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Membrane Topography of the Photosynthetic Reaction Center Polypeptides of *Rhodopseudomonas sphaeroides*†

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ABSTRACT: The topography of the photosynthetic reaction center (RC) polypeptides (H, M, and L) was investigated by proteolysis and radioiodination of membrane vesicles isolated from *Rhodopseudomonas sphaeroides*. Chromatophores, obtained from French-pressed cell lysates, are closed vesicles and oriented inside out with respect to the cytoplasmic membrane (cytoplasmic side out). Spheroplast-derived vesicles (SDVs), obtained after osmotic lysis of lysozyme-treated cells, are oriented right side in (periplasmic side out). α-Chymotrypsin treatment of chromatophores and trypsin treatment of SDVs resulted in cleavage of H. α-Chymotrypsin treatment of SDVs did not cleave H, and trypsin treatment of chromatophores did not consistently cleave this polypeptide. M and L of both vesicles were apparently not affected by these proteases. The SDV trypsin cleavage product of H was identified by α-chymotryptic ^{125}I-labeled peptide mapping and had a molecular weight of 26 000. Membrane surface radioiodination with chloroglycoluril coated on glass tubes resulted in preferential labeling of H and M of SDVs and chromatophores. The radiospecific activities of H, M, and L were higher with labeling of SDVs as compared to labeling of chromatophores. α-Chymotryptic ^{125}I-labeled peptide maps of H, M, and L from surface-radioiodinated SDVs differed from the corresponding maps of these polypeptides from surface-radioiodinated chromatophores. The results indicate the asymmetric exposure of H, M, and L on opposite surfaces of the *R. sphaeroides* membrane. Exposed iodination sites of these polypeptides are more abundant on the periplasmic surface than on the cytoplasmic surface of this membrane.

The photosynthetic membrane system of *Rhodopseudomonas sphaeroides* consists of a network of intracellular vesicles formed as an extension of the cytoplasmic membrane (Lascelles, 1968; Oelze & Drews, 1972). Isolated photosynthetic membrane fractions contain several major polypeptides involved in the harvesting and utilization of light energy. Among these are three polypeptides with estimated molecular weights of 28 000, 24 000, and 21 000, designated H, M, and L, respectively. Together with bacteriochlorophyll *a*, bacteriopheophytin, ubiquinone, and iron, these polypeptides comprise the photochemical reaction center (Feher, 1971; Clayton & Haselkorn, 1972; Okamura et al., 1974). The membranes also contain several polypeptides (at least four) with molecular weights between 8000 and 12 000 (Broglie et al., 1980) which in association with bacteriochlorophyll *a* and carotenoids function in harvesting light.

The topographical arrangement of the reaction center (RC)[1]

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polypeptides within the membrane system has been the subject of several recent studies. Valkirs et al. (1976) reported the binding of antibodies prepared against isolated RCs and against RC polypeptide H to membrane fragments of *R. sphaeroides*, strain R-26 (a mutant incapable of synthesizing carotenoids). Feher & Okamura (1976) reported the binding of antibodies prepared against a complex of RC polypeptides M and L (LM complex) to both sides of strain R-26 spheroplast membranes and the binding of antibodies against H to only the inner surface of these membranes. However, earlier studies by Steiner et al. (1974) indicated no exposure of RC polypeptides M and L antigenic sites on the surface of membrane vesicles isolated from this organism. Also, Reed et al. (1975) could not bind anti-RC ferritin-conjugated antibodies to membranes isolated from strain R-26 unless the membranes were first treated with ethylenediaminetetraacetic acid. Hall et al. (1978) showed that Pronase digested polypeptide H, but not M and L, presumably on the surfaces of vesicles derived from French-pressed cell extracts. Recent studies by Francis & Richards (1980) revealed that polypeptide H and to a lesser extent L are accessible to radioactive labeling with KB^3H_4 in the presence of pyridoxal 5′-phosphate on the cytoplasmic side of the membrane. In studies on a related organism, *Rhodospirillum rubrum*, Zurrer et al. (1977) enzymatically iodinated membranes (with lactoperoxidase) and recovered the iodine label predominantly in the RC polypeptide H subunit of isolated RCs. In summary, the studies have not yet provided a consensus on the arrangement of the RC polypeptides in the membrane or on the exposure of these polypeptides on the membrane surfaces. At best, the results indicate that RC polypeptide H is probably exposed on or in close proximity to the surfaces of membranes isolated from *R. sphaeroides* and *R. rubrum* and that polypeptides M and L may possibly be on both sides of the photosynthetic membrane of *R. sphaeroides*.

