ON THE LOCALIZATION OF A BACTERIOCHLOROPHYLL-ASSOCIATED POLYPEPTIDE IN THE CHROMATOPHORE MEMBRANE OF *RHODOSPIRILLUM RUBRUM* G-9

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1. Introduction

A bacteriochlorophyll–protein complex was isolated by detergent solubilization from reaction-center-depleted chromatophores of *Rhodospirillum rubrum* G-9$^+$ [1]. This complex, proposed to correspond to the light-harvesting (LH) antenna, contains mainly one subunit peptide (LH-polypeptide, LHP) mol.wt ~14 000. LHP is soluble in a mixture of chloroform/methanol and can also be extracted into organic solvent from intact chromatophores. This polypeptide which contributes up to about 50% total chromatophore membrane protein [2] is expected to play an important structural as well as functional role in the photosynthetic membrane. Knowledge of the position of LHP in the membrane should help to understand the mechanism of excitation transfer within the antenna pigment and to the photoactive reaction centers.

In this investigation enzymatic iodination [3–5] has been used to localize the LHP within the chromatophore membrane. The sequential release of this polypeptide with successive detergent extractions of labeled chromatophores was also investigated. The results of these experiments suggest that the LHP is partially exposed at the chromatophore surface. A preliminary report is in [6]. Results [7] on reaction center localization are complementary to this work.

Abbreviations: LH, light-harvesting; LHP, light-harvesting polypeptide; LDAO, lauryldimethylamine *N*-oxide; SDS–PAGE, sodium dodecyl sulfate–polyacrylamide gel electrophoresis; BSA, bovine serum albumin

2. Materials and methods

All reagents were analytical grade. The carotenoidless mutant G-9$^+$ of *Rhodospirillum rubrum* was grown in the light in 10 liter bottles at 30°C in the medium of [8] from which yeast extract and peptone were omitted. Chromatophores were obtained from washed cells by sonic disruption [9].

Iodination of intact chromatophores with KI and H_2O_2 was controlled by lactoperoxidase which inhibits the diffusion of H_2O_2 through the membrane. Chromatophores were suspended in 10 μM phosphate buffer, pH 7.0, to protein conc. 10 mg/ml. The suspension was then made 1.3 μM in lactoperoxidase and 0.1 μM in KI (~5 mCi $^{131}I^-$). Addition of H_2O_2 initiated the reaction.

The method of sequential additions [4] was employed to maintain a low concentration of H_2O_2. Each addition contained enough H_2O_2 to give final conc. 8 μM in the reaction mixture. At the end of the reaction the chromatophores were washed and dialyzed overnight. Free LHP was treated similarly in the presence of 0.3% LDAO, using the same iodine concentration relative to the protein. From the iodinated, freeze-dried chromatophores LHP was extracted with a mixture of equal volumes of chloroform/methanol. The polypeptide was separated from bacteriochlorophyll and lipid by gel filtration on Sephadex LH-60 [1].

The detergent extraction methods used to examine the sequential release of labeled LHP will be summarized here. They are detailed [1,7]. After treatment with 0.225% LDAO (extraction 1) the iodinated chromatophore suspension was centrifuged. The

supernatant is the same as the crude reaction center preparation in [7]. Pelleted reaction-center-depleted chromatophores were then extracted with 0.1% deoxycholate (extraction 2). The supernatant obtained after centrifugation still contains reaction centers [1]. The membrane sediment was then extracted twice with 0.1% LDAO (extractions 3, 4). Almost pure LH-complexes were obtained in supernatant 4. All 4 supernatants were analyzed by SDS–PAGE on 12% gels as in [10]. The gels were cut into slices of equal thickness and the radioactivity determined for each slice.

In addition to the LHP extracted into organic solvent from chromatophores, the polypeptide was also isolated from freeze-dried detergent supernatants. Identity of these preparations was checked by SDS–PAGE and amino acid analysis. This was carried out on a Beckman 121 C analyzer on samples which had been hydrolyzed for 24 h in 6 N HCl.

Protein was assayed as in [11] using BSA as a standard with SDS present in the samples.

3. Results and discussion

At first LHP was isolated from unlabeled chromatophores and then exposed to the labeling system. This polypeptide which is poor in reactive labeling sites (it contains no tyrosine and only one histidine (table 1)) was found to be labeled (fig.1a). Assuming mol.wt 14 000 and 1 histidine/polypeptide, 25–30% potentially available reaction sites became iodinated. The radioactive profile in fig.1b shows that LHP isolated from iodinated chromatophores was also labeled. Specific labeling, cpm/mg protein, of iodinated LHP isolated from labeled chromatophores is only 3–4% of that found when LHP is first extracted and then labeled. The amino acid composition of both protein preparations is identical (table 1). From the reproducible results in fig.1 it can be concluded that LHP is at least partially exposed at the chromatophore surface (i.e., the cytoplasmic face, since chromatophores are derived from vesicular intracytoplasmic membranes).

