BChlb-containing core-antenna should be larger. This is in good agreement with the RC-to-RC distance of 102 Å for the BChla-containing core-complex of *Rp. marina* compared to the RC-to-RC distance of 130 Å for the BChlb-containing bacterium *Rp. viridis* [3].

The same center to center distance of 102 Å for the native membranes of *Rp. marina* is found in the artificial protein vesicles of the RC less B880-preparation. This indicates that the isolated B880-complex has the same structure as in the native membrane even without the

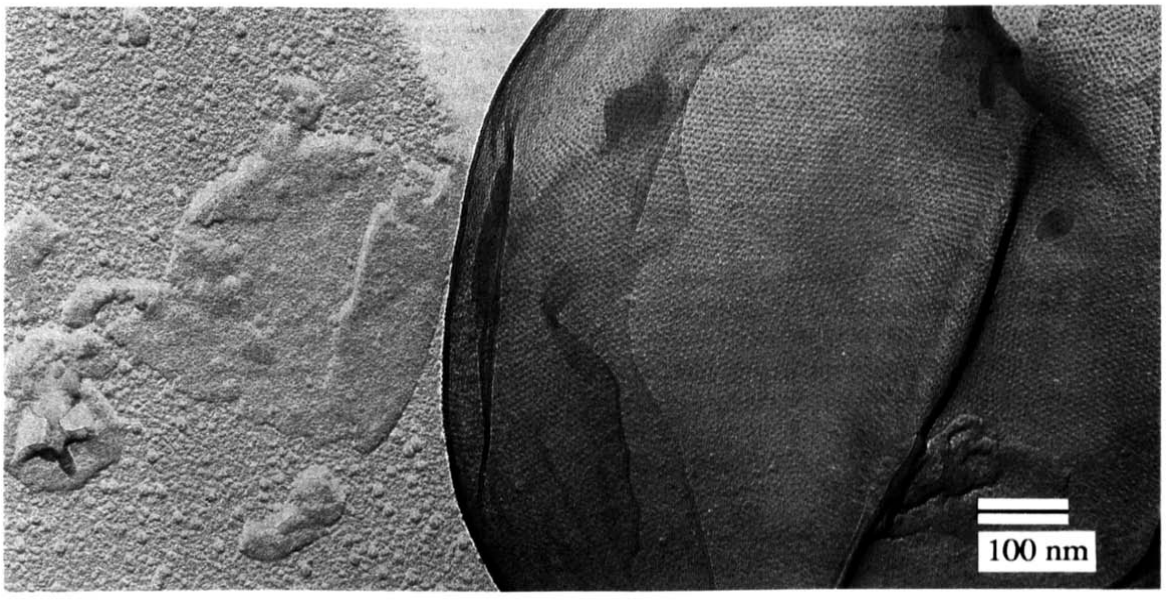

Fig. 1. Unidirectional shadowed photosynthetic membranes of *Rp. marina*.