A further approach to the determination of RC polypeptide topography in this membrane would involve the use of isolated membrane vesicle populations with defined orientations and biochemical properties. Recently, membrane vesicle preparations of wild-type *R. sphaeroides* have been investigated in regard to direction and uniformity of orientation (Hellingwerf et al., 1975; Matsuura & Nishimura, 1977; Michels & Konings, 1978; Lommen & Takemoto, 1978; Takemoto & Bachmann, 1979). Closed membrane vesicles (termed chromatophores) derived from French-pressed cell lysates are known to be oriented similarly to the in vivo intracellular vesicles and thus inside out with respect to the cytoplasmic membrane (Scholes et al., 1969; Prince et al., 1975; Matsuura & Nishimura, 1977). In contrast, spheroplast-derived vesicles (SDVs) obtained by osmotic lysis of lysozyme-treated cells are oriented right side in with respect to the cytoplasmic membrane (Hellingwerf et al., 1975; Matsuura et al., 1977; Michels & Konings, 1978; Lommen & Takemoto, 1978; Takemoto & Bachmann, 1979). Chromatophores and SDVs can be obtained with greater than 95% and 80% orientation uniformity (Lommen & Takemoto, 1978; Elferink et al., 1979; Takemoto & Bachmann, 1979). Such vesicle preparations permit investigations on the exposure and topography of proteins and other components on the respective membrane surfaces of the *R. sphaeroides* photosynthetic membrane.

In the present report, we describe results which reveal the exposure of RC polypeptides H, M, and L on both surfaces of the photosynthetic membrane. The results were obtained from experiments using limited proteolytic treatments with trypsin and α-chymotrypsin and membrane surface radioiodination techniques on chromatophores and SDVs.

Materials and Methods

Organism, Growth, and Preparation of Membrane Vesicles. Cells of *R. sphaeroides* (NCIB 8253) were grown, and SDVs, and chromatophores were prepared by methods we have described previously (Takemoto & Bachmann, 1979). SDVs and chromatophores were stored at 5 °C and used within 7 days.

Protease Treatments. Protease treatments were performed in mixtures containing 0.1 mg dicyclohexylcarbodiimide-treated trypsin or α-chymotrypsin, SDVs or chromatophores (1 mg of protein), and 40 mM Tris-HCl, pH 8.0, in a total volume of 0.5 mL. The mixtures were incubated at 37 °C between 5 and 60 min. Proteolyses by trypsin and α-chymotrypsin were terminated by the additions of 0.5 mg of soybean trypsin inhibitor or 5 μg of phenylmethanesulfonyl fluoride, respectively, at 0 °C. Ten milliliters of 10 mM Tris-HCl, pH 7.5, was added, and the samples were centrifuged at 150000*g* for 45 min. The supernatants were discarded, and the membrane pellets were immediately solubilized for $NaDodSO_4$–polyacrylamide gel electrophoresis.

Radioiodination of Chromatophores and SDVs. Borosilicate glass culture tubes (12 × 75 mm, Kimax) were each coated with 0.01 mg of 1,3,4,6-tetrachloro-3α,6α-diphenylglycoluracil (chloroglycoluril) according to methods described by Fraker & Speck (1978). The coated tubes were stored desiccated with Drierite at −20 °C up to 8 weeks. For iodination, aliquots of chromatophores or SDVs (5 mg of protein) in 0.5 mL of 10 mM Tris-HCl, pH 8.0, were deposited into coated tubes. Iodination was initiated with the addition of 10 μL carrier-free $Na^{125}I$ solution (~400 μCi, pH 8–11). The tubes were gently shaken and incubated at room temperature. At designated times, the reactions were terminated with the addition of 50 μL of sodium hydrosulfite solution (10 mg/mL) and 0.1 mL of 0.4 M NaI. The mixtures were applied to Sephadex G-25 (coarse) columns (5 × 180 mm) and eluted with 10 mM sodium phosphate buffer, pH 7.5. The pigmented material free of unbound I was collected in the void volume.

$NaDodSO_4$–Polyacrylamide Gel Electrophoresis. Vesicle samples were solubilized in a solution containing 2% (w/v) $NaDodSO_4$, 10% (v/v) glycerol, and 40 mM Tris-HCl, pH 7.5, and heated in boiling water for 30 s. Between 20 and 110 μg (5–30 μL) of solubilized membrane protein was applied to each well of $NaDodSO_4$–polyacrylamide (12% w/v) slab gels (thickness 1.5 mm). The acrylamide to bis(acrylamide) concentration ratio was 38:1. Procedures for electrophoresis, staining with Coomasie Brilliant Blue R-250, gel autoradiography, and gel densitometric scanning were performed as previously described (Takemoto & Huang Kao, 1977). Radioactivities of H, M, and L polypeptides separated on $NaDodSO_4$ gels were determined by slicing out the respective stained gel bands and counting the slices in a γ counter (Abbot, Model 221).

