Localization of the N-terminal regions of the B870α,β and of reaction center L polypeptides on the cytoplasmic surface of the chromatophores of *Rhodopseudomonas capsulata*

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Chromatophores (intracytoplasmic inside-out membrane vesicles) from the mutant strain Ala[+] of *Rhodopseudomonas capsulata* were treated with proteinase K in order to determine the orientation of the pigment-binding polypeptides of the photochemical reaction center and of the light-harvesting complex B870. Nine amino acid residues of the B870α polypeptide were cleaved up to position Leu[9]-Val[10] of the N-terminus and 22 residues up to position Val[22]-Tyr[23] of the β-chain N-terminus. From the reaction center L-chain the N-terminus up to position 27 (Val) was split off. The C-termini of the B870α and β polypeptides including the hydrophobic α-helical transmembrane portion remained intact. It is concluded that the N-terminal region of the pigment-binding polypeptides α and β of the B870 light-harvesting complex and of the L-chain of the reaction center point to the cytoplasm while the C-termini of the B870α,β polypeptides are exposed or pointing toward the periplasmic membrane surface.

Bacteriochlorophyll-binding polypeptide *Membrane topography* *Protease treatment* *Photosynthetic apparatus* *Membrane particle*

1. INTRODUCTION

The photosynthetic apparatus of *Rhodopseudomonas capsulata* contains 3 membrane-bound bacteriochlorophyll-carotenoid-protein complexes, the photochemical reaction center (RC) and the light-harvesting complexes B870 and B800-850 (reviews [1,2]). All 3 complexes have been isolated and characterized [2–5]. The B870 complex, which surrounds the RC [6], consists of two low-M_r polypeptides, bacteriochlorophyll (Bchl) and carotenoid in a molar ratio of 1:1:2:1 [2]. From the primary structure [7,8] and its evaluation [2] it has been suggested that each polypeptide spans the membrane once with the α-helical, hydrophobic region. Similar results have been obtained with B870 and B800-850 pigment-binding polypeptides from *Rps. capsulata* and other species [2,9–12]. The amino acid sequence of the RC polypeptides of *Rps. capsulata* has been deduced from the DNA sequence [13]. For understanding of the membrane topography and the assembly process it is important to know which portions of the polypeptides are exposed on the membrane surface. Here, the orientation of the B870α,β polypeptides and of the reaction center L-chain will be described.

Dedicated to Hans Grisebach on the occasion of his 60th birthday

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2. MATERIALS AND METHODS

2.1. *Organisms and culture conditions*

Rps. capsulata strain Ala[+] (B800-850[−],crt[−]) [3] was grown anaerobically in the light.

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2.2. *Preparation of chromatophores*

Chromatophores were prepared as in [14]. Their integrity to proteinase K was controlled by measuring the content of cytochrome c_2 trapped inside the membrane vesicles [15].

2.3. *Protease digestion*

2 ml of chromatophores (0.94 mg Bchl/ml and 12 mg/ml of protein as judged by the Lowry test) were diluted with 8 ml Tris-HCl (20 mm, pH 7.8) buffer and incubated at 30°C with 0.4 ml proteinase K solution (2.2 mg/ml). The enzyme:protein weight ratio was 1:25. An incubation time of 55 min was chosen. Enzyme digestion was terminated by adding 25 μl of 220 mM phenylmethylsulfonyl fluoride in ethanol to 1 ml of the incubation mixture.

2.4. *Isolation and purification of polypeptides from protease-treated chromatophores*

The protease-treated and untreated chromatophores were freeze-dried and extracted with chloroform-methanol-ammonium acetate (4 ml per 70 mg material) and the extract subjected to a Sephadex LH60 (Pharmacia) column (2.6 × 98 cm) and eluted at 10°C with the same solvent as described [16].

2.5. *Polyacrylamide gel electrophoresis*

Samples were solubilized by heating in dodecyl sulfate sample buffe (30 min at 70°C) and electrophoresed on dodecyl sulfate/polyacrylamide slab gels using an 11.5–16.5% acrylamide gradient [7].

