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Localisation of reaction centre and light harvesting complexes in the photosynthetic unit of *Rhodopseudomonas viridis*

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Abstract. The photosynthetic unit of *Rhodopseudomonas viridis* contains a reaction centre (P960) and a light harvesting complex (B1015). Immune electron microscopy combined with image processing has allowed the central core of the photosynthetic unit to be identified as the reaction centre and the surrounding protein ring as the light harvesting complex. This light harvesting complex, subdivided into twelve subunits was shown to contain 24 bacteriochlorophyll b molecules. A model is presented which may account for the far red shift of the Qy absorption of the bacteriochlorophyll b molecules in vivo.

Key words: Bacteriochlorophyll b – Immuno-electron microscopy – Light harvesting complex – Pigment-protein complex – *Rhodopseudomonas viridis*

Immuno-electron microscopy has been shown to be a powerful tool for the localisation of polypeptides within membrane systems. In 1977 Aebi et al. showed, that it is possible to improve the relatively poor resolution by avoiding electron dense markers like ferritin or colloidal gold and using image analysis methods to directly visualise bound Fab fragments of antibodies. The nature of the fourier filtering technique, which enhances all the regular features in the picture such as antibody binding sites and eliminates all the irregular parts in the picture, allows it to detect even smaller distances than the actual size of the labelling antibody. The application of this technique to the photosynthetic membrane of *Rhodopseudomonas viridis* seems to be an appropriate method to identify the different pigment-protein complexes in the photosynthetic unit.

Electron microscopy combined with fourier image analysis has shown that the photosynthetic unit consists of a central core which is surrounded by a ring (Stark et al. 1984). This photosynthetic unit only contains seven polypeptides (Jay et al. 1984). The four largest are known to be part of the reaction centre (Thornber et al. 1980), which includes the cytochrome complex. The molecular structure has been reported in several publications by Michel et al. (1983), Michel and Deisenhofer (1986) and Deisenhofer et al. (1984, 1985, 1985b). At present there is, unfortunately, no method available to isolate and purify the light harvesting complex from this organism with a native absorption spectrum. However, it is most likely that all the three low molecular weight polypeptides are part of the light harvesting complex. Antibodies are available to six of the seven polypeptides and have been characterised in studies with either ferritin conjugates or the protein A gold technique, providing information about the lateral distribution of the polypeptides in the membranes (Jay et al. 1983, 1985).

The combination of antibody labelling and the image analysis methodology allowed us to show clearly the arrangement of the reaction centre and the light harvesting complex.

These results, combined with a variety of results found by other authors, are the foundation of the model of the pigments in the light harvesting complex which may be the key to the far red shift in the absorption spectrum. This red shift in the absorption spectrum allows these bacteria to exist at extremely low light intensities.

Materials and methods

The growth conditions of *Rhodopseudomonas viridis* (ATCC 19567) and the isolation of thylakoid membranes and single membrane sheets was carried out according to previously published methods (Stark et al. 1984). The isolation of the photosynthetic units used the procedure of Jay et al. (1984). For the protein determination the method of Lowry et al. (1951) was used. Two different methods were applied for the determination of the bacteriochlorophyll content. One method used the absorption in the near infra red range at about 1010 nm with a millimolar extinction coefficient (ε = 100/cm) which was described by Thornber (1971) for in vivo determination. A second, in vitro, method was reported by Garcia et al. (1968). After extraction of the bacteriochlorophyll with acetone/methanol (7:2) the absorption at 790 nm was measured and the bacteriochlorophyll amount was calculated using the millimolar extinction coefficient (ε = 75/cm) described by Jones and Saunders (1972). The molecular weight used for the calculation of the number of bacteriochlorophyll molecules per photosynthetic unit was estimated to be 430,000.

The preparation and characterisation of the monospecific and monoclonal antibodies and their respective Fab' fragments has been previously described by Jay et al. (1983, 1985). The membranes were labelled with antibodies following the scheme described in Jay et al. (1983) with the exception that the ferritin marker was omitted and higher amounts of antibodies (0.05–0.1 mg/ml) were used in order to obtain maximal binding.

