The transverse membrane orientation of the light-harvesting and reaction centre polypeptides of *Rhodopseudomonas capsulata*, investigated by surface iodination

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The transverse orientation of photosynthetic polypeptides in the membrane of *Rhodopseudomonas capsulata* was investigated using surface radioiodination of chromatophores and spheroplasts with the reagent 1,3,4,6-tetrachloro-3α, 6α-diphenylglycoluril. Native pigment–protein complexes were isolated by Triton X-100–polyacrylamide gel electrophoresis to identify and distinguish polypeptides not separated by sodium dodecylsulphate–polyacrylamide gel electrophoresis. The two antenna light-harvesting complexes were found to be asymmetrically oriented with polypeptides 14 kDa and 8 kDa of complex B800–850 and 12 kDa of B870 being exposed on either membrane surface. Subunits H and M of the photochemical reaction centre, and probably L, span the membrane.

Rhodopseudomonas capsulata *Transverse membrane topography* *Bacteriochlorophyll–protein* *Surface iodination*

1. INTRODUCTION

The photosynthetic apparatus of *Rhodopseudomonas (R.) capsulata* contains 3 different bacteriochlorophyll *a*–carotenoid–protein components, the light-harvesting (LH) complexes I (near IR absorption at 870 nm) and LH II (near IR absorption at 800 and 850 nm) and the photochemical reaction centre (RC) [1]. The LH I complex has polypeptide subunits of app. M_r 12000 (12 kDa) and 7500 (7.5 kDa) [2]. LH II contains polypeptides of app. M_r of 14000 (14 kDa), 10000 (10 kDa), and 8000 (8 kDa) [3]. The topography of these pigment–protein complexes has been investigated in order to understand the mechanism of energy transfer. Biophysical investigations [4,5] as well as chemical cross-linking studies [6,7] have revealed specific lateral relationships. In studies of the transverse topography of the constituent polypeptides, exposure of RC subunits H and M [8] and light-harvesting polypeptides 8 kDa and 12 kDa [9] were reported on both sides of the cytoplasmic membrane, as concluded from surface iodination data. However, data on the 8 kDa polypeptide are ambiguous, as this component was not separated from the 7.5 kDa subunit of LH I complex.

We have separated the two native LH complexes prior to analysis on SDS–polyacrylamide gels and were thus able to resolve and identify all of the light-harvesting polypeptides of *R. capsulata* on the gel. The transverse orientation of polypeptides was investigated using radioiodination with Iodo-Gen [10] of chromatophores and intact spheroplasts.

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Abbreviations: EDTA, ethylenediamine tetraacetic acid; LH, light-harvesting; *R.*, *Rhodopseudomonas*; RC, reaction centre; SDS, sodium dodecylsulphate

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2. MATERIALS AND METHODS

2.1. *Culture conditions and membrane preparations*

Cells of *R. capsulata* strain St Louis (ATCC 23782) were grown anaerobically in malate-based mineral medium [11] at 30°C in light (200 lux) and harvested in the late logarithmic phase.

Purified chromatophores were prepared as in [12]. For preparation of spheroplasts fresh cells from the same batch (20 mg wet wt/ml), suspended in Tris/HCl (20 mM, pH 8.0)–sucrose (300 mg/ml) buffer, were incubated at 30°C for 5 min with 3 mM EDTA. Then lysozyme (Sigma) (0.2 mg/ml) was added and spheroplast formation followed microscopically. When about 70% of cells had been converted into spheroplasts, the label was added.

2.2. *Radioiodination*

Glass beads (2 mm diam.) were coated with 1,3,4,6-tetrachloro-3a, 6a-diphenylglycoluracil (Iodo-Gen, Pierce) by immersing the beads in chloroform containing 1 mg/ml reagent, decanting the supernatant and drying the beads under a gentle stream of nitrogen on aluminium foil. Immediately before labeling, 10 beads were added to 200 μl sample in Eppendorf vials. After addition of ^{125}I (20 μCi, Amersham) the reaction was allowed to proceed for 2 min at 20°C and the supernatant removed and mixed with 20 μl 1 M NaI, 50 mM $NaHSO_3$ in 50 mM Tris/HCl buffer at pH 8.0.