Table 1
Identity of the LHP isolated from the detergent supernatants and from chromatophores

Amino acid	Chromatophores[a]	Extraction 2[a] 0.1% DOC	Extraction 3[a] 0.1% LDAO	Extraction 4[a] 0.1% LDAO
Lys	3	3	3	3
His	1	1	1	1
Arg	7	7	7	8
Asp	6	6	7	5
Thr	8	8	8	9
Ser	7	6	8	7
Glu	13	12	13	14
Pro	5	6	5	6
Gly	6	7	6	6
Ala	9	10	9	9
Cys	0	0	0	0
Val	6	6	6	6
Met	1	1	0	1
Ile	6	6	5	5
Leu	17	15	16	17
Tyr	0	1	1	0
Phe	6	8	7	8
Trp	ND	ND	ND	ND

[a] Nearest integer of uncorrected values

These protein preparations yielded a single band on SDS–PAGE (fig.1) with the same mobility as the fast-moving component in the gels in fig.2

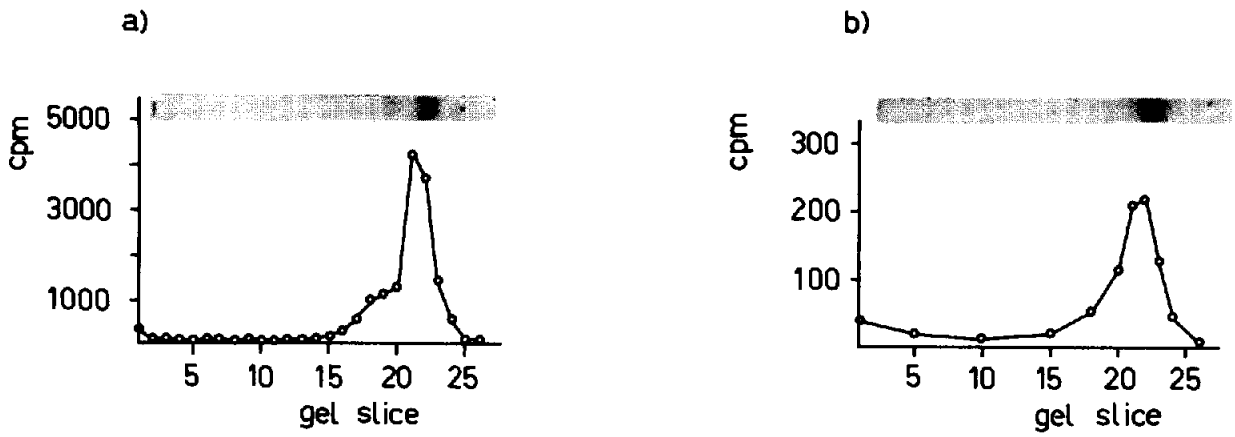

Fig.1. (a) Iodinated LHP. Specific labeling 9.7 × 10^4 cpm/mg protein. Assuming that only one iodine atom can be incorporated into each LHP molecule, 28% of the polypeptides are iodinated. (b) LHP isolated from iodinated chromatophores of *R. rubrum* G-9$^+$. Specific labeling 3.4 × 10^5 cpm/mg protein. This value is only 3.5% of the one obtained for the labeled free LHP.

The difference between the specific labeling of free and chromatophore LHP (fig.1) shows that only part of the LHP present in the intact membrane reacted with the label. The low content of specific labeling sites and the sterically-hindered accessibility of the single histidyl group to the large-mol.wt peroxidase are possible reasons for the low incorporation of ^{131}I into the LHP. This is consistent with the findings in [4,12] on the exposure of proteins at the surface of erythrocyte membranes. Using the iodine/lactoperoxidase technique as well as proteolysis it was found that only the major glycoprotein and 90 000 mol.wt polypeptide were labeled. Also a large portion of potential labeling sites on the membrane surface appeared to be inaccessible to iodine/lactoperoxidase. Calculations of the number of iodinated sites in intact membranes indicated that only ~2% of the specific reaction sites became labeled.

The data of the detergent extractions of iodinated chromatophores are presented in fig.2. Of all the polypeptides separated by SDS–PAGE the LHP band is found to contain the highest radioactivity. Identity of LHP and the fast moving polypeptide band in the gels is demonstrated in table 1. As estimated from the

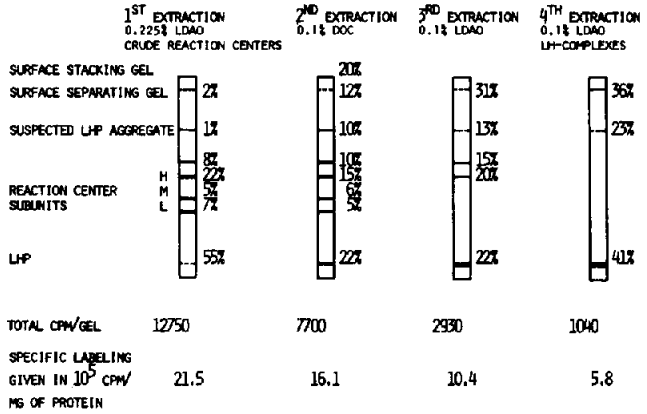

Fig.2. SDS–PAGE of the supernatants obtained after successive detergent extractions of iodinated chromatophores of *R. rubrum* G-9$^+$. Distribution of the radioactivity in the protein bands in % total counts in the gels (mean of background substracted).

density of the Coomassie brilliant blue the amount of solubilized LHP increases with the number of extractions. However, the specific labeling of the supernatants decreases with the number of extractions. This means that in the later extractions portions of unlabeled LHP are also released. This confirms the assumption that only part of the LHP present in the membrane reacts with the label. Furthermore, evidence of the surface specifity of the lactoperoxidase iodination procedure is provided.

These studies and the present data suggest that LHP is partially exposed at the cytoplasmic surface of the chromatophore membrane. This is in agreement with the successful action of α-chymotrypsin on LHP in chromatophores of the same organism as in [13]. The large portions of more weakly labeled LHP obtained after sequential detergent treatments indicate a heterogenous arrangement of LHP in the membrane surface. It is also demonstrated that selected regions of the chromatophore membrane can be released by mild detergent extractions.

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