Orientation of the B800–850, B870, and Reaction Center Polypeptides on the Cytoplasmic and Periplasmic Surfaces of *Rhodobacter capsulatus* Membranes†

Monier Habib Tadros,*,‡,§ Rainer Frank,§ Bernhard Dörge,‡ Nasser Gad'on,‡ Jon Y. Takemoto,∥ and Gerhart Drews‡

Institute of Biology 2, Microbiology, Albert Ludwigs University, D-7800 Freiburg, Federal Republic of Germany, European Molecular Biology Laboratory, D-6900 Heidelberg, Federal Republic of Germany, and Department of Biology, Utah State University, Logan, Utah 84322-0300

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ABSTRACT: Chromatophores (inside-out vesicles) and spheroplasts (right-side-out vesicles) of the photosynthetic bacterium *Rhodobacter capsulatus* were treated with proteinase K. The pigment-binding membrane polypeptides of the photochemical reaction center (RC) and of the light-harvesting (LH) complexes B870 and B800–850 were isolated from untreated and protease-treated membranes, and the N- and C-terminal amino acid sequences were determined. Under conditions of proteinase K treatment, which left the in vivo absorption spectrum and the membrane intact, specific portions of the N- and C-termini of the polypeptides were removed. Twenty-six and 48 N-terminal amino acyl residues were cleaved off of RC-L and RC-M, respectively, and RC-H was digested when the chromatophores were treated with proteinase K. It was concluded that the H-subunit of RC and the N-terminal regions of the L- and M-subunits of RC are exposed on the cytoplasmic side of the membrane. Sixteen amino acyl residues were removed from C-terminal regions of both the B870 α and B800–850 α polypeptides by protease digestion of spheroplasts. The C-terminal regions of these polypeptides therefore seem to be exposed on the periplasmic side of the membrane. The N-terminal regions of the α polypeptides were protected from protease treatment on the cytoplasmic side of the membrane. Four and nine amino acyl residues from the β polypeptides of both LH complexes were removed from the cytoplasmic side of the membrane. No amino acyl residues were removed from the C-terminal regions, presumably because the C-terminal regions of the β polypeptides are buried in the membrane or are otherwise protected from protease digestion. From these and published experimental data it is concluded that the N-termini of all pigment-binding polypeptides of LH complexes are exposed or localized to the cytoplasmic surface of the membrane, while the C-termini are exposed or localized to the periplasmic membrane surface. The importance of that for organization and assembly of the pigment–protein complexes is discussed.

The photosynthetic apparatus of *Rhodobacter capsulatus* is localized within an intracytoplasmic membrane system that consists of interconnected vesicles (chromatophores), formed by invagination of the cytoplasmic membrane (Drews & Oelze, 1981). These membranes contain several bacteriochlorophyll (Bchl)–carotenoid–protein complexes. The light-harvesting (LH) complexes are named by their near infrared in vivo absorption maxima as B800–850 and B870, respectively (Drews & Oelze, 1981; Drews, 1985). Light energy absorbed by the B800–850 antenna complexes creates mobile electronic singlet states, called excitons, which migrate by random walk through other antennae via B870 and are trapped in reaction centers (RC) (Monger & Parson 1977; Drews, 1985) where charge separation takes place. The pigments in each of the complexes are bound stoichiometrically to two different polypeptides, which are small in the LH complexes (M_r 4000–8000). These pigment-binding polypeptides, designated α and β, interact with two or three Bchl and one or two carotenoid molecules (Drews, 1985). The B870 complexes are believed to surround RC's, while aggregates of B800–850 complexes surround and interconnect the RC–B870 complexes (Monger & Parson, 1977; Drews, 1985; Takemoto et al., 1985). Recently, the amino acid sequences of the α and β polypeptides of the B870 and B800–850 complexes have been determined (Tadros et al., 1983, 1984, 1985a,b), and the amino acid sequence of RC polypeptides was deduced from the DNA sequences (Youvan et al., 1984). The polypeptides of the light-harvesting complexes each have one central domain consisting of 20 hydrophobic amino acids that are believed to form a transmembrane α-helix (Drews, 1985; Breton & Nabedryk, 1984; Cogdell & Scheer, 1985).

Bchl is bound by its Mg to a histidine residue (Robert & Lutz, 1985) and by other noncovalent bonds to the protein. The flanking N- and C-terminal domains contain hydrophilic, hydrophobic, and charged amino acids that are presumed to be exposed and localized to the membrane surfaces (Drews, 1985). Support for this proposed topography was provided by a recent study using hydrophobic photolabeling of *Rhodospirillum rubrum* G-9 chromatophores (Meister et al., 1985). Labeling occurred both within the N-terminal segments and within the hydrophobic transmembrane stretches of these polypeptides. These observations indicated that association of the transmembrane helices of the B870 α and β chains occurred and further suggested that an amphipathic domain was present at the N-terminal region on the cytoplasmic side of the membrane. The latter presumably interacts with the water–lipid interface of the membrane (Meister et al., 1985). Recent studies with the mutant strains Y5 (RC, B870-negative) and Ala$^+$ (B800–850-negative) of *Rhodobacter capsulatus* showed that the N-termini of the B800–850 and B870 α and β polypeptides were exposed on the cytoplasmic surface

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* Address correspondence to this author at Albert Ludwigs University.
‡ Albert Ludwigs University.
§ European Molecular Biology Laboratory.
∥ Utah State University.

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of chromatophores (Tadros et al., 1986a,b). The basic units of the LH complexes assemble to form oligomeric structures (Drews, 1985).

The RC also contains two pigment-binding polypeptides having M_r's of about 30 000 (Youvan et al., 1984). These L and M polypeptides span the membrane 5 times by hydrophobic α-helical domains (Youvan et al., 1984; Deisenhofer et al., 1985). The L and M chains are believed to be exposed on the cytoplasmic membrane surface, as suggested by surface labeling (Peters & Drews, 1983) and protease treatment (Peters & Drews, 1984). A third polypeptide in the RC, the H subunit, does not bind pigments and is predominantly hydrophilic (Youvan et al., 1984).