Radioiodination and α-Chymotrypsin Digestion of Unlabeled Polypeptides in $NaDodSO_4$ Gel Slices. Unlabeled polypeptides which were electrophoretically resolved on $NaDodSO_4$ gels were radioiodinated with chloramine-T and treated with α-chymotrypsin, and the peptides were extracted by using the methods described by Elder et al. (1977). $NaDodSO_4$ gels stained with Coomasie Brilliant Blue R-250 were used.

α-Chymotrypsin Digestion of RC Polypeptides Separated in $NaDodSO_4$ Gels of Surface-Radioiodinated Vesicles. RC

[1] Abbreviations used: RC, reaction center; LH, light harvesting; SDV, spheroplast-derived vesicle; Tris, tris(hydroxymethyl)aminomethane; $NaDodSO_4$, sodium dodecyl sulfate.

polypeptides H, M, and L of vesicles which were surface radioiodinated with chloroglycoluril and separated on $NaDodSO_4$ gels had relatively low radiospecific activities. When treated with α-chymotrypsin and extracted by the methods of Elder et al. (1977), the resultant peptide extracts had radioactivities which were insufficient for peptide mapping analyses. The following procedure was developed and used to increase the efficiency of gel extraction and to obtain larger quantities of digested sample. Stained gel slices (~20) of H, M, or L from ~500 μg of vesicle protein originally applied to the gels were pooled and lyophilized to dryness in glass tubes (12 × 75 mm). α-Chymotrypsin (25 μg) in 1 mL of 0.05 M sodium bicarbonate was added to each tube, and the mixtures were incubated at 37 °C for 15 h. The gels were extracted twice with 1-mL aliquots of water by heating at 50–60 °C for 2 h. The solutions were dispensed into 5-mL-capacity conical centrifuge tubes which had been siliconized. The extracts were lyophilized, suspended in 200 μL of 5% ammonium hydroxide, and centrifuged in a swinging-bucket rotor at 1500*g*. The supernatants were lyophilized and dissolved in 20 μL of 5% ammonium hydroxide. Extraction efficiency varied between 50% for H and 30% for M and L.

Peptide Mapping. α-Chymotrypsin digests (10–20 μL) containing ~10 000 cpm were applied to cellulose thin-layer chromatography plates (20 × 20 cm) (EM Laboratories, Elmsford, NY). Electrophoresis was performed with acetic acid/formic acid/water (15:5:80) or acetic acid/pyridine/water (5:5:90) at a constant voltage of 900 V at 5 °C for 45 min. An apparatus described by Gracy (1977) was used. The apparatus permitted simultaneous electrophoresis of two plates. The plates were dried and then chromatographed in the second dimension with butanol/pyridine/acetic acid/water (35.5:25:5:20) at room temperature. The plates were dried and exposed to Kodak X-Omat R X-ray film using Kodak (Lanex Regular) or Du Pont (Lightening Plus) intensifying screens at −90 °C for 3–6 days. The cellulose coat was in direct contact with the film emulsion. The optical densities of the developed film spots were determined to be linearly proportional to radioactivity up to an absorbance of 2.0 measured at 580 nm. Schematics of the peptide maps were constructed by computer plotting with manual assignment of intensity levels (1 to 4) and x, y coordinates to each developed film spot.

Protein Assay. The amounts of protein in chromatophore and SDV preparations were determined by the method of Markwell et al. (1978) using bovine serum albumin as standard.

Chemicals. Trypsin (type XI, DCCD treated) and chloramine-T were obtained from Sigma Chemical Co. α-Chymotrypsin (3× crystallized) was obtained from Worthington Biochemicals or Sigma Chemical Co. Carrier-free $Na^{125}I$ (~40 mCi/mg) in NaOH was obtained from ICN Chemical and Radioisotope Division. Chloroglycoluril (Iodogen) was purchased from Pierce Chemical Co. $NaDodSO_4$ was purchased from Bio-Rad Laboratories. Acrylamide and bis(acrylamide) were purchased from Eastman Organic Chemicals.

Results

Protease Treatments of Chromatophores and SDVs. When chromatophores (1 mg of protein) were incubated with chymotrypsin (0.1 mg) at 37 °C for 1 h, RC polypeptide H was cleaved as revealed by the $NaDodSO_4$–polyacrylamide gel electrophoretic patterns (Figure 1, slots B and C). A new polypeptide band appeared in the gel migrating in a position intermediate between the normal migration positions of H and

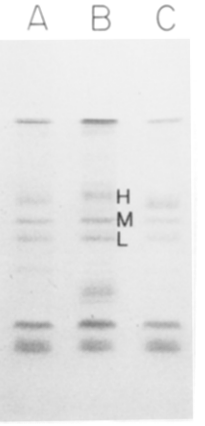

FIGURE 1: Effects of trypsin and α-chymotrypsin treatments of chromatophores. Treated and washed chromatophores were solubilized in 2% (w/v) $NaDodSO_4$ and electrophoresed in $NaDodSO_4$–12% (w/v) polyacrylamide gels (anode, bottom). Aliquots (30 μg of protein) of trypsin-treated (A), α-chymotrypsin-treated (C), and untreated (B) chromatophores were electrophoresed. The gel was stained with Coomasie brilliant blue.