2.6. *Amino acid analysis*

Proteins were hydrolyzed [16] and the amino acid composition determined using an automatic amino acid analyser (Durrum D-500). Tryptophan and cysteine were determined by established methods [17,18].

2.7. *Digestion with carboxypeptidase*

To determine the carboxyl-terminal sequences, carboxypeptidase Y, A or B (Boehringer, Mannheim) was used as described [7,9].

2.8. *Sequence analysis*

Automated Edman degradation was performed in a self-constructed gas-liquid phase microsequencer [19]. The sequencer was equipped with an on-line HPLC system which allowed direct identification of the PTH-amino acids [20]. All peaks containing proteins obtained from the column were analyzed.

3. RESULTS

3.1. *Protease digestion*

To determine the conditions for protease K treatment, which allows near complete digestion of the membrane surface-exposed portion of the membrane without disturbing membrane integrity, small- scale experiments were performed. Chromatophores were treated with protease K as described in section 2 for 0–75 min. The area of the 800 nm absorption peak decreased during this period from 3.1 to 1 (not shown). Up to 95% of B870α and β polypeptides were cleaved after 55 min as revealed by Edman degradation and SDS-polyacrylamide gel electrophoresis patterns (not shown). Large-scale experiments were subsequently performed as described in section 2 and the degradation products isolated by chromatography.

3.2. *Characterization of peak I fraction*

Fractionation of organic solvent extract of untreated chromatophores on Sephadex LH60 yielded a fraction in the void volume (peak I in fig.1a). This peak contains polypeptides H, L and M of the RC [9,10]. Fractionation of the organic solvent extract of chromatophores treated with proteinase K yielded a void volume peak (peak I, fig.1b). This peak contains mainly a polypeptide which migrates as a major band in SDS-polyacrylamide gel electrophoresis with an apparent M_r of 16000. The N-terminal amino acid sequence of this peptide was determined by automated Edman degradation as Gly-Pro-Phe-Tyr-Val-Gly-Phe-Phe-Gly-Val---. This sequence is identical to a segment (starting with position 28) of the N-terminus of the L-chain of the RC [13]. Peak I (fig.1b) contained besides this major sequence another very minor sequence of 5 other peptides for which no clear amino acid sequence could be identified.

3.3. *Characterization of peak II and III fractions*

Fractions II and III of the chromatographed solvent extract from protease K-treated chromatophores were identified as degradation products of

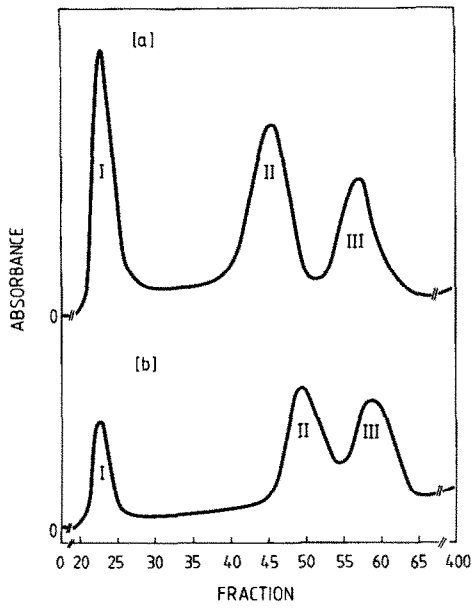

Fig.1. Elution diagram of the chloroform-methanol-ammonium acetate extracts from proteinase K-treated or untreated membrane (see section 2) applied to a Sephadex LH60 column (2.6×98 cm). The extract was prepared from lyophilized chromatophores of *Rps. capsulata*, strain Ala$^+$, which had previously been treated with proteinase K (b); control profile, untreated chromatophores (a). For identification of each polypeptide peak in a and b, see text.

the B870α and β polypeptides. The N-terminal sequence of the peak II fraction was Val-Phe-Asp-Pro-Arg-Arg-Val-Phe-Val-Ala---. This is the N-terminal sequence of the B870α polypeptide beginning with position 10 [8]. The amino acid composition of the fraction II (fig.1b) indicated that all remaining 49 amino acid residues were present. To confirm these data C-terminal analysis of the fragment (peak II, fig.1b) and the whole polypeptide (peak II, fig.1a) was performed as described in section 2. Carboxypeptidase Y treatment resulted in the same degradation patterns revealing the C-terminal sequence Ala-Gln which was characterized as the C-terminal end of the B870α polypeptide [8].

