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LOCALISATION OF THE SUBUNITS OF THE PHOTOSYNTHETIC REACTION CENTERS IN THE CHROMATOPHORE MEMBRANE OF *RHODOSPIRILLUM RUBRUM*

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SUMMARY

Reaction centers were isolated with the detergent lauryl dimethyl amine oxide from chromatophore membranes of *Rhodospirillum rubrum*. The subunit composition of these reaction centers is similar to the one obtained from *Rhodopseudomonas spheroides*: three subunits with the molecular weights of 21000, 24000 and 29000. Reaction centers prepared from chromatophores labeled with ^{131}I were heavely labeled in their large subunit (H). The smaller subunits (L and M) contained only little label. Sonication during labeling yielded a slightly higher incorporation of ^{131}I in subunit H compared to the smaller ones. It is concluded that the H protein is largely exposed at the cytoplasmic side of the membrane but might also be accessible for iodination on the inside of the membrane while the L and M proteins are almost completely embedded in the membrane. Iodination of spheroplasts results in only a slight binding of ^{131}I to chromatophores and reaction centers.

INTRODUCTION

Isolation of reaction centers from chromatophores have been described for several species of photosynthetic bacteria [1, 2]. These pigment-protein complexes are at least partially embedded in the chromatophore membrane and can be isolated from the membrane with detergents [3–5]. The isolated pigment-protein complex contains besides bacteriochlorophyll *a* and bacteriopheophytin *a* a special bacteriochlorophyll (*P*-865). This reaction center pigment is oxidized in the light and reduced in the dark. The protein part of the reaction center is composed of three subunits differing in their molecular weights, H for heavy, M for medium and L for light [6].

Knowledge of the orientation of this protein in the membrane might be of importance for an understanding of the mechanisms of coupling between light absorption and redox potential differences in electron transport. Localisation of the reaction centers in the chromatophore membranes from *Rhodopseudomonas spheroides*

Abbreviations: LDAO, lauryl dimethyl amine oxide; SDS, sodium dodecyl sulphate.

was investigated with immunological methods by Steiner et al. [7], Reed et al. [8] and Valkirs et al. [9]. Their results give evidence that the H subunit is at least partially exposed at the cytoplasmic surface of the chromatophore membrane. According to Reed et al. [8] reaction centers are located at the membrane surface, below ATPase particles.

Another means of localizing membrane proteins is labeling with enzymatic methods. A well established procedure is iodination with ^{131}I [10–12].

In the present paper we describe the localisation of the reaction center proteins and its subunits in the chromatophore membrane employing enzymatic iodination.

MATERIALS AND METHODS

The carotinoidless mutant G-9$^+$ of *Rhodospirillum rubrum* was grown in the light in 10-l bottles at 30 °C in the medium of Ormerod et al. [13] omitting yeast extract and pepton. Chromatophores were obtained from washed cells by sonic disruption. Reaction centers were prepared according to a modification of the method of Okamura et al. [14]. After treatment with 5 mM EDTA the chromatophores (absorbance at 870 nm = 85) were slowly mixed with LDAO (0.45 %) under anaerobic conditions to a final concentration of approx. 0.225 %. The exact optimal concentration was determined for each batch of cells. After removing the reaction center-depleted membranes by centrifugation (60 min, 304 000 × *g*), the reaction centers in the supernatant were concentrated by ultrafiltration (Amicon UM 20 E) and further purified by gel filtration on Sepharose 6B (elution buffer 10 mM Tris · HCl, pH 8.0) and DEAE cellulose (NaCl step gradient as described by Okamura et al. [14]). All elution buffers contained 0.025 % LDAO [15]. Purity was checked by SDS gel electrophoresis at constant voltage with the procedure of Davis [16], modified for SDS gels. Spectroscopic analysis was done on an Aminco DW-2 or Cary spectrophotometer equipped with side illumination. Proteins were determined after Lowry et al. [17] using bovine serum albumin as standard with SDS present as in the samples. Spheroplasts were prepared with EDTA and lysozyme as described for *Escherichia coli* [18]. *R. rubrum* cells were suspended in 20 mM Tris · HCl buffer, pH 8.0, and 10 % sucrose, EDTA was added to a concentration of 4 mM followed by lysozyme (0.25 mg/ml). The preparation was controlled with the microscope for the morphology of the spheroplasts. Osmotic fragility was tested by observing the absorption change at 660 nm after a 10-fold dilution with water. For the iodination the chromatophores, suspended in 10 mM phosphate buffer (pH 7.0, 10 mg protein/ml), were treated with KI (final concentration 10^{-7} M, total 4 mCi ^{131}I) and lactoperoxidase (0.1 mg/ml). Addition of H_2O_2 (8 μM) initiated the reaction. To remove the unbound ^{131}I, the chromatophores were washed twice and dialysed overnight. Spheroplasts and reaction centers were treated similarly with iodide and lactoperoxidase using the same concentration relative to the protein. Spheroplasts were iodinated in the presence of sucrose (20 %). Determination of the radioactivity was done in a commerical γ-counter. Labeling patterns in gels were determined from slices of equal thickness which were counted without further treatment. Controls without enzyme or substrate proved the strong dependence of the protein iodination reaction on lactoperoxidase and H_2O_2. The iodination of protein-bound chlorophyll was negligible compared to the amount fixed on the proteins (3–5 %). Strong illumination