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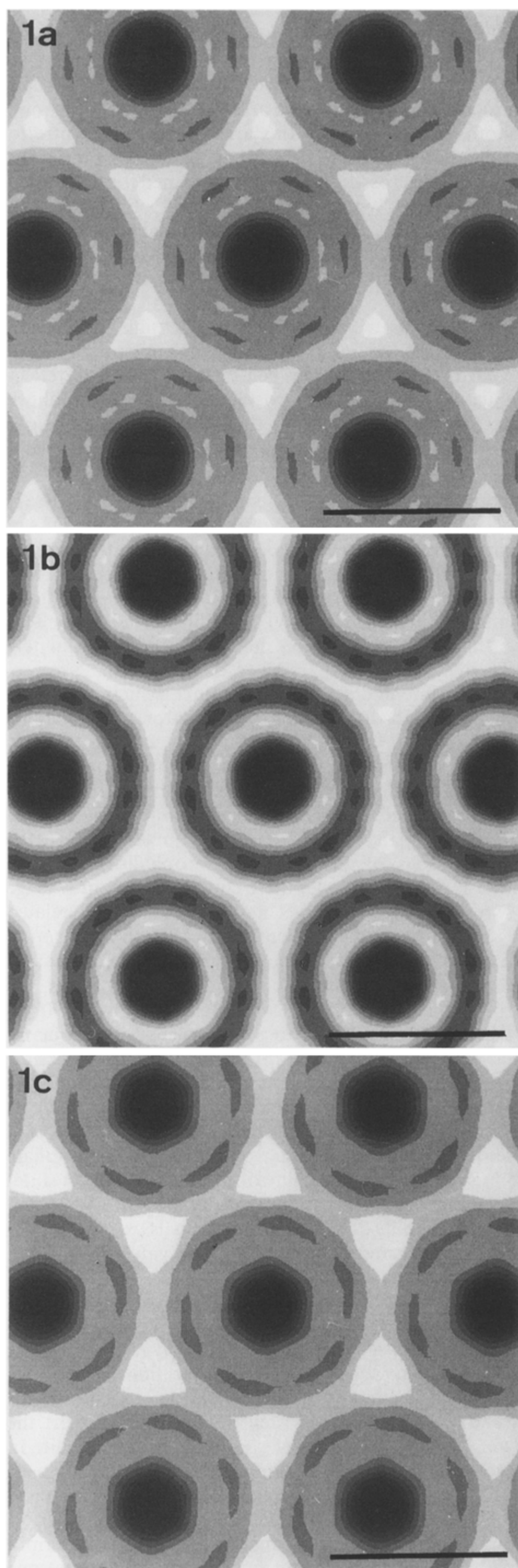

All specimens were contrasted with a 2% uranyl acetate solution and air-dried. The electron microscopic images were recorded and image analysis was carried out as reported by Stark et al. (1984).

Results

The localisation of the reaction centre was carried out using a monospecific antibody directed against the P960 H-polypeptide (the polypeptides are named according to Cogdell et al. 1985) and the filtered image of the labelled single membrane sheets compared with the unlabelled control. The antibody labelled membranes (Fig. 1a) show a relatively large and very prominent central core, when compared with the control (Fig. 1b). The comparatively flat groove between the core and the ring may be caused by the increased mass of the central core and a more diffuse image, while the interunit depressions appear to remain unchanged. The location of the reaction centre is therefore shown to be in the central core of the photosynthetic unit.

For the labelling of the light harvesting complex we used a monoclonal antibody directed against the B1015-α polypeptide. Monoclonal antibodies have an advantage over polyclonal sera in that they only bind to a single antigenic determinant. In this case the result (Fig. 1c) is less prominent than obtained with the reaction centre serum. The filtered image shows a relatively flat environment surrounding the unaltered core. With the exception of the reaction centre core region the membrane appears to be uniformly labelled. The antibody binding sites for the light harvesting polypeptides are therefore thought to be located in the outer ring. However, a definite localisation of the antigens in the ring is not possible due to resolution problems.