A similar analysis of peak fraction III (fig.1b) revealed the following sequence: Tyr-Met-Ser-Gly-Leu-Ser-Ala-Phe-Ile-Ala-Val-Ala-Val--- which corresponds to the sequence of polypeptide B870β beginning with position 23 [7]. The amino acid composition of the degraded polypeptide yielded the amino acid composition of the B870β polypeptide minus its cleaved N-terminal fragment. To confirm these data, C-terminal analysis of the fragment (peak III, fig.1b) and the whole polypeptide (peak III, fig.1a) by carboxypeptidase A and/or B treatment was performed and resulted in the same degradation patterns revealing the C-terminal sequence Trp-Phe which was characterized as the C-terminal end of the B870β polypeptide [7].

These results demonstrate that the B870α and β polypeptides were not attacked by proteinase K within the hydrophobic membrane-spanning including the C-terminal region under our experimental conditions.

4. DISCUSSION

Studies on the arrangement of the light-harvesting B870α and β and the RC polypeptides became possible after their primary structure had been elucidated [7,9,10–13]. Proteinase K experiments showed that at least 90% of the proteins was cleaved. Nine and 22 amino acid residues are removed from the N-terminal regions of the B870α and β polypeptides, respectively. Amino acid determinations and carboxypeptidase digestion demonstrated that the central hydrophobic stretch including the C-terminal regions of the two polypeptides (B870α and β) were not affected by proteinase K treatment. This shows clearly that the N-termini of B870α and β subunits are exposed at the cytoplasmic side of the membrane and that the C-terminal segments are on or pointed toward the periplasmic side. These data confirm the hypothesis that during assembly of the B870 complex the polypeptide chains are folded and rearranged in such a way that the N-termini become exposed on the cytoplasmic surface [2]. Interestingly, this is not only the case for both polypeptides of the B870 complex of *Rps. capsulata* but also for *Rhodospirillum rubrum* B870 polypeptides [21]. The exposed N-terminal regions of the *Rps. capsulata* B870α and β polypeptides contain charged amino acids, and it is possible that the basic residues of the B870α [8] could interact with the acidic amino acids of the B870β providing a strong force for association of the α,β-chains, formation of oligomers of B870 subunits and B870-RC association [2,5,6]. The absorption spectrum of the membrane

was still intact after proteinase K treatment although the sensitive NIR peaks were lowered. This might indicate that the conformation of the B870α and β polypeptides is not markedly affected by the removal of the 9 N-terminal amino acids in the B870α subunits and the 22 N-terminal amino acids in the B870β subunit. The conserved histidines (His^{32} in B870α [8] and His^{38} in B870β [7]), which are buried within the membrane, are proposed to function as a ligand for the Mg^{2+} of the Bchl ring [22]. Furthermore, a recent X-ray structure analysis of the *Rps. viridis* RC indicates that protein side chains are bound to the special pair of Bchls [23]. In both the B800-850 and B870 complexes [7–12,21], a third histidine (His^{20} in the B870 β-chain [7]) is conserved. It could coordinate a third Bchl which, according to the proteinase K digestion, is located on the cytoplasmic side of the membrane and surrounded by protein and/or lipid near the phosphate group cytoplasmic interface. Its orientation would be different from that of the remaining two chlorophylls which are buried within the membrane.

From the RC we were able to identify a fragment which corresponds to the L-chain after protease K treatment. 27 residues were removed from the N-terminal region which are supposed to be on the cytoplasmic surface of the membrane.

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