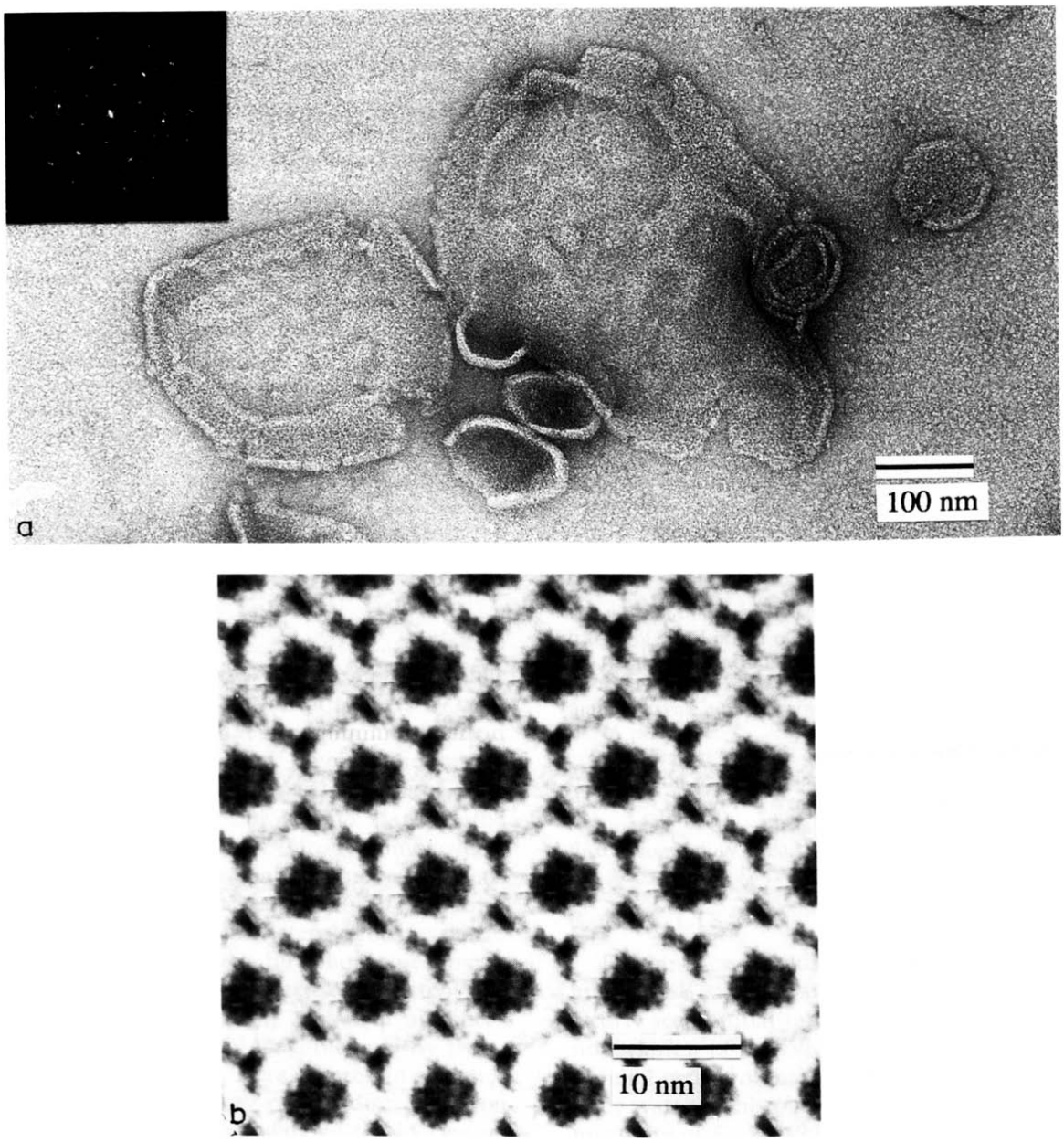

Fig. 2. (a) Artificial protein vesicles of purified B880-complex. The insert shows the Fourier transformed image which indicates a resolution of 26 Å. (b) Image processing of a B880-vesicle with a lattice of 150 B880-molecules.

probably stabilising RC. It is here shown for the first time that an isolated core-complex without RC has the same ring-like structure as in the membrane.

The image processing of the micrographs of the B880-vesicles reveals that the B880-complex consists of several subunits which organise themselves to the ring-like structure. The image processing technique permitting an averaging over a number of single molecules is based on the cross correlation function between the crystal image and a small reference extracted from it. It does not take into account that the single particles could be distorted to each other and therefore the information is blurred. Because of the small signal-to-noise ratio still remaining in the averaged data which is probably due to the imperfect order of the complexes relatively to each other it is not clear whether the B880-complex consists of 5 or 6 subunits. However, the biochemical data (see Part I) and the close evolutionary relationship of *Rp. marina* to *Rp. viridis* where the core-complex consists of 6 subunits [3], suggest that the core-complex of *Rp. marina* also consists of 6 subunits which could be B820-subunits [see part I].

Two-dimensional crystallisation of the B880-complex, which is in progress, should result in better resolutions and enable us to determine the number of subunits per B880-complex. Preliminary results with two dimensional crystals confirm the six-fold symmetry of the B880-complex.

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