Based on the bacteriochlorophyll determination after extraction with organic solvents, the total amount of bacteriochlorophyll b molecules per photosynthetic unit was calculated to be 26.7. Using the in vivo absorption at 1015 nm a light harvesting bacteriochlorophyll per photosynthetic unit ratio of 24.7 was calculated.

Discussion

Labelling single membrane sheets with antibodies specific for the H-chain of the reaction centre followed by image processing, results in an increase in the mass of the central protein core of the photosynthetic unit. The localisation of the H-polypeptide within the central core is in agreement with the X-ray data obtained by Deisenhofer et al. (1984, 1985b) and Michel and Deisenhofer (1986). They indicate an asymmetric lateral distribution of the H-polypeptide on the cytoplasmic membrane surface. In addition, the length of the reaction centre is reported to be 13 nm (Deisenhofer et al. 1985b) which can only be accommodated in the central region perpendicular to the membrane.

The labelling of the light harvesting complex is less prominent than the reaction centre labelling. No distinct

Fig. 1a–c. Fourier filtered image of negatively stained membranes. **a** labelled with monospecific antibodies against the H-polypeptide of the reaction centre **b** unlabelled and **c** labelled with a monoclonal antibody against the B1015-α polypeptide of the light harvesting complex (scale bar = 10 nm)

elevations can be detected within the outer ring, a homogeneous distribution of the antibodies is observed with the exception of the central core, which does not appear to be labelled.

Since the localisation of the B1015-α and B1015-β light harvesting polypeptides appears to be highly repetitive (Ludwig and Jay 1985), it can be assumed that the antigen determinants of these polypeptides are distributed in a similar fashion and the distances between the polypeptides are insufficiently resolved even with Fab' fragments of monoclonal antibodies.

Based on a crude hollow cylindrical model of the outer ring with estimated dimensions (inner diameter = 7 nm; outer diameter = 11 nm; height = 7 nm), a volume of 396 nm^3 was calculated. Using a factor of 1.3 nm^3/kd (this value was derived from the investigations of Henderson 1975 and Henderson and Unwin 1975 for rhodopsin) a maximal molecular weight for the outer ring, composed of 12 subunits, of about 300,000 was calculated. The maximal molecular weight of one subunit is then 25,000. This is sufficient to accommodate one set of light harvesting polypeptides (B1015-α: MW = 6,848: B1015-β: MW = 6,138; B1015-γ: MW = 4,000) which are known to be in a 1:1:1 ratio (Brunisholz et al. 1985) and the related chromophores. According to the six-fold symmetry, observed using electron microscopy, a regular distribution of the components in the outer ring is required. The space between two subunits would thereby approximate to 2.2 nm. If we regard the dimensions of the antibodies and their Fab' fragments it is obvious that such small distances cannot be resolved, particularly if the flexibility of the antibody is taken into consideration. The distance between the antibody binding sites should be at least larger than 10 nm to obtain a reasonable resolution. It seems unlikely that this method will therefore be suitable for resolving structures within the outer ring. We have, however, clear proof that the reaction centre is located in the central core.

The amino acid sequences of the light harvesting polypeptides (Brunisholz et al. 1985) showed the presence of one histidine residue in the B1015-α apoprotein and two histidine residues in the B1015-β apoprotein. Histidine residues located in the hydrophobic part of the B1015-α and B1015-β polypeptides are postulated to represent the bacteriochlorophyll b-binding sites. These histidine residues are in positions highly conserved amongst the light harvesting polypeptides of purple bacteria, so far investigated (Theiler et al. 1984). No histidine residues are present in the B1015-γ polypeptide. According to the structural information on the twelve subunits in the outer ring (each subunit is thought to contain all three polypeptides which, together, can accommodate two bacteriochlorophyll b molecules) 24 light harvesting bacteriochlorophyll b molecules are postulated per photosynthetic unit. 24.7 light harvesting bacteriochlorophylls were, in fact, calculated for each photosynthetic unit. The value obtained for the total amount of the bacteriochlorophyll per photosynthetic unit was calculated to be 26.7. The second method includes the four reaction centre bacteriochlorophylls and should therefore account for four additional bacteriochlorophylls per photosynthetic unit. Considering the inaccuracy of the determination, the insecurity in the precise molecular weight of the photosynthetic unit and the obtained symmetry, a value of 24 light harvesting bacteriochlorophylls b molecules per photosynthetic unit seems to be an acceptable average.

Only little information is available on the arrangement of the chromophores in the light harvesting complexes in *Rhodopseudomonas viridis*. Theoretically, the proposed 24 bacteriochlorophylls could be arranged in a circle around the photosynthetic unit. In this arrangement the centre to centre distance would be in the range of 1.1 to 1.4 nm. This correlates of the cross-linking results obtained for the B1015-α and B1015-β polypeptides using linkers of 1.2 nm span (Ludwig and Jay 1985). This arrangement of the chromophores would allow a conjugated π-system spanning around the whole light harvesting ring. The bacteriochlorophyll b-containing organisms all show an extreme far red shifted absorption of the light harvesting pigments and a similar macromolecular structure (Engelhardt et al. 1983). This red shift is thought to reflect a conjugated π-system. The postulated inter-chromophore distance is within the range of that found in another light harvesting complex (B800 – 850) of *Rhodobacter sphaeroides* by Kramer et al. (1984).

In order to obtain a red shifted absorption, it is necessary to have a sequential alignment of the transition moments of the light harvesting bacteriochlorophylls. The angles of the transition moments to the membrane plane in *Rhodopseudomonas viridis* were reported to be about 70 degrees (Qx) and about 20 degrees (Qy) by Paillotin et al. 1979. This shows that the Qy transition moments, all approximately lying in the same plane parallel to the membrane, are predestined for interactions with each other, which produce a red shift in the absorption spectrum. Cantor and Schimmel (1980) published a formula for the calculations of hypochromic shifts of an absorption maximum induced by polymerisation of chromophores. This formula can be extended to a closed ring of 24 chromophores. In order to obtain a model, the distances and the angles between the tetrapyrrol rings of the bacteriochlorophylls have been assumed as constant. Under these conditions the knowledge of two absorption wavelengths and their related aggregational state are sufficient to calculate the absorption of the other aggregational states. During the solubilisation of the photosynthetic unit, under reducing conditions, the absorption maximum of 1015 nm shifts gradually to 890 nm and then changes radically to 810 nm. This behaviour indicates that the 890 nm form is the smallest possible aggregational state of the light harvesting pigments. This 890 nm absorbing form, obtained with lithium dodecyl sulphate, probably corresponds to the 880 nm form reported by Thornber (1971) using sodium dodecyl sulphate solubilisation. Taking the value of 810 nm (monomer state) and assuming 890 nm is the dimer state, then the absorption of a ring containing 24 bacteriochlorophylls extrapolates to 1018 nm. This corresponds closely to the value observed in native membranes.

Independent data, provided by the localisation of the antigen binding sites (Jay et al. 1983), iodination of vesicles (Jay and Lambillotte 1985), the cross-linking results (Ludwig and Jay 1985, Peters et al. 1984), photoselection data (Breton et al. 1985) and circular dichroism and polarized infrared spectroscopy data (Nabedryk et al. 1985), all support the following structural model of the photosynthetic unit. In this model the light harvesting ring consists of a closed ring of 24 transmembrane α-helices. Each of these α-helices belongs to a B1015-α or B1015-β polypeptide and their N-termini are located on the plasmatic surface. On the periphery of the photosynthetic unit (within the membrane) one

bacteriochlorophyll-b molecule is connected to the histidine residues located near the C-terminal end of each α-helix. All these bacteriochlorophyll molecules are then able to build an overlapping π-system around the protein ring.

This model fits the presently available data, yet requires confirmation at the molecular level and does not therefore exclude other possibilities.

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