In the present study the wild-type strain was used in order to study interactions between the polypeptide chains when both LH complexes were present. In addition to the topography of the N-terminal regions, the localization of the C-terminal portions of the chains was investigated by proteinase K treatment of chromatophores (inside-out vesicles) and spheroplasts (right-side-out vesicles).

Materials and Methods

Cultivation of Bacteria and Preparation of Chromatophores. The wild-type strain 37b4 of *Rhodobacter* (*R.*) *capsulatus* (formerly *Rhodopseudomonas capsulata*), DSM938, was used in this study. The cells were grown phototrophically in a malate–yeast extract–mineral medium (Drews, 1983). A bench-top 14 l fermentor (Microferm, New Brunswick), filled with 12 l RÄH medium (Drews, 1983), was inoculated with a preculture from a 50-mL bottle, gassed with pure nitrogen (99.99%) for 5 min, and incubated for 24 h at 33 °C under illumination with 6 × 200 incandescent bulbs. The cells were harvested in the late exponential growth phase, washed with 50 mM tris(hydroxymethyl)aminomethane (Tris) buffer (pH 7.8), and disrupted in a French pressure cell at 92 MPa in the presence of 10 μg/mL deoxyribonuclease and 0.1 mM phenylmethanesulfonyl fluoride (PMSF). Cells and cell debris were removed from the extract by centrifugation in a Sorvall RC2-B centrifuge (25 min, 23000*g*). The supernatant was layered on top of a discontinuous sucrose gradient (0.6–1.5 M sucrose in the Tris buffer). After 17 h of centrifugation at 130000*g* in the Ti 60 rotor, the chromatophores fraction was isolated with a hypodermic syringe. The chromatophores were washed in 20 mM Tris buffer and used immediately.

Preparation of Spheroplasts. Freshly harvested cells were suspended in 50 mM Tris-HCl buffer, pH 7.6, containing 1.5 M sucrose and 3 mM ethylenediaminetetraacetic acid (EDTA). The cells were treated with lysozyme (Fluka, 10 μg/μg of Bchl) for 30 min at 37 °C. Proteinase K (Merck, Darmstadt) was added to a final concentration of 1.5 mg/mg of Bchl, and the sample was incubated at 30 °C. After various time intervals, portions were removed, and the reaction was stopped with 1 μg of PMSF/mg of Bchl. Absorption spectra were recorded with material diluted in 1.5 M sucrose buffer.

After proteinase treatment, spheroplasts were sedimented at 6000 rpm (rotor SS34, Sorval) and disrupted by osmotic shock. Membrane-derived vesicles were obtained by ultrasonic treatment of the material and separated by sucrose density gradient (30–60% sucrose in Tris buffer) centrifugation for 18 h at 35 000 rpm (Ti 60 rotor, Beckman).

Treatment of Chromatophores with Proteinase K. Chromatophores in Tris-HCl buffer (20 mM, pH 7.6) were mixed with proteinase K (final concentration 1 mg of protease/1.22 mg of Bchl) and incubated at 30 °C. After various time intervals, aliquots were removed, and the reaction was stopped with PMSF (383 μg of PMSF/62 μg of protease K).

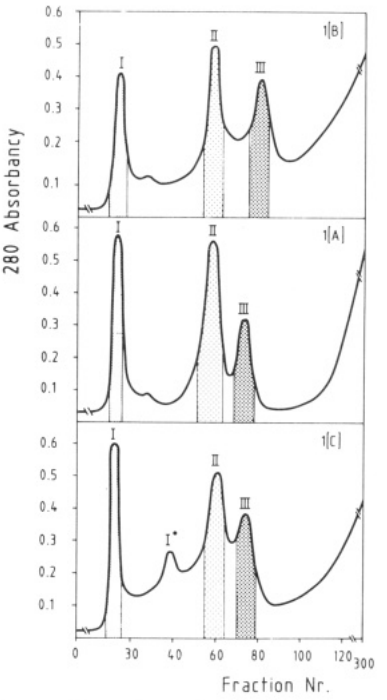

FIGURE 1: Column chromatography of chloroform–methanol–ammonium acetate extract of lyophilized membranes on Sephadex LH60 using the same solvent as for extraction (see Material and Methods): (A) membranes of untreated chromatophores (control); (B) membranes of chromatophores treated with proteinase K; (C) membranes of spheroplast-derived vesicles treated with proteinase K. Flow rate, 8 mL/h; 400 fractions of 6 mL were collected. Three major fractions were obtained. Fraction I contained RC polypeptides, fraction II contained the α polypeptides of B870 and B800–850, and fraction III contained the β polypeptides of B870 and B800–850. Fraction I* is an unknown protein.

Analytical Measurements. Bchl concentrations were calculated from absorption measurements in methanol–acetone (7:2 v/v) extracts at 770 nm by using the absorption coefficient of 76 cm^{-1} mM^{-1} (Clayton, 1966). Membrane proteins were separated by sodium dodecyl sulfate (SDS)–polyacrylamide gel electrophoresis on 1-mm slab gels (Laemmli, 1970) with a 11.5–16.5% continuous gradient of acrylamide. Gels were stained with Coomassie brilliant blue.

Isolation and Purification of B800–850 and B870 α and β Polypeptides. Untreated and proteinase K treated chromatophores and spheroplasts were freeze-dried and extracted with 15–20 mL of chloroform–methanol (1:1)–ammonium acetate (0.1 M) per 70 mg of protein. The extract was applied to a Sephadex LH60 column (2.6 × 120 cm), equilibrated with chloroform–methanol–ammonium acetate, and eluted with the same solvent. Three major fractions (absorption at 280 nm) were isolated (Figure 1). The mixture of polypeptides present in fraction II (about 40 mL) was concentrated to 2.2 mL by rotary evaporation and mixed with chloroform–methanol (1:1 v/v) containing 40 vol % acetic acid at 0 °C. The mixture was applied to a Sephadex LH60 column (2.6 × 120 cm). The column was equilibrated and eluted with chloroform–methanol (1:1 v/v) containing 0.1% ammonium acetate (w/v) and 20% (v/v) acetic acid.