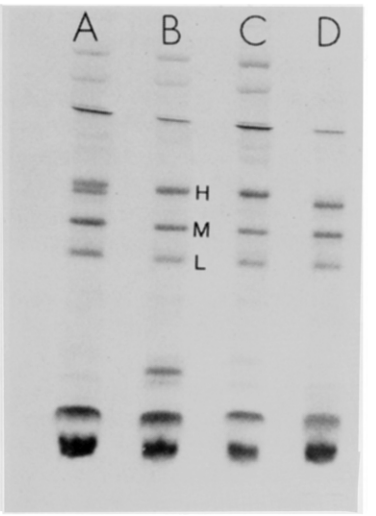

FIGURE 2: Effects of trypsin and α-chymotrypsin treatments of SDVs. Procedures were the same as described for Figure 1. Shown are gel profiles of a mixture of untreated and trypsin-treated (A), untreated (B), α-chymotrypsin-treated (C), and trypsin-treated (D) SDV samples.

RC polypeptide M. Other minor polypeptides in the 50 000–60 000 and 14 000–18 000 molecular weight ranges of the gel were cleaved as judged by their disappearance from the gels. Equivalent trypsin treatment inconsistently resulted in a very slight cleavage of H (Figure 1, slots A and B). The chymotrypsin and trypsin treatments of chromatophores did not appear to result in cleavage of M and RC polypeptide L.

In contrast, chymotrypsin (0.1 mg) treatment of SDVs (1 mg of protein) for 1 h did not appear to result in H cleavage (Figure 2, slots B and C). However, a 1-h incubation with the same amount of trypsin consistently resulted in cleavage of this polypeptide (Figure 2, slots A, B, and D). With trypsin, a new polypeptide band appeared on the gels migrating to a position slightly below the normal position of H. Again, polypeptides M and L were apparently not cleaved by these proteases.

These observations indicate differences between chromatophores and SDVs in regard to the susceptibility of H to trypsin and chymotrypsin cleavage. If it is assumed that the proteases did not penetrate the membranes, the results would further suggest that different regions of H are exposed on opposite sides of the membrane.

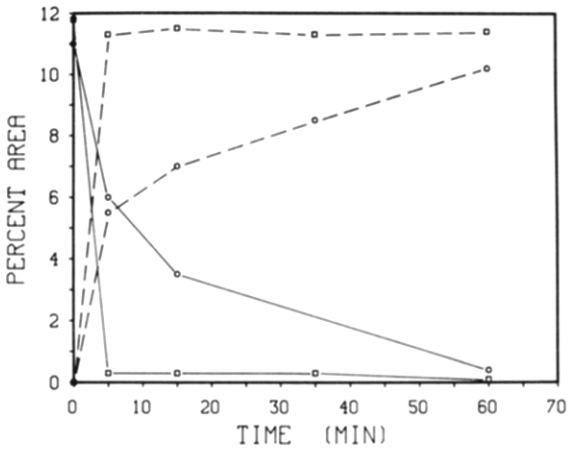

FIGURE 3: Kinetics of the cleavage of RC polypeptide H (—) and the appearance of new polypeptides (---) in $NaDodSO_4$ gels after trypsin and α-chymotrypsin treatments of SDVs (○) and chromatophores (□), respectively. Coomasie Brilliant Blue stained gels were scanned densitometrically at 580 nm, and the percent peak areas of the polypeptides over the entire gel were calculated.

The kinetics of trypsin and chymotrypsin cleavage of H of SDVs and chromatophores, respectively, and the occurrence of the corresponding new polypeptide bands are shown in Figure 3. The kinetics of H cleavage by trypsin with SDVs was nearly identical with the appearance of the new polypeptide, suggesting that the latter is the cleavage product of H. Complete cleavage occurred after 1 h of trypsin treatment. In contrast, nearly all of the chromatophore H was cleaved by chymotrypsin in 5 min. The corresponding new polypeptide in chromatophores appeared with the disappearance of H, but the kinetic data are not sufficient to indicate a precursor–product relationship.

Representative ^{125}I-labeled chymotryptic peptide maps of H and the new polypeptides are shown in Figure 4. The maps of H and the new polypeptide from trypsin-treated SDVs were very similar. Approximately 80% of the peptide spots was reproducibly observed to be common to both polypeptide maps. At least two major peptides identified on maps of H were missing from the maps of the new polypeptide. The similarities indicate that the new polypeptide was a trypsin cleavage product of H, as also suggested by the kinetic data shown in Figure 3. A few spots of the new polypeptide maps were not found on the maps of H. Reasons for this are not clear. A trypsin cleavage product would be expected to give rise to one and possibly more unique peptides. Also the degree and sites of iodination may differ slightly with the cleavage product. In contrast, maps of the chromatophore new polypeptide did not resemble maps of intact H but instead were similar to those of chymotrypsin. Thus, the new polypeptide of chymotrypsin-treated chromatophores was chymotrypsin itself. Apparently, chymotrypsin adhered to the chromatophores during incubation and was not removed during the washing procedures.