increased the labeling in the chlorophyll, but was without significant effect on the protein.

RESULTS

Fig. 1 shows the spectrum of purified reaction centers of *R. rubrum* in the reduced and oxidized state. As judged from the absorbance ratio $A_{280\,nm}/A_{802\,nm} = 1.25$, our preparation is of the same purity as those described in the literature [14]. Actinic illumination results in the complete bleaching of the pigment species *P*-865.

The results of an iodination experiment are shown in Table I. Isolated reaction centers, chromatophores and spheroplasts were iodinated with the same amount of ^{131}I relative to the protein. Specific labeling, cpm/mg of protein, of reaction centers is about 50 % higher than that of chromatophores. Reaction centers isolated from iodinated chromatophores as well as the remaining reaction center-depleted membrane fragments are also highly labeled. Small but strongly labeled surface proteins which are also removed from the membrane by LDAO are lost during purification. Spheroplasts are labeled to a much lower degree on a protein basis, because less of their proteins are exposed to the marker. Chromatophores prepared from labeled spheroplasts bear only little label as well as reaction centers prepared from this material. The specific labeling of these chromatophores and reaction center proteins is only a few percent of the labeling obtained after iodine treatment of chromatophores or reaction centers directly. It has been described that treatment with EDTA removes loosely bound surface proteins from the chromatophore membrane, especially the coupling factor of phosphorylation [19–21]. One would expect that after this treatment the more intrinsic proteins could become accessible to iodination with lactoperoxidase. In Table I, however, no significant difference is seen between the specific labeling of reaction centers prepared from chromatophores which where iodinated either before or after washing with EDTA.

Subunits of reaction centers, either directly iodinated in the reaction center or prepared from iodinated chromatophores or spheroplasts were separated on SDS polyacrylamide gels. The three subunits of *R. rubrum* reaction centers have an apparent molecular weight of 21 000, 24 000 and 29 000, similar to the values published

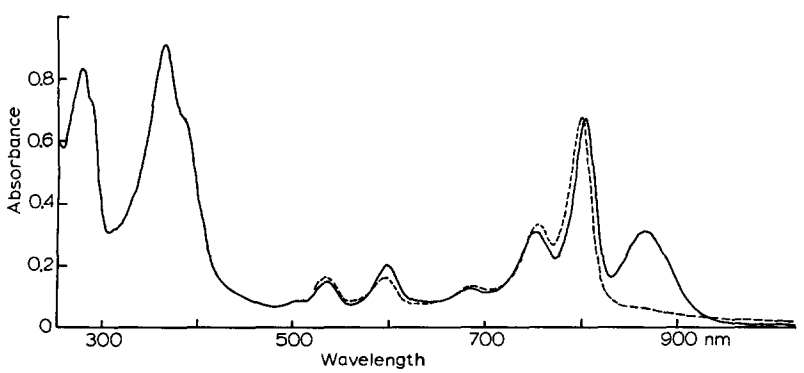

Fig. 1. Spectrum of a reaction center preparation from *R. rubrum* in its reduced and oxidized state. Photooxidation was achieved by illumination at 368 nm with a light intensity of $8 \cdot 10^4$ erg/cm^2 per s.