2.3. *Native pigment–protein complex fractionation and analysis*

Membranes (0.5 mg bacteriochlorophyll/ml) were suspended in Tris/HCl buffer containing 1 mM phenylmethyl sulfonyl fluoride and treated dropwise with Triton X-100 (100 mg/ml) to 25 mg Triton X-100/ml final conc. at 0°C and left on ice for 45 min. Spectrally intact pigment–protein complexes were isolated and identified on 4–7% acrylamide gradient gels using the system of Laemmli as modified in [13] with the following modifications: 10 mg SDS/ml in the gel were replaced by 5 mg Triton X-100/ml, the stacking layer was omitted, and gel electrophoresis was conducted at 4°C in the dark. Native pigmented bands corresponding spectrally to RC/LH I (near IR absorption maxima at 800 and 870 nm, reversible photoinduced bleaching at 865 nm) and LH II (B800–850) were excised and treated with 200 mM Tris/HCl (pH 6.8) 40 mg SDS/ml, 10 mm dithiothreitol and 100 μl glycerol/ml for 5 h at 37°C. The extracts were applied to 11.5–16.5% SDS–acrylamide gradient gels [13]. For evaluation, gels were dried under vacuum and exposed on Kodak X-Omat film. For quantitation of labeling rates, excised bands were subjected to liquid scintillation counting.

2.4. *Test for non-penetration of ^{125}I into the membrane and for spheroplast integrity*

Purified chromatophores were iodinated for 2 min as in the labeling studies. An aliquot was treated with 5 mg SDS/ml for 10 min to disrupt the membrane and iodinated in the same way. After termination of the reaction as above, SDS was added to 20 mg/ml final conc. and the samples were heated at 60°C for 10 min and subjected to immunofractionation with antibodies directed against cytochrome c_2 (a gift from Dr Roland Dierstein), using the method in [6]. Bands corresponding to cytochrome c_2 were excised and ^{125}I-activities evaluated.

To investigate the integrity of osmotically sensitive spheroplasts the incorporation of ^{125}I into the nucleic acid fraction during labeling was studied by subjecting 200 μl sample to deproteination with $CHCl_3$/phenol as described in [14] and separating nucleic acids from free ^{125}I on 7–16.5% polyacrylamide gels. Vacuum-dried gels were screened using autoradiography and evaluated by liquid scintillation counting.

3. RESULTS AND DISCUSSION

Whereas chromatophores were assumed to be about 95% inside-out oriented, studies with vesicles prepared by osmotic lysis of spheroplasts from *R. sphaeroides* yielded only 80% right-side-out orientation [15–17]. As judged by the amount of ^{125}I incorporated into nucleic acids with respect to spheroplasts lysed by brief ultrasonication, the spheroplasts used here were 98% intact. This also indicates that active ^{125}I does not penetrate the cytoplasmic membrane. The impermeability of reversely oriented chromatophores to ^{125}I was ascertained by isolating cytochrome c_2 as above

and measuring the amount of ^{125}I incorporated in comparison with detergent-disrupted vesicles. As cytochrome c_2 is located in the interior of chromatophores [18], the rate of ^{125}I insertion into cytochrome c_2 may be taken as a measure of ^{125}I membrane penetration. Only 3% of the label were found in cytochrome c_2 compared to the value of disrupted vesicles (taken as 100%). Thus, both with chromatophores and spheroplasts, ^{125}I labels the cytoplasmic surface of chromatophores and the exoplasmic surface of spheroplasts.

For the identification of labeled polypeptides, one-dimensional SDS-polyacrylamide gel electrophoresis as in [9] is not sufficient, as the light-harvesting polypeptides 7.5 kDa and 8 kDa are not resolved. Also, minor polypeptides, occurring at the positions of photosynthetic polypeptides in the gel, may become heavily labeled and lead to erroneous results as pointed out in [19]. We have therefore separated native pigment–protein complexes using Triton X-100–polyacrylamide gel electrophoresis. Pigmented bands identified as RC/LH I and LH II by their infra-red absorption spectra were analyzed on SDS–polyacrylamide gels.

Fig.1 shows tracks of SDS–polyacrylamide gels stained with Coomassie as well as autoradiograms of the RC/LH I and the LH II fractions. Whereas 7.5 kDa of LH I and 8 kDa of LH II are not resolved without preceding Triton fractionation (see track A) and whereas strongly iodinated minor polypeptides severely hamper the identification of labeled photosynthetic polypeptides (not shown), tracks D and E reveal clear labeling patterns that may be quantitatively evaluated. The results of such an evaluation are shown in fig.2. Besides intact chromatophores (cytoplasmic face exposed) and spheroplasts (periplasmic face exposed) Triton-solubilized chromatophores were also iodinated in order to investigate the availability of reactive sites on native pigment–protein complexes. The bottom set of data shows that all polypeptides studied are susceptible to iodination, but L of RC and 14 kDa of LH II are only weakly labeled with respect to the other components. The failure of membrane-bound 10 kDa to incorporate ^{125}I in contrast to iodination within solubilized LH II indicates that active ^{125}I does not penetrate significantly into the bulk of the membrane. The following conclusions are derived from fig.2:

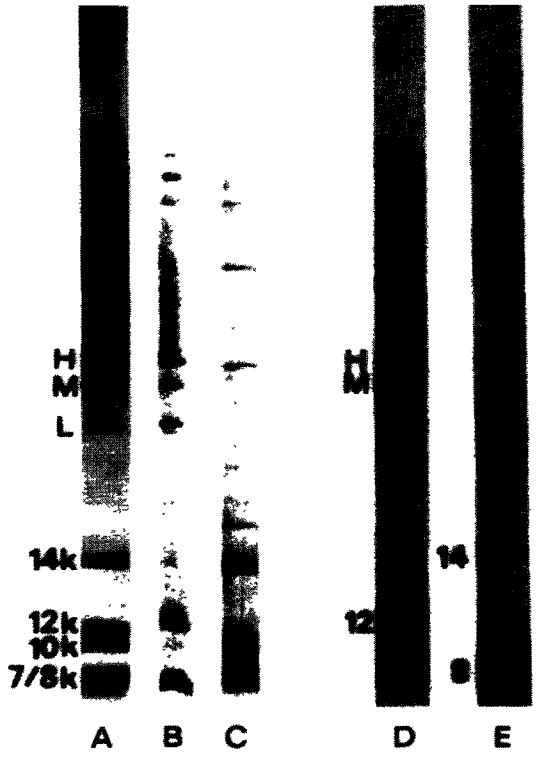

Fig.1. Autoradiograms of native pigment–protein fractions obtained from ^{125}I surface-labeled chromatophores, separated on SDS–polyacrylamide gels: Coomassie-stained gel tracks of chromatophores (A); the RC/LH I fraction (B); and the LH II fraction (C), obtained by Triton fractionation as in section 2. Autoradiograms of the RC/LH I fraction (D) and the LH II fraction (E), labeled in situ with ^{125}I.

(1) RC polypeptides H and M, and probably also L, are exposed on either side of the membrane as previously found with *R. sphaeroides* [20].

(2) The 8 kDa polypeptides of the LH II complex and 12 kDa of LH I are the principally labeled light-harvesting polypeptides (fig.2 and [9]).

(3) Whereas the LH II-fraction incorporates more label than LH I on the cytoplasmic surface, the reverse is true for the periplasmic side. Thus, the membrane is asymmetrical with respect to the light-harvesting complexes.

(4) In comparison with the control experiment, 14 kDa of LH II is significantly labeled on both sides of the membrane and is thus considered a transmembrane polypeptide.

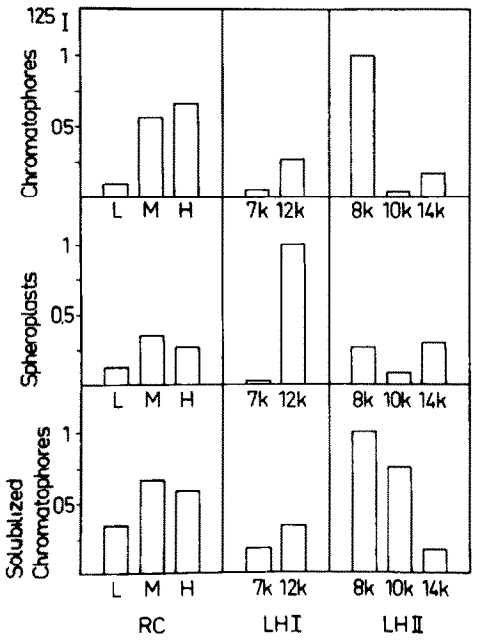

Fig.2. Rates of ^{125}I-incorporation into the photosynthetic polypeptides of *R. capsulata*. The data obtained with chromatophores (top row) and spheroplasts (middle row) are compared with iodination rates of Triton X-100-solubilized pigment–protein complexes. Iodination rates were obtained by evaluating SDS–polyacrylamide gel patterns as in fig.1. (D,E). For each of the three experiments, ^{125}I-activities were related to the highest labeling rate, which was arbitrarily set to one.

(5) The failure of the 10 kDa polypeptide to incorporate label on either side of the membrane is in accordance with the results of protease digestion studies which revealed a pronounced protease resistance of this polypeptide ([21], unpublished).

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