When the estimated sizes of H, M, and L were considered (Okamura et al., 1974) and a linear relation between electrophoretic migration distance and logarithm of polypeptide molecular weight in $NaDodSO_4$ gels was assumed, the trypsin cleavage product of SDVs was calculated to be approximately 26 000 daltons. Consequently, a portion of H equivalent to 2000 daltons was removed on the SDV surface.

Membrane Surface Radioiodination of Chromatophores and SDVs. Membrane surface exposed proteins can be specifically radioiodinated with ^{125}I by using the oxidizing agent chloroglycoluril (1,3,4,6-tetrachloro-3α,6α-diphenylglycoluril)

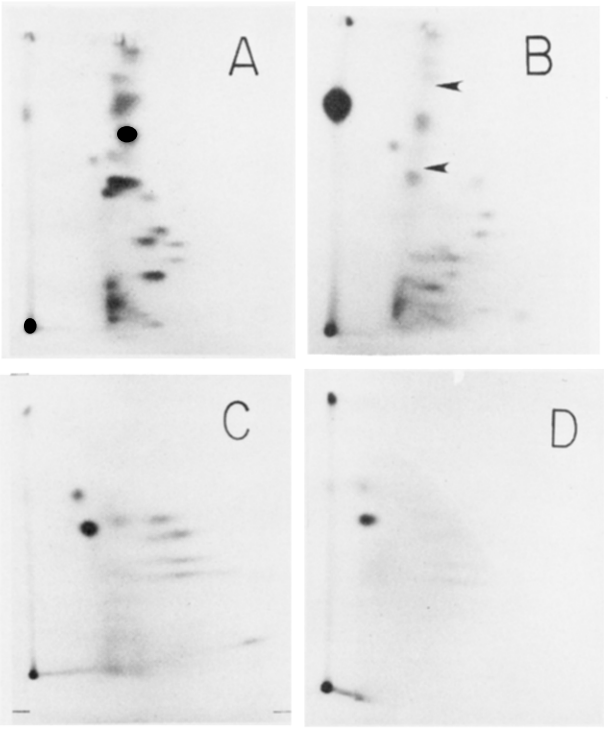

FIGURE 4: Autoradiograms of α-chymotryptic ^{125}I-labeled peptide maps of polypeptide H (A), the new polypeptide from trypsin-treated SDVs (B), the new polypeptide from α-chymotrypsin-treated chromatophores (C), and α-chymotrypsin (D). The arrows in (B) indicate the positions of peptides normally found in maps of intact polypeptide H. The electrophoresis buffer was acetic acid/formic acid/water (15:5:80).

(Fraker & Speck, 1978; Markwell & Fox, 1978). Chloroglycoluril is water insoluble and can be coated on the inner walls of glass reaction vessels. This permits solid phase, nonenzymatic radioiodination of membrane vesicles without membrane penetration.

Chromatophores and SDVs were each radioiodinated in chloroglycoluril-coated glass tubes for 0, 10, 40, 80, and 120 min and then subjected to $NaDodSO_4$ gel electrophoresis and autoradiography (Figure 5). The autoradiograms reveal that H and M were preferentially labeled in comparison to the labeling of L with both chromatophores and SDVs. Clear differences in the relative labeling of other polypeptides were also observed. For example, with chromatophores, the upper protein band of the light-harvesting (LH) complex was not labeled as heavily as the lower protein band of this complex. With SDVs, these two fractions appeared more equally labeled. Although the gel system used did not resolve the four polypeptides of the LH complex, the upper protein band probably corresponds to the B875 complex (Broglie et al., 1980).

Determinations of the radiospecific activities (counts per minute per milligram of vesicle protein) of H, M, and L at the various labeling times are shown in Figure 6. With chromatophores, H, M, and L showed radiospecific activity ratios of 1.00:0.89:0.47, respectively, after labeling for 120 min. With SDVs, the radios were 1.00:0.72:0.35, respectively, at 120 min. When chromatophores were initially solubilized with 2% (w/v) $NaDodSO_4$ and then radioiodinated, H, M, and L had radiospecific activity ratios of 1.00:0.95:0.60. The radiospecific activities of each of the three RC polypeptides were higher with SDV labeling as compared to labeling of chromatophores.