TABLE I

LABELING OF THE MEMBRANE SURFACE WITH ^{131}I

Labeling given as specific labeling in 10^4 cpm/mg protein. Initial activity = $434 \cdot 10^4$ cpm/mg protein. Column 3 is a mean of five experiments.

Label found in	Spheroplasts	Chromatophores		Reaction centers
		before EDTA treatment	after EDTA treatment	
Spheroplasts	37.2	–	–	–
Chromatophores	4.4	264	255	–
Reaction centers	4.8	210	276	389
Reaction center depleted chromatophores	3.7	180	204	–

for *Rps. sheroides* [22]. According to Okamura et al. [14] the stoichiometry of these subunits is 1 : 1 : 1.

Fig. 2 shows the gel electrophoretic separation of a crude reaction center preparation and the distribution of radioactivity. The three subunits are about equally labeled. It was not possible to treat highly purified reaction centers with $^{131}I^-$, lactoperoxidase and H_2O_2, since this treatment resulted in a complete denaturation of the subunit structure. The subunits of reaction centers isolated from labeled chromatophores or spheroplasts were labeled differently. The smaller subunits L and M contained only a few percent of the activity compared to the heavy subunit (Figs.

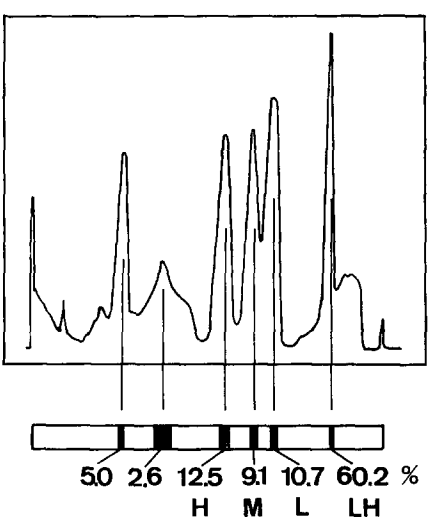

Fig. 2. Separation of a crude reaction center preparation on SDS gels after iodination. Upper curve: densitometric analysis of the gel at 600 nm after staining with Coomassie Blue; lower part of the figure: distribution of ^{131}I radioactivity in gel slices of equal thickness corresponding to the protein bands, expressed in percent of the total counts in the gel (total counts = 22 400 cpm, mean of background subtracted).

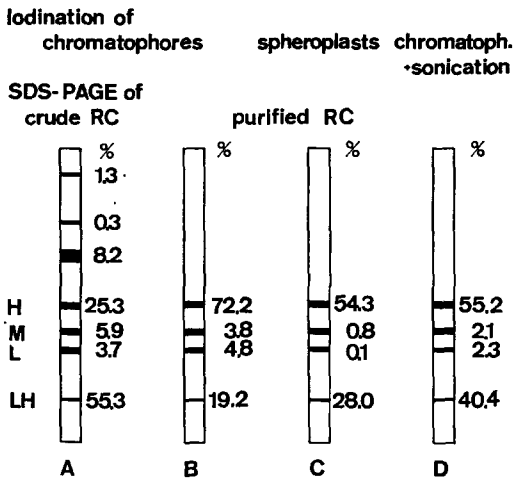

Fig. 3. Distribution of ^{131}I radioactivity in SDS polyacrylamide gels (SDS-PAGE) of reaction center (RC) preparations of *R. rubrum*. (A) Gel of crude reaction centers isolated from iodinated chromatophores. Distribution of radioactivity in the protein bands in percent of the total counts of the gel (= 53 300 cpm). (B) Gel of purified reaction centers isolated from iodinated chromatophores, radioactivity expressed as in A. Total counts = 5225 cpm. (C) Gel of purified reaction centers isolated from iodinated spheroplasts, radioactivity expressed as in A. Total counts = 625 cpm. (D) Gel of purified reaction centers isolated from iodinated chromatophores when treated with sonic oscillations during the iodination process. Radioactivity expressed as in A. Total counts = 4990 cpm.

3A–3C). Furthermore, there is a band from a small protein running close to the front which is labeled heavily, probably the light harvesting protein complex (LH) [23]. Estimated from the density of the Coomassie Blue, the specific labeling of this fraction seems to be at least as high as the one of the heavy subunit of the reaction centers. In crude reaction center preparations (Fig. 2, Fig. 3A) which contain more than traces of this light harvesting complex, the radioactivity in this band is high.

In another set of experiments chromatophores as well as spheroplasts were subjected to ultrasonic vibration during the iodination process with the purpose of breaking up the chromatophore membrane temporarily and allowing the iodinating reagents to get into contact with the inner surface of the membrane. In general, ultrasonic treatment reduced the enzymatic iodination of the membrane proteins to about 10 % of the untreated sample. In Fig. 3D the distribution of the radioactivity in the subunits of the reaction centers is shown for reaction centers isolated from iodinated and sonicated chromatophores. As in the experiments described before most of the activity is found in the heavy band. Furthermore, the activity in the L and M band is decreased compared to the H band after sonication.

DISCUSSION

The results presented show that part of the reaction center protein is located on the cytoplasmic side of the chromatophore membrane. These proteins are accessible to iodination without removal of extrinsic proteins. In contrary Reed et al.

[8] described that ferritin-labeled antibodies against whole reaction centers from *Rps. spheroides* react with the chromatophore membrane only after removal of surface proteins with EDTA. It may be that the surface proteins prevented the relatively large ferritin-antibody complex to react with the accessible part of the reaction centers while in our experiment a reaction with iodine and lactoperoxidaxe was still possible, since both agents are of smaller size. On the other hand a slightly different membrane topology of *R. rubrum* compared to *Rps. spheroides* cannot be excluded.

Comparison of iodinated isolated reaction centers with those extracted from iodinated chromatophores show that differences in labeling between the H subunit compared to the L and M is not due to chemical differences between the three proteins. At least in *Rps. spheroides* reaction centers there are only small differences in the number of tyrosine and histidine residues in the subunits L, M and H [24]. In isolated reaction centers of *R. rubrum* these subunits show about an equal iodide binding relative to their molecular weight (Fig. 2). The specific activity of all the subunits after direct iodination is larger than in reaction centers isolated from labeled chromatophores. The distribution of radioactivity among the three subunits indicate that only the H subunit is accessible to the cytoplasm. Similar conclusions were presented by Steiner et al. [7] from experiments with antibodies against whole reaction centers. Furthermore they observed a reaction of these antibodies with membranes of a non-photosynthetic mutant of *Rps. spheroides* lacking the subunits L and M of the reaction center (mutant 8-17 of Takemoto and Lascelles [25]). This was taken as evidence for the location of subunit H on the membrane surface. Finally the same conclusion was reached by Valkirs et al. [9] with antibodies specifically prepared against the H subunit in *Rps. spheroides*.

The activity ratio between H and L+M is increased slightly when the chromatophores were sonicated during the labeling process. Under the assumption that the ultrasonic treatment allows the reagents of the iodination process to enter the chromatophore vesicles and also to react on the inside of the membrane, these results would suggest that the H subunit is accessible from both sides of the membrane while the L and M subunits seem almost completely buried in the membrane.

Iodination of spheroplasts yields only slightly labeled chromatophores and reaction centers. It seems probable that both chromatophores and reaction centers are not accessible from the outside of the spheroplast, the small incorporation observed being due to the presence of some broken cells. A comparison between our results from *R. rubrum* and the data published on *Rps. spheroides* indicate that the organisation of both membranes are similar. The orientation of the reaction centers in the chromatophore membrane is not significantly different as judged from the evidence so far.

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