Two fractions were pooled (Figure 2 and Results). Fraction 1 contained the B870 α polypeptide, while fraction 2 contained the B800–850 α polypeptide. Both fractions were rechromatographed on Sephadex columns.

(Diethylaminoethyl)cellulose (DEAE-cellulose) (Whatman DE 52) was treated with concentrated acetic acid and then with concentrated NH_4OH. The precycled DEAE-cellulose was washed with chloroform–methanol (1:1) and chloroform–

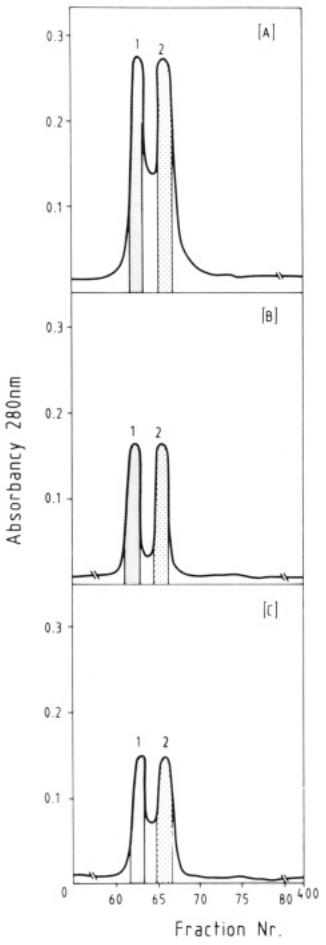

FIGURE 2: Separation of the α polypeptide (peak fraction II, Figure 1) by chromatography on LH60 using chloroform–methanol–ammonium acetate–acetic acid (20 vol %). Flow rate, 7 mL/h; 400 fractions of 5 mL were collected. Peak 1, B870 α; peak 2, B800–850 α.

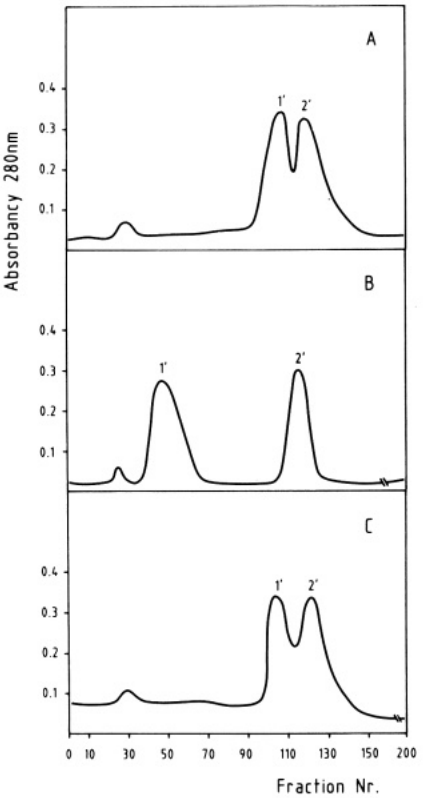

FIGURE 3: Purification of polypeptides (peak fraction III, Figure 1A–C) employing DEAE-cellulose column eluted with chloroform–methanol–ammonium acetate (fraction 1′) or the solvent containing 5% acetic acid (fraction 2′). Fraction 1′, purified B800–850 β polypeptide, uncleaved (A, C) and cleaved (B, Figure 1). Fraction 2′, purified B870 β polypeptide, uncleaved (A, C) and cleaved (B, Figure 1).

methanol–ammonium acetate (0.1 M). Columns (2.2 × 35 cm) were packed with a pressure of 70 kPa. They were regenerated with 3 column volumes of chloroform–methanol–ammonium acetate containing 20 vol % acetic acid and equilibrated with the same solvent without acetic acid. All columns were run with hydrostatic pressure. Flow rates were adjusted with the operating pressure.

Fraction III from the first Sephadex LH60 column (Figure 1A) was applied to a DEAE-cellulose column. The column was washed with 1 column volume of chloroform–methanol–ammonium acetate. Elution with the same solvent containing 5% acetic acid resulted in the protein fractions 1′ and 2′ (Figure 3A). This purification scheme was also used for proteinase K treated chromatophores and spheroplasts; however, peak 1 eluted during the wash, while peak fraction 2 eluted after the addition of 5% acetic acid to the solution (Figure 3B,C). Fraction 1′ contained the B800–850 β polypeptide, while fraction 2′ contained the B870 β polypeptides. Both fractions were rechromatographed on Sephadex LH60 columns using chloroform–methanol–ammonium acetate containing 20% acetic acid until purity was achieved.

Amino Acid Composition and Sequence Analysis. The proteins were hydrolyzed (Tadros et al., 1985b), and the amino acid composition was determined by using a Durrum D-500 automatic amino acid analyzer. Automated Edman degradation was performed in a gas–liquid phase microsequencer (Tadros et al., 1986b). The sequencer was equipped with an on-line high-performance liquid chromatography (HPLC) system and a Triuector chromatography data system which allowed the direct identification and quantitative determination of the PTH amino acids. Digestion of polypeptides with carboxypeptidase Y, A, and B (Boehringer, Mannheim) was performed as described previously (Tadros et al., 1984).

RESULTS

Proteinase K Treatment of Chromatophores and Spheroplasts: Effects on Absorption Spectra and Protein Patterns in SDS–Polyacrylamide Gels. In chromatophores treated with proteinase K the B800–850 γ polypeptide was degraded within the first 10 min (Figure 4A). The absorption spectrum showed only minimal changes in the peak heights after 25 min of treatment but no shift in the position of the maxima (Figure 5A). This result and earlier observation suggest that the γ polypeptide of the B800–850 complex does not bind pigment (Feick & Drews, 1979). After 25 min of proteinase K treatment, the α polypeptides of the B800–850 and B870 antenna complexes still migrated at the same positions in the SDS–polyacrylamide gel. A small broadening of bands was observed. The β polypeptides of both complexes seemed to be partially degraded, as visualized by broadening of the protein bands (Figure 4A). The absorption spectrum showed a relative decrease but no shift of the 800-nm peak to shorter wavelengths (Figure 5A). The membranes were still intact as shown by the criteria described in Tadros et al. (1986a,b). These conditions were selected for large-scale preparations.

The RC polypeptides were also degraded by proteinase K. In accordance with earlier observations (Peters & Drews, 1984) a decrease of H- and M-subunit bands and the formation of new bands were observed (Figure 4A). The L-

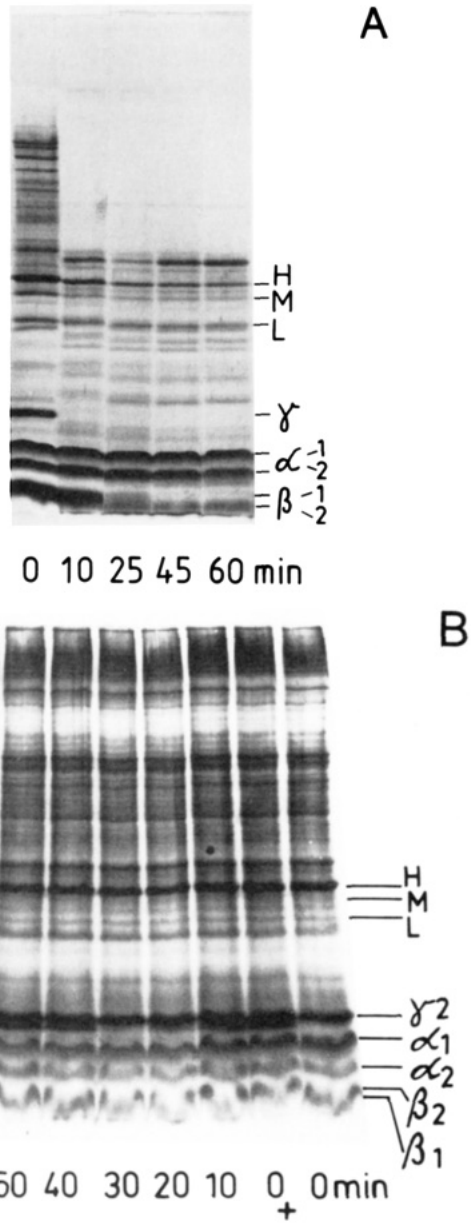

FIGURE 4: SDS–polyacrylamide gel electrophoresis of membranes treated with proteinase K (see Materials and Methods). (A) Chromatophores treated for different times (abscissa) with proteinase K; H, L, and M RC subunits; α and β polypeptides of light-harvesting complexes, 1 = B870, 2 = B800–850. (B) Spheroplasts, explanation as in (A).

subunit was only slightly modified in size (Figure 4A).

When spheroplasts (right-side-out vesicles) were treated with proteinase K, the B800–850 α and β polypeptides seemed to be nearly unchanged during an exposure period of 25 min. The β polypeptides of both complexes were not well resolved on polyacrylamide gels, but there seemed to be no clear change in the mobility over 25 min of treatment (Figure 4B). The RC polypeptides appeared to be unchanged. The absorption spectrum of spheroplasts showed changes similar to those in chromatophores: a small decrease in the height of the 800- and 855-nm peaks but no shift in the maxima (Figure 5B). For large-scale preparations at 25-min period of protease K treatment was selected. The pigment-binding polypeptides were completely digested by proteinase K when the membranes were solubilized by Triton X-100 (not shown). This is a control that the proteins are accessible to proteinase K digestion. If the proteins are not cleaved, they must be protected from protease attack.

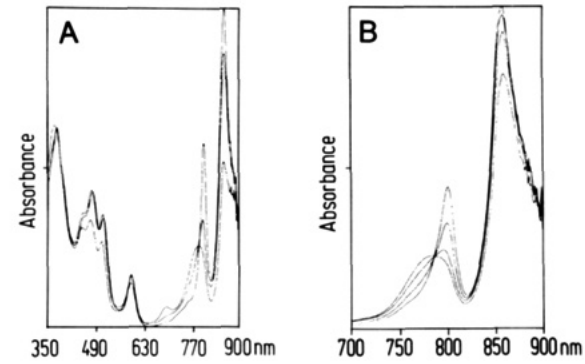

FIGURE 5: Absorption spectra of chromatophores and spheroplasts treated for different times with proteinase K: (A) chromatophores treated for 1, 25, and 75 min with proteinase K; (B) spheroplasts treated for 0, 25, 35, and 60 min with proteinase K. The most sensitive peaks to proteinase K digestion are the peaks at 800 and 850 nm.

Identification of the Degradation Products. Characterization of Peak Fraction I. Chromatophores and spheroplasts, untreated or treated with proteinase K, were freeze-dried and extracted with chloroform–methanol–ammonium acetate. The organic solvent extract of untreated chromatophores was fractionated by column chromatography on Sephadex LH60 as described under Materials and Methods. The peak I fraction from the void volume (Figure 1) was analyzed by automatic sequence determination. Two major components having the N-terminal sequence Ala-(Leu/Glu)-(Leu/Tyr)-(Ser/Gln)-(Phe/Asn)-(Glu/Phe)-(Arg/Phe)-(Lys/Asn)-(Tyr/Gln)-(Arg/Val)... and a very minor component having the sequence Met-Val-Gly-Val-Asn-Phe-Phe-Gly-Asp... were obtained. The published sequence data from reaction center polypeptides (Youvan et al., 1984) allowed identification of peak fraction I fragments as belonging to the L-, M-, and H-subunits of the RC. These proteins have the N-terminal sequence

L: NH_2-Ala-Leu-Leu-Ser-Phe-Glu-Arg-Lys-Tyr-Arg...

M: NH_2-Ala-Glu-Tyr-Gln-Asn-Phe-Phe-Asn-Gln-Val...

H: NH_2-Met-Val-Gly-Val-Asn-Phe-Phe-Gly-Asp-Phe...

From a comparison with the DNA sequence data (Youvan et al., 1984), it is evident that the N-terminal Met residues of the L- and M-subunits are removed from the polypeptides before assembly. Since the H-subunit is poorly soluble in the organic solvent, only a minor amount of this protein was obtained from peak fraction I.

Fractionation of the organic solvent extract from proteinase K treated spheroplasts by column chromatography yielded a peak I fraction in the void volume (Figure 1C) that showed N-terminal sequences identical with those of the intact L-, M-, and H-polypeptides of RC isolated from untreated chromatophores. We conclude from these results that the N-terminal regions of the RC polypeptides were not digested by proteinase K during the 25-min incubation period under the conditions used when exposed on the periplasmic side of the membrane (right-side-out vesicles).

Completely different results were obtained when chromatophores (inside-out vesicles) were treated with proteinase K. The peak fraction I (void volume of LH60 chromatography) contained two major fragments having the sequence Gly^{27}-Pro-Phe-Tyr-Val-Gly-Phe-Phe-Gly-Val-Thr.... This sequence is identical with a segment starting with position 27 of the N-terminal region of the L-chain of the RC. The second major sequence was $Ile\text{-}Tyr^{49}$-Leu-Gly-Ile-Ala-Gly-Thr-Val-Ser-Leu..., which is identical with a segment starting with position

Table I: Amino Acid Composition (Molar Ratios) of Pigment-Binding Polypeptides B870 α and B800–850 α Isolated from Protease K Treated and Untreated Chromatophores (Uncleaved Proteins) and Proteinase K Treated (Cleaved Proteins) and Untreated Spheroplasts[a]

	chromatophores B870 α uncleaved			spheroplasts B870 α cleaved			chromatophores B800–850 α uncleaved			spheroplasts B800–850 α cleaved		
	A	*B*	*C*	*A*	*B*	*D*	*A*	*B*	*C*	*A*	*B*	*D*
Asp	1.98	2	1	2.02	2	0	5.86	6	0	4.08	4	2
Asn			1						6			
Thr	2.82	3	3	0.86	1	2	5.82	6	0	4.84	5	1
Ser	1.62	2	2	1.76	2	0	0.71	1	1	0.60	1	0
Glu	1.94	2	0	1.12	1	1	0.98	1	0	0.48	0–1	0–1
Gln			2						1			
Pro	1.76	2	2	1.94	2	0	3.74	4	4	2.23	2	2
Gly	1.98	2	2	0.87	1	1	4.66	5	5	3.76	4	1
Ala	7.99	8	8	2.92	3	5	10.89	11	11	7.02	7	4
Val	6.64	7	7	4.78	5	2	6.78	7	7	3.71	4	3
Met	0.98	1	1	0.88	1	0	1.82	2	2	0.84	1	1
Ile	2.59	3	3	2.79	3	0	3.74	4	4	3.82	4	0
Leu	8.64	9	9	7.72	8	1	4.68	5	5	4.76	5	0
Tyr	1.76	2	2	0.82	1	1	0.89	1	1	0.82	1	0
Phe	5.86	6	6	5.94	6	0	1.00[b]	1	1	0.76	1	0
Trp	1.87	2	2	1.44	1–2	1–0	2.87	3	3	1.00	1	0
Lys	2.88	3	3	1.92	2	1	1.94	2	2	1.51	1–2	1–0
Arg	2.00[b]	2	2	2.00[b]	2	0	[c]	[c]	0	[c]	0	0
His	1.56	2	2	0.61	1	1	0.59	1	1	0.62	1	
Cys	ND		0				ND		0	ND		0
total			58		42–43	15–16		60			44–45	15–16
polarity (%)			28					28.3				
net charge			4+		3+			2+			2+	

[a] Tryptophan was determined by the method of Liu and Chan (1971); cysteine was not determined. The values for Ala, Val, Ile, Leu, Phe, and His were obtained after 80 h of hydrolysis. All other determinations are the mean values obtained following hydrolysis for 24/48 h. *A* = molar ratio; *B* = integral number; *C* = the number of residues found by sequencing of undigested polypeptides; *D* = differences between uncleaved polypeptides and proteinase K cleaved polypeptides; ND = not determined. [b] The calculation of molar ratios is based on the indicated amino acyl residue (Arg = 2.00). [c] Below 0.1 nmol.

49 of the N-terminus of the M-subunit of RC. Besides these major components, two other minor components were found in peak fraction I, but no amino acid sequence could be identified. We conclude from these results and the kinetics of digestion shown in Figure 4A that the N-terminal regions of the H-, L-, and M-subunits of RC are exposed on the cytoplasmic side of the membrane.

Fraction I* (Figure 1C) in extracts from proteinase K treated spheroplasts has the N-terminal sequence (Asn/Ile)-(Tyr/Arg)-(Lys/Tyr)-Asn-(Val/Pro).... The polypeptide in this fraction has not been identified.

Characterization of Peak Fraction II. Material from peak fraction II (Figure 1A), obtained from LH60 chromatography of organic solvent extract of untreated chromatophores, had the N-terminal amino acyl sequence NH_2-Met-Asn-Asn-Ala-Lys-Ile-Trp-Thr-Val..., which is characteristic of the B800–850 α polypeptide (Tadros et al., 1983). After fraction II was deblocked with HCl–chloroform–methanol (Tadros et al., 1985a), the N-terminal sequence was Met-(Asn/Ser)-(Asn/Lys)-(Ala/Phe)-(Lys/Tyr)-(Ile/Lys)-(Trp/Ile)-(Thr/Trp)-(Val/Leu), of which the second sequence corresponds to the N-terminal sequence of the B870 α polypeptide (Tadros et al., 1985a). Fraction II was applied to a Sephadex LH60 column and separated into fractions 1 and 2 as described. Rechromatography of peak fractions 1 and 2 yielded pure fractions of the B870 α and B800–850 α polypeptides as revealed by N-terminal sequence analysis. The amino acid composition of the two polypeptides is given in Table I.

The same method as described in the previous paragraph was used to purify the α polypeptides from proteinase K treated spheroplasts (Figure 2C). Identical N-terminal sequences as shown for the peak fractions 1 and 3 from untreated chromatophores were obtained. Thus the N-terminal regions of B870 α and B800–850 α polypeptides were not accessible to proteinase K digestion from the periplasmic side of the membrane.

The polypeptides B870 α and B800–850 α of proteinase K treated chromatophores were isolated by the same method as described in the previous paragraph. We were surprised to find that the N-terminal sequences of treated and untreated chromatophores wer identical, suggesting that the N-terminal regions of these α polypeptides were not degraded.

These results differ from those obtained with mutant strains of *R. capsulatus* (Tadros et al., 1986a,b).

C-Terminal analysis (see Materials and Methods) of peak fraction 1 from untreated chromatophores and from chromatophores treated with proteinase K revealed the C-terminal sequence Ala-Gln, which is the C-terminus of B870 α (Tadros et al., 1985b). The results showed that the C-terminus of B870 α is not degraded by proteinase K when isolated from chromatophores. If, however, fraction 1, isolated from proteinase K treated spheroplasts, was analyzed by treatment with carboxypeptidase Y, A, and/or B, the C-terminal sequence Ala-Phe-Asn was obtained. Also, small amounts of tryptophan and leucine were liberated. It is concluded that the C-terminus of polypeptide B870 α is accessible to proteinase K digestion and is therefore exposed on the periplasmic side of the spheroplast-derived vesicle.

Fraction 2 (B800–850 α polypeptide) was also subjected to C-terminal analysis. Untreated and treated chromatophores exhibited the same C-terminal sequence of Ala-Gln, characteristic of intact B800–850 α polypeptide. If, however, the same polypeptide, isolated from proteinase K treated spheroplast, was digested with carboxypeptidase Y, A, and/or B, the C-terminal sequence Ala-Asn-Tyr (A:N:Y = 0.9:0.95:1) was obtained. Small amounts of the amino acids Trp and Asn were also liberated by carboxypeptidase treatment. The results show that in spheroplasts the C-terminus of the B800–850 protein is exposed on the periplasmic surface of the membrane. The results from the amino acid analysis of B800–850 α polypeptide also confirmed the proposed digestion patterns of proteinase K (Table I).

Table II: Amino Acid Composition (Molar Ratio) of the Polypeptides B807 β and B800–850 β Isolated from Untreated and Proteinase K Treated (Cleaved) Chromatophores[a]

	chromatophores B800–850 β uncleaved			chromatophores B800–850 β cleaved			chromatophores B870 β uncleaved			chromatophores B870 β cleaved		
	A	*B*	*C*	*A*	*B*	*D*	*A*	*B*	*C*	*A*	*B*	*D*
Asp	2.89	3	3	1.08	1	2	3.92	4	3	1.86	2	2
Asn			0						1			
Thr	2.96	3	3	1.92	2	1	1.86	2	2	1.92	2	0
Ser	4.82	5	5	4.44	4–5	1–0	2.68	3	3	2.67	3	0
Glu	2.87	3	3	2.98	3	0	3.96	4	2	4.08	4	0
Gln			0						2			
Pro	1.69	2	2	0.89	1	1	1.19	1	1	0.94	1	0
Gly	5.21	5	5	4.52	4–5	1–0	2.56	2–3	2	1.92	2	0
Ala	6.82	7	7	5.92	6	1	7.54	8	8	6.86	7	1
Val	1.74	2	2	1.86	2	0	3.72	4	4	3.68	4	0
Met	1.12	1	1	0.16	0	1	1.76	2	2	1.82	2	0
Ile	3.48	3–4	4	3.89	4	0	1.87	2	2	1.98	2	0
Leu	5.62	6	6	5.82	6	0	5.62	6	6	5.76	6	0
Tyr	0.92	1	1	0.94	1	0	0.88	1	1	0.92	1	0
Phe	0.97	1	1	1.12	1	0	2.84	3	3	2.94	3	0
Trp	0.86	1	1	1.04	1	0	1.86	2	2	1.79	2	0
Lys	1.82	2	2	0.94	1	1	1.04	1	1	0.16	0	1
His	1.64	2	2	1.84	2	0	1.55	2	2	1.74	2	0
Arg	1.00	1	1	1.00[b]	1	0	1.00[b]	1	1	1.00	1	0
Cys	ND		0	ND								
total			49		40–42	7–9		48			44	
polarity (%)			36					35				
net charge			3(−)			3(−)		3(−)			3(−)	

[a] Tryptophan was determined by the method of Liu and Chan (1971). Cysteine was not determined. The values for Ala, Val, Ile, Leu, Phe, and His were obtained after 80 h of hydrolysis. *A* = molar ratio, *B* = integral number; *C* = the number of residues found by sequencing the uncleaved polypeptides; *D* = differences between uncleaved and proteinase K cleaved polypeptides; ND = not determined. [b] All molar ratios are calculated on the basis of Arg = 1.00.

The N-terminal regions of polypeptides B800–850 α and B870 α remained intact when chromatophores were treated with proteinase K. However, 16 amino acids were cleaved from the C-terminus of both polypeptides if spheroplasts were treated with proteinase K. The N-terminal regions remained intact. We conclude that the C-terminal domains of both polypeptides are exposed on the periplasmic surface of the membrane. The N-terminal domains are exposed on the cytoplasmic surface of the membrane as shown with mutant strains Y5 and Ala$^+$ (Tadros et al., 1986a,b). In the wild-type strain the N-terminal regions seem to be protected by protein–protein interactions between the polypeptides of the pigment–protein complexes.

Characterization of Peak Fraction III. Sequence analysis of fraction III (Figure 3A), isolated from untreated chromatophores, revealed the major sequences (Ala/Met)-(Asp/Thr)-(Lys/Asp)-(Asn/Asp)-(Asp/Lys)-(Leu/Ala)..., which correspond to the N-terminal sequences of B870 β and B800–850 β, respectively (Tadros et al., 1984, 1985a). The material of peak fraction III was separated into two major fractions by DEAE-cellulose chromatography as described under Materials and Methods. From peak fraction 1 the sequence Met-Thr-Asp-Asp-Lys-Ala-Gly-Pro... was obtained, which is the N-terminus of the B800–850 β polypeptide. The amino acid composition is identical with that of the B800–850 β polypeptide (Table II).

The sequence of peak fraction 2 was NH_2-Ala-Asp-Lys-Asn-Asp-Leu-Ser-Phe..., which corresponds to the N-terminal sequence of the B870 β polypeptide (Tadros et al., 1984). The amino acid composition was identical with that of the B870 β protein (Tadros et al., 1984; Table II).

Analysis of peak fraction 1, isolated from chromatophores treated with proteinase K, revealed the sequence Gly-Leu^{10}-Ser-Leu-Lys-Glu-Ala-Glu-Glu-Ile..., which is the N-terminal sequence of B800–850 β starting from position 10. From fraction 2 the sequence Asp-Leu^5-Ser-Phe-Thr-Gly-Leu^{10}-Thr-Asp-Glu-Gln-Ala-Gln... was obtained, which is the N-terminal sequence of the B870 β polypeptide starting from position 5 (Tadros et al., 1984). About 98% of the B800–850 β and B870 β proteins were cleaved at the N-terminal region.

The N-terminal sequences of the purified peak fractions 1 and 2, isolated from proteinase K treated spheroplasts (Figure 3C), were identical with the N-terminal sequences of the intact proteins. The results showed that from the polypeptides of B870 β and B800–850 β four and nine N-terminal amino acyl residues were removed by proteinase K digestion when isolated from chromatophores, respectively. No N-terminal amino acid residues were digested by protease K when the polypeptides were isolated from spheroplasts.

C-Terminal analysis of the B870 β polypeptide (peak 2, Figure 3A–C) isolated from untreated or proteinase K treated chromatophores or spheroplast-derived vesicles revealed the same degradation pattern showing Trp-Phe in the ratio of 0.8:1, which is the C-terminus of B870 β protein (Tadros et al., 1984). These results demonstrate that the C-terminal regions including the hydrophobic transmembrane domain of the B870 β and B800–850 β polypeptides are not accessible to proteinase K regardless of whether chromatophores or spheroplasts were used.

Discussion

The results of the present study provide evidence that the N-terminal regions of the H-, L-, and M-subunits of RC are exposed on the cytoplasmic side of the membrane. Twenty-six and 48 residues were cleaved off of the L- and M-subunits when chromatophores were exposed to proteinase K. The polypeptides remained unchanged, however, when spheroplasts were treated. The hydrophobic transmembrane portion of the L-subunit begins in *Rhodopseudomonas viridis* and *R. capsulatus* at residue 30 and in the M-subunit at residue 50 (Youvan et al., 1984; Michel et al., 1986). These observations fit very well with the number of amino acid residues clipped

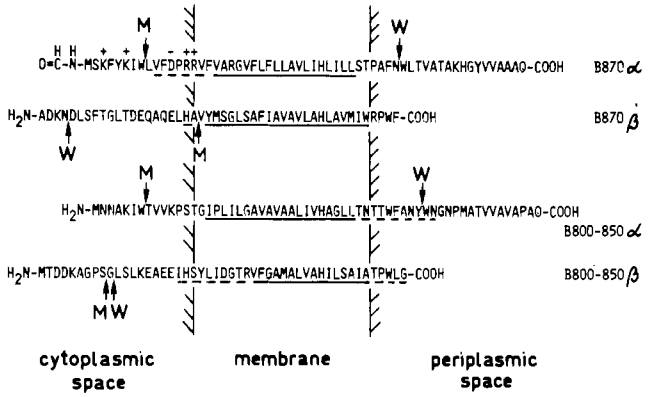

FIGURE 6: Amino acid sequence of the pigment-binding transmembrane polypeptides and the proteolytic attack on these proteins on the cytoplasmic and periplasmic side of the membrane. The arrows point to the positions up to which the polypeptides were cleaved off by proteinase K from the N-terminal or C-terminal regions. M = cleavage in mutant strains; W = cleavage in wild-type strain.

off by proteinase K as shown in this study. The H-subunit was not completely extracted by the used method, but it was shown that the polypeptide is exposed on the cytoplasmic surface. From the hydropathy plot (Youvan et al., 1984) and X-ray studies (Deisenhofer et al., 1985) it was suggested that the H-subunit is anchored with one hydrophobic α-helix in the membrane and that most of the H-subunit is localized on the cytoplasmic side of the membrane. The exposed N-terminal regions of all RC polypeptides contain charged and hydrophobic amino acid that may be of importance for incorporation, assembly, protein–protein interaction of the RC polypeptides, and interactions between RC and other pigment–protein complexes (Peters et al., 1983). The pigment-binding polypeptides L and M of RC form five membrane-spanning α-helices each, as shown by X-ray crystallographic analysis in *Rhodopseudomonas viridis* (Deisenhofer et al., 1985) and by hydropathy plots of the amino acid sequences in *R. capsulatus* (Youvan et al., 1984). Some of the loops between the α-helices may also be exposed on the membrane surface.

Light-harvesting complexes have an organization different from that of RC. The pigment-binding polypeptides of B870 and B800–850 LH complexes span the membrane only once, as shown by hydropathy plots of primary structure and ultraviolet-circular dichroism measurements (Breton & Nabedryk, 1984; Tadros et al., 1983, 1984, 1985a,b; Codgell & Scheer, 1985; Drews, 1985). The LH complexes form oligomers of the basic units (Shiozawa et al., 1982; Drews, 1985). It has been shown that in mutant strains of *R. capsulatus* which are defective in RC-B870 as in B800–850 complexes the N-terminal regions of the B870 α and β polypeptides are exposed on the cytoplasmic side of the chromatophore membrane (Tadros et al., 1986a,b). Eight and 21 amino acid residues were cleaved off from the B800–850 α and β polypeptides, respectively (Tadros et al., 1986a,b; Figure 6). Under the same experimental conditions proteinase K treatment of wild-type strain chromatophores removed only four and nine amino acid residues from the B870 β and β800–850 β polypeptides, respectively, and left the B870 α and B800–850 α polypeptides unchanged (Figure 6). The same digestion pattern was obtained when the proteolytic treatment was extended to 50 min. The N-terminal regions of all B870 and B800–850 polypeptides remained intact when isolated from spheroplasts. However, the polypeptides were completely digested in a short period by proteinase K when the membranes were solubilized by addition of 1% Triton X-100. It is evident from these results that the N-terminal regions of the β polypeptides are exposed on the cytoplasmic surface of the membrane irrespective of whether mutant or wild-type chromatophores were investigated. Although the N-terminal regions of the α polypeptides were not digested by proteinase K in wild-type chromatophores, we postulate that they have the same orientation as in mutant strains A1a$^+$ and Y5, where they have been found to be exposed on the cytoplasmic surface of the membrane. The following observations support this hypothesis: (i) The primary structures of α and β polypeptides in mutant strains A1a$^+$ and Y5 are the same as in the wild-type strain (Youvan et al., 1984; Youvan & Ismail, 1985; Tadros et al., 1983, 1984, 1985a,b). (ii) The absorption spectra of B870 and B800–850 in mutant and wild-type strains are the same in the near infrared region, which reacts very sensitively to conformation changes. (iii) As will be discussed later, the C-terminal region of α polypeptides of B870 and B800–850 is exposed on the periplasmic surface. (iv) In all models of LH complexes the hydrophobic α-helices are oriented perpendicular to or at an angle of about 85° to the plane of the membrane (Kramer et al., 1984; Zuber 1986). We speculate that in the wild-type membrane the N-terminal regions of the α polypeptides point to the cytoplasm and are more protected from protease digestion than in mutant membranes because two LH complexes and the RC are present and the pigment–protein complexes are more densely packed, as concluded from determination of particle density by freeze fracture electron microscopy (J. Golecki, H. Reidl, and G. Drews, unpublished results). The higher particle density increased protein–protein and protein–lipid interactions.

C-Terminal analysis of pigment-binding polypeptides after proteinase K treatment revealed that in chromatophores the C-termini are protected from protease attack, but in material isolated from spheroplasts, 16 amino acyl residues were removed from the C-terminal regions of the B870 α and B800–850 α polypeptides. This is a direct proof that the C-termini of the α polypeptides are exposed on the periplasmic surface of the membrane (Figure 6).

The C-termini of the β chains remained undigested, possibly because they are buried in the hydrophobic zone of the membrane. This is suggested by the hydropathy plot showing that the domain of hydrophobic amino acids extends to the C-terminus of the β polypeptides (Drews, 1985). The β polypeptides are asymmetrically organized (Drews, 1985). Their N-terminal regions are exposed on the cytoplasmic surface (Figure 6). It is concluded that their C-termini point to the periplasmic space but are not exposed on the periplasmic membrane surface. The second conserved histidine in the β chains is close to the C-terminal end of the hydrophobic α-helix (Drews, 1985; Zuber, 1986). Only the α polypeptides have amphipathic and charged domains at the N- and C-terminal regions.

All the α and β polypeptides of LH complexes and RC-L and -M polypeptides so far studied exhibit N-terminal regions localized to the cytoplasmic side of the intracytoplasmic membrane (Brunisholz et al., 1984, 1986; Deisenhofer et al., 1985; Tadros et al., 1986a,b). Transmembrane charge asymmetry as described for many bacterial inner membrane proteins (Von Heijne, 1986) might possibly be of importance for insertion and assembly of the integral membrane proteins.

It has been shown in this paper that the N-terminal regions of α polypeptides of wild-type chromatophores were not digested by proteinase K, while in chromatophores of mutant strains they were digested. Similar results were obtained with *Rhodospirillum rubrum*, which has only the B890 LH complex (Brunisholz et al., 1984, 1986). These authors speculated

that carotenoids have an influence on the conformation of the proteins during assembly and therefore on the accessibility of the N-termini of α chains to proteinase K. *R. capsulatus* strain $A1a^+$ is also a carotenoid-free mutant, and the absence of carotenoids may be of importance for the secondary structure of the B870 polypeptides. However, the mutant Y5, which synthesizes only the B800–850 complex, contains carotenoids, and the complex does not differ from the same complex of the wild-type strain 37b4 (Shiozawa et al., 1982). We believe that different factors influence the organization of the complexes and the exposition of their polypeptides on the membrane surface. A mutant defective in B870, having a functional RC, which, however, is not correctly oriented in the membrane, demonstrates the importance of interactions between the pigment-binding polypeptides and complexes (Jackson et al., 1986).

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