These results indicate that H and M, and to a lesser extent L, have iodination sites exposed on both sides of the membrane. All three polypeptides appear to have more sites exposed on

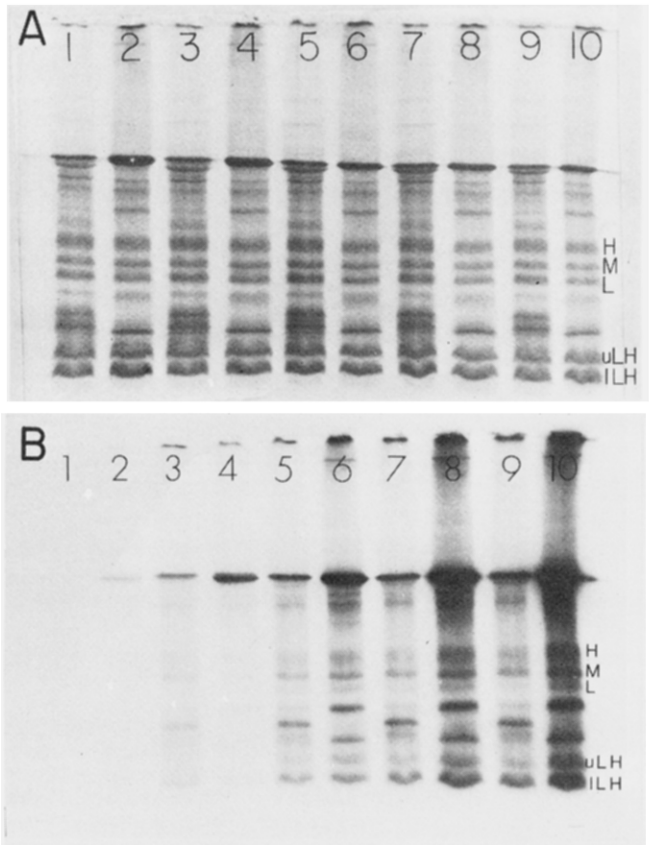

FIGURE 5: Coomasie brilliant blue stained $NaDodSO_4$ slab gel (A) and the corresponding autoradiogram (B) of chromatophores (slots 1, 3, 5, 7, 9) and SDVs (slots 2, 4, 6, 8, and 10) which were surface radioiodinated with chloroglycoluril. Labeling was terminated after 0 (slots 1 and 2), 10 (slots 3 and 4), 40 (slots 5 and 6), 80 (slots 7 and 8), and 120 (slots 9 and 10) min. Approximately 60 μg of protein was applied to each slot. RC polypeptides H, M, and L and upper (uLH) and lower (lLH) light-harvesting protein fractions are designated. Anode is on the bottom.

the outer SDV surfaces than on the chromatophore surfaces.

α-Chymotryptic ^{125}I-Labeled Peptide Maps of RC Polypeptides from Radioiodinated Vesicles. Gel slices of H, M, and L were obtained from stained $NaDodSO_4$ gels of chromatophores and SDVs which were previously radioiodinated in chloroglycoluril-coated glass tubes. The polypeptides were treated with α-chymotrypsin and the peptides extracted for ^{125}I-labeled peptide mapping. Typical autoradiograms and schematic representations of the peptide maps of each polypeptide are shown in Figure 7. For direct comparison of maps, individual polypeptides obtained from radioiodinated chromatophores and SDVs were treated, electrophoresed, and chromatographed in parallel. The maps for each polypeptide are significantly different when comparing extracts derived from labeled chromatophores and SDVs. For example, in the maps shown, H from surface-labeled chromatophores showed two autoradiogram spots (schematic x, y coordinates 6.2, 7.5; 6.5, 6.5) which were not evident on equivalent maps of H from surface-labeled SDVs. Conversely, SDV-labeled H showed a major spot (9.5, 0.8) not appearing on maps of chromatophore-labeled H. Several spots appearing on both H maps were clearly more prominent on the maps of either chromatophore-labeled H (7.3, 8.3; 4.8, 6.4; 5.5, 7.1; 5.4, 8.0) or SDV-labeled H (7.4, 12.2; 7.9 8.5; 8.5, 8.2; 10.2, 5.2). Similarly, maps of M had spots which were unique (5.7, 3.8) or more prominent (12.5, 4.8; 6.0, 8.3) on maps of chromatophore-labeled M and unique (9.8, 7.2) or more prominent (9.0, 10.8; 7.0, 13.5) on maps of SDV-labeled M. With L, unique

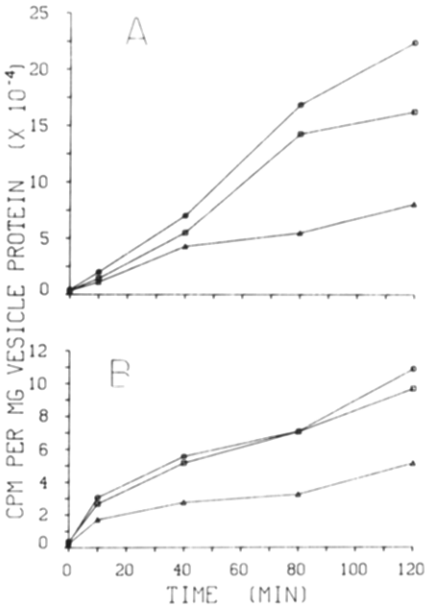

FIGURE 6: Time course of ^{125}I labeling of RC polypeptides H (○), M (□), and L (△) from SDVs (A) and chromatophores (B) which were surface radioiodinated by using chloroglycoluril. Coomassie Brilliant Blue stained bands of H, M, and L were sliced from $NaDodSO_4$ gels (Figure 5A) and counted in a γ counter. The data shown are expressed as cpm/mg of vesicle protein applied to the gel.

(8.7, 8.6; 5.8, 4.0; 4.5, 9.0) and prominent (6.7, 4,8) spots on chromatophore-labeled maps and unique (8.9, 8.9; 5.8, 14.0) and prominent (7.9, 12.4) spots on SDV-labeled maps were also observed. Certain spots appeared equally labeled with either SDV or chromatophore maps of H (5.8, 12.5), M (9.0, 6.4), and L (6.4, 6.5). Although in separate experiments the x, y coordinates for particular peptides varied slightly, the overall patterns were reproducible. Overall, these results show that different portions of H, M, and L are preferentially labeled with radioiodination of opposite surfaces of the membrane.

Discussion

The combined results of protease digestion and radioiodination of SDVs and chromatophores indicate the asymmetric exposure of RC polypeptides H, M, and L on both surfaces of the photosynthetic membrane of *R. sphaeroides*. Iodination sites of these polypeptides occur on both membrane surfaces and are more abundant on the periplasmic surface (outer SDV surface). Estimates of the degree of exposure of these polypeptides are limited by the specificities of the proteases used and the sites susceptible to iodination. Tyrosine, phenylalanine, and histidine residues are most susceptible to iodination (Koshland et al., 1963). However, since the amino acid sequences of the RC polypeptides are not known, it is impossible to draw conclusions about the relative exposure of the unlabeled portions of these polypeptides on the respective membrane surfaces. Likewise, the absence of sequence information limits conclusions about the relationship between the exposure of H on the periplasmic surface and the positions of lysine or arginine residues with peptide bonds susceptible to trypsin hydrolysis.

The extent of surface exposure of L is less certain. Although L is radioiodinated when chloroglycoluril and vesicles are used, it is labeled significantly less than H and M. However, L is also labeled less than H and M in $NaDodSO_4$-solubilized chromatophores in which presumably all or most potential labeling sites are exposed. Reasons for the lower level of labeling of L in solubilized membranes are unclear since amino

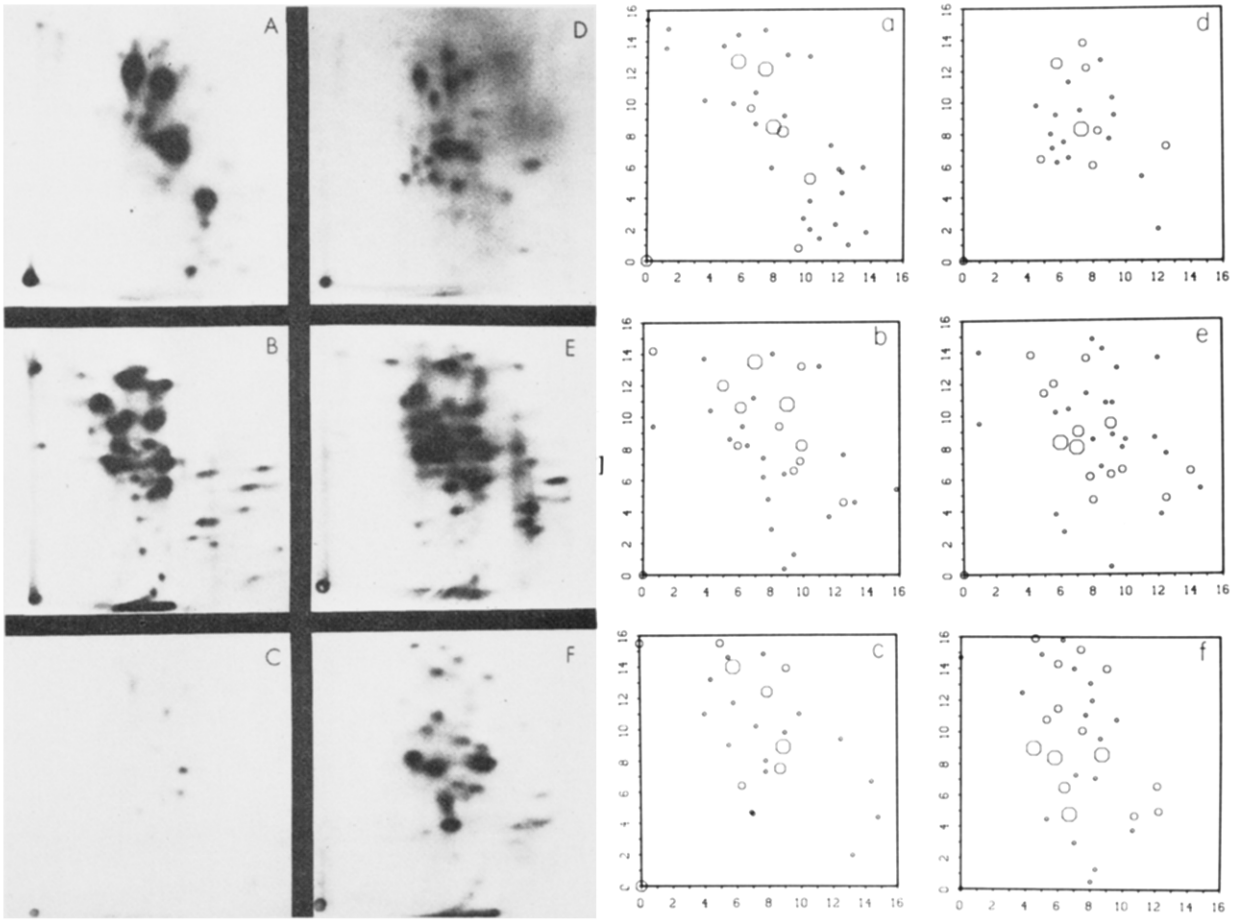

FIGURE 7: Autoradiograms (left) and schematic representations (right) of α-chymotryptic ^{125}I-labeled peptide maps of H, M, and L of chromatophores and SDVs which were surface radioiodinated with chloroglycoluril. (A), (B), and (C) and (a), (b), and (c) are maps of H, M, and L, respectively, from labeled SDVs. (D), (E), and (F) and (d), (e), and (f) are maps of H, M, and L, respectively, from labeled chromatophores. The diameters of the spots on the schematic representations are roughly proportional to the intensities of the corresponding autoradiogram spots. Each spot was assigned one of four possible diameters. The electrophoresis buffer was acetic acid/pyridine/water (5:5:90).

acid composition analyses show that L has approximately the same number of potential iodination sites (tyrosine, phenylalanine, and histidine residues) as M and L (Steiner et al., 1974).

The α-chymotrypsin cleavage product of H from chromatophores could not be identified. Conceivably, H was hydrolyzed to small peptides (less than 10000 daltons) not resolvable on the $NaDodSO_4$ gels, or α-chymotrypsin treatment resulted in the release of the cleavage products from the membranes. Pronase treatment of chromatophores also appears to result in complete removal of H from the membrane without affecting M and L (Hall et al., 1978).

Different sets of labeled peptides were observed on the respective peptide maps of surface-labeled SDV and chromatophore polypeptides. This observation strongly suggests that radioiodination was specific for surface-exposed sites of the polypeptides. It also confirms that the majority of vesicles in the SDV preparations are oppositely oriented to the majority of chromatophore vesicles. The occurrence of peptides commonly labeled with SDVs and chromatophores (Figure 7) could be attributed to the occurrence of α-chymotryptic peptides representing portions of the RC polypeptides which span the membrane or to the occurrence of small fractions of reversely oriented vesicles in the SDV and chromatophore preparations (Lommen & Takemoto, 1978; Takemoto & Bachmann, 1979).

Trypsin and α-chymotrypsin apparently did not penetrate the membrane under the experimental conditions employed. The asymmetric effects of these proteases on H of chromatophores and SDVs argue against their penetration into these vesicles. With penetration, proteolytic cleavages of equal extent would be expected with both kinds of vesicles. The apparent lack of effect on RC polypeptides H and M also suggests that these enzymes did not penetrate or destroy the structural integrity of the membrane. α-Chymotrypsin is clearly capable of hydrolyzing M and L to smaller peptides when extracted from the membrane, as shown by the α-chymotryptic peptide maps (Figure 7). Corresponding tryptic peptide maps have also been made (data not presented), indicating the susceptibility of these polypeptides to trypsin hydrolysis. Independent evidence for the impermeability of trypsin in the SDV membrane has been reported previously (Takemoto & Bachmann, 1979).

Radioiodination with glass-coated chloroglycoluril was used to give membrane surface specific labeling. The advantages of this method over other iodination techniques for surface labeling have been discussed (Fraker & Speck, 1978; Markwell & Fox, 1978). With SDVs and chromatophores, we have found that the labeling reaction must be terminated with addition of a reducing agent such as sodium hydrosulfite. Removal of the sample from the reaction vessel without addition of a reducing agent [as suggested by Markwell & Fox (1978)] was not sufficient for stopping the labeling reaction. Attempts to radioiodinate these vesