# Topographical relationships of polypeptides in the photosynthetic membrane of *Rhodopseudomonas viridis* investigated by reversible chemical cross-linking

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The B1020 antenna pigment–protein complex of *Rhodopseudomonas viridis* was isolated and found to contain two polypeptide subunits of apparent $M_r$ 8000 and 12000. Near-neighbour relationships between both antenna 8-kDa and 12-kDa polypeptides were revealed by reversible chemical cross-linking. Cross-linked homooligomers, probably up to the respective hexamer, were observed. Subunit M of the photochemical reaction center (RC) and the membrane-bound cytochrome *c*-553/558 were cross-linked in high yield at a 1:1 stoichiometry. The heavy subunit H of the RC was cross-linked to the 8-kDa polypeptide.

*Topographical relationship* *Reversible chemical cross-linking* Rhodopseudomonas viridis
*Bacteriochlorophyll* b *Photosynthetic apparatus* *B1020 antenna complex*

## 1. INTRODUCTION

Photosynthetic bacteria harvest incident light energy by antennae comprised of bacteriochlorophyll (Bchl) and carotenoid non-covalently bound to low-molecular-mass polypeptides. Energy is transferred via excited singlet states of the pigments to the photochemical reaction center (RC) for conversion into a charge-separated state (ref. in [1]). The efficiency of these events depends on the spatial relationships of pigment molecules, which are arranged in the photosynthetic membrane by polypeptide components of the pigment–protein complexes. To obtain insight into the mode of functioning of the photosynthetic apparatus, it is therefore important to study the topographical relationships of the polypeptide components.

Specific near-neighbour relationships among polypeptides of the Bchl *a*-containing photosynthetic apparatus of *Rhodopseudomonas capsulata* have been revealed by reversible chemical cross-linking [2–4]. The organization of photosynthetic membranes containing Bchl *b* has been studied mainly by freeze-fracture electron microscopy. In contrast to Bchl *a*-containing Rhodospirillaceae, extensive hexagonal lattices have been observed [5–10]. Also, the isolated RC contains a membrane-bound cytochrome *c*-553/558 [11].

The crystallization of the RC of the Bchl *b*-containing bacterium *R. viridis* has been reported [12]. However, the subunit composition of the antenna and the molecular organization of the photosynthetic apparatus are still unknown. It was therefore of considerable interest to identify the antenna light-harvesting polypeptides and investigate the spatial relationships of antenna and reaction center polypeptides of *R. viridis*.

## 2. MATERIALS AND METHODS

### 2.1. *Isolation of the B1020 antenna complex of* R. viridis

Cells of *R. viridis* were cultivated and in-

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tracytoplasmic membranes prepared as in [8]. The protein concentration was adjusted to 2 mg/ml [13]. Two different ways of isolating the light-harvesting complex were used.

(i) The pH was adjusted to 10.5 and 10 mM glutathione was added. After addition of 25 mg lithium dodecyl sulfate (LDS) (detergent:protein weight ratio 0.75) the sample was incubated at 0°C for 30 min. Polyethyleneimine (250 mg/ml) adjusted to pH 7.0 with 10 mM HCl was added to a final concentration of 40 mg polyethyleneimine/ml. The sample was then centrifuged at 1000 × *g* and 4°C for 5 min. The supernatant contained the B1020 antenna complex. For analysis by SDS-polyacrylamide gel electrophoresis (SDS–PAGE), polyethyleneimine has to be removed by repeated washing and centrifugation at 27000 × *g*. The LDS:protein weight ratio is the most critical parameter for obtaining a native B1020 preparation.

(ii) Intracytoplasmic membranes (1 ml, 2 mg protein/ml) were layered on top of a sucrose step gradient containing in 50 mM Tris–HCl (pH 8.8), 5 mM glutathione, 1 mM phenylmethanesulfonyl fluoride: 500 mg sucrose/ml (1 ml), 300 mg sucrose/ml (3 ml), 150 mg sucrose/ml (3 ml), 50 mg sucrose/ml + 1.5 mg LDS/ml. After centrifugation (Beckman Ti 75 rotor, 30000 rpm, 4°C, 16 h), the precipitated material on the 50% sucrose cushion was removed and solubilized with Triton X-100 (2 mg Triton/mg protein). This procedure is not suited for preparative purposes, as the yields of B1020 complex are 10% or lower.

### 2.2. *Electrophoresis procedures*

Native Triton X-100 PAGE at constant pH 8.8 and isoelectric focusing were carried out as in [4]. SDS–PAGE under denaturing conditions was performed as in [14] using 11.5–18% acrylamide gradient gels.

### 2.3. *Cross-linking procedure*

Reversible cross-linking with dithiobis(succinimidyl propionate) was carried out as in [3]. The pH was 8.0 or 8.9. The concentration of the cross-linking agent was 1 mM, protein concentration 1 mg/ml, and reaction time 30 min.

### 2.4. *Staining procedures*

Gels were stained with Coomassie brilliant blue. Two-dimensional gels were subsequently silver-stained as in [15].

## 3. RESULTS

### 3.1. *Identification of B1020 antenna polypeptides*

Apart from the RC absorption at 830 nm and the cytochrome region around 550 nm the absorption spectrum of the isolated B1020 antenna complex of *R. viridis* is identical with that of the membrane fraction (fig.1). The B1020 complex isolated by one of the two methods described in section 2.1 migrated as a single band, both at constant pH 8.8 and in isoelectric focusing. It contained the polypeptides of apparent $M_r$ 8000 and 12000 (fig.1). These two polypeptides are thus identified as subunits of the B1020 antenna complex of *R. viridis*.

A 6-kDa polypeptide considered as a possible constituent of the B1020 complex [16] was not present in thylakoids purified by 2-fold sucrose gradient centrifugation (see fig.1). The 8-kDa polypeptide was found to be associated with the RC when purified thylakoids were solubilized with dodecyl dimethylamine *N*-oxide and separated by

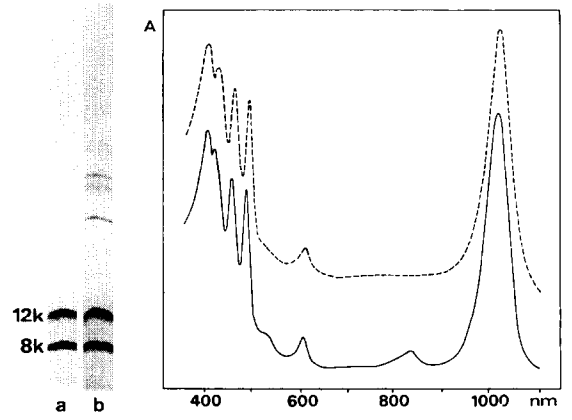


Fig.1. Absorption spectrum and polypeptide composition of the isolated B1020 antenna complex of *R. viridis*. (– – –) Isolated B1020 complex [method (ii) in section 2.1] solubilized with Triton X-100, (——) purified thylakoids of *R. viridis*. Tracks a and b: separation of the isolated complex (a) and purified thylakoids (b) on 11.5–18% SDS-polyacrylamide gels. k, kDa.

isoelectric focusing. This RC–8-kDa complex still contained antenna Bchl *b*. The 8-kDa subunit also comigrated with Bchl *b* (absorption peaks at 796 and 683 nm) when thylakoids were solubilized with 1 mg SDS/mg protein at room temperature and subjected to SDS–PAGE. Thus, the 8-kDa subunit seems to be involved in the binding of Bchl *b* within the B1020 complex.

### 3.2. *Cross-linking of intracytoplasmic membranes*

Two-dimensional mapping of cross-linked thylakoids at pH 8.9 gave results as shown in fig.2. Both B1020 subunits appear in spots below the diagonal formed by unmodified polypeptides. As these spots are not vertically superimposed, they are assigned to homooligomers of 8 and 12 kDa, respectively. Both polypeptides seem to cross-link at least up to the stage of the hexamer. These data indicate that the membrane-bound B1020 complex contains hexamers or, possibly, higher oligomers of both subunits.

The apparent $M_r$ values of cross-linked 8- and 12-kDa oligomers are much smaller than expected. For instance, the supposed hexamer of 8 kDa appears at apparent $M_r$ 33000 as opposed to 48000. As studies with the isolated and aggregated 10-kDa subunit of the B800-850 antenna complex of *R. capsulata* have revealed that the $(10\text{-kDa})_6$ hexamer has an apparent $M_r$ of 34000 [3], the assignment of oligomers in fig.2b is not contradicted by the apparent $M_r$ scale.

Cytochrome *c*-553/558, which is the largest polypeptide of the RC ($M_r$ 38000 [16]), was found to cross-link to the 27-kDa polypeptide [16] of the RC in very high yield. From the apparent $M_r$ of 74000 of the cross-linked product a stoichiometry of 1:1 may be deduced. As there are only small amounts of these two polypeptides left on the diagonal, it is suggested that M and cytochrome *c*-553/558 are present in equimolar amounts in the membrane.

The 33-kDa polypeptide of RC (H, apparent $M_r$ 33000 [16]) also appeared in spots below the diagonal which were probably derived from cross-linked products of H and oligomers of 8 kDa. As vertical relationships may not be distinguished in fig.1 because of extensive homooligomer formation of the antenna components, cross-linking was also carried out at pH 8.0. Fig.3 shows that the yield of cross-linking among the antenna polypeptides decreases strongly with successively higher oligomerization. The spots below the diagonal belonging to RC subunit H show a similar behaviour. It is concluded from this figure that the 8-kDa polypeptide cross-links specifically to subunit H. Spots of H at apparent $M_r$ 38000–64000 below the diagonal in fig.2b are probably attributable to oligomers (monomers

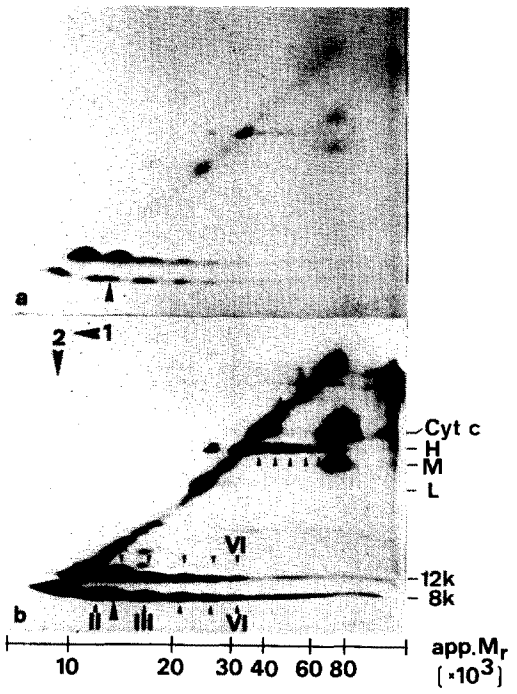


Fig.2. Two-dimensional mapping of reversibly cross-linked polypeptides of *R. viridis* membranes. (a) Coomassie-stained gel, (b) the same gel after silver staining and partial destaining as in [15]. The directions of electrophoresis are indicated by arrows in (b). Spots below a diagonal formed by unmodified polypeptides are derived from cross-linked products cleaved into their components before separation in the second dimension. Homooligomers of 8 and 12 kDa are designated by small arrows (II–VI). The large arrow points to a possible vertical relationship of 8- and 12-kDa polypeptides, which would probably correspond to 8–12 kDa. Spots below the diagonal corresponding to RC subunit H at apparent $M_r$ 38000–64000 are also denoted by small arrows. A vertical relationship of M and cytochrome *c*-553/558 is observed at apparent $M_r$ 74000, indicating cross-linking of these polypeptides at a 1:1 stoichiometry. Cross-linking was carried out at pH 8.9. k, kDa.

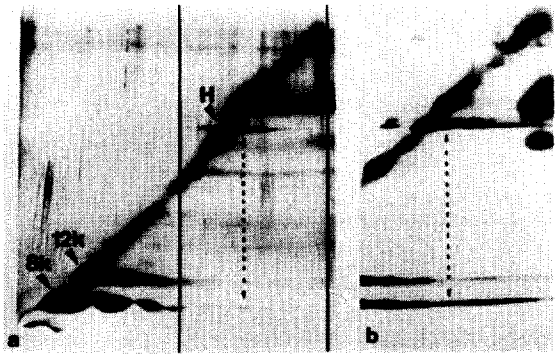


Fig.3. Cross-linking of *R. viridis* membranes at different pH values. (a) Conditions were the same as in fig.2, except that the pH was changed from 8.9 to 8.0. In contrast to fig.2, the yields of cross-linked homoligomers of 8 and 12 kDa decrease strongly with increasing oligomer size. Thus, a vertical relationship between H and 8-kDa polypeptide becomes apparent (dotted vertical line). (b) For comparison a section of fig.2b corresponding to the section in (a) designated by vertical lines is shown. The vertical superimposition of H and 8-kDa polypeptide seen in (a) is again indicated. k, kDa.

through pentamers) of 8-kDa polypeptide cross-linked to H.

## 4. DISCUSSION

Recent electron microscopic studies on the lateral topography of the photosynthetic apparatus of *R. viridis* [5–10] have revealed hexagonal lattices of particles in the membrane which have been interpreted as light-harvesting complexes surrounding reaction centers [6,7]. If this interpretation is correct, the peripheral particles must consist of oligomers of light-harvesting polypeptides because of the particle size. This is supported by the present data.

*R. capsulata* and *R. viridis* contain an RC-bound antenna complex, i.e., B870 in *R. capsulata* and B1020 in *R. viridis*. The polypeptides of both complexes have apparent $M_r$ values of about 12000 and 7000–8000 [17]. In both cases the smaller of the two polypeptides was found to cross-link to subunit H of the RC. These similarities indicate that the heavy subunit (H) of *R. viridus* is comparable to that of *R. capsulata*. As with *R. capsulata* [18], H may be removed from the RC without affecting the spectral properties of the reaction center (unpublished). The data support the idea that in Rhodospirillaceae the pigment molecules of the RC-bound antenna complex and the primary donor of the RC are sterically coordinated by interactions between the smaller polypeptide of the antenna complex and polypeptide H of RC to provide efficient exciton transfer.

The association of RC subunit M and cytochrome *c*-553/558 in *R. viridis* may reflect a functional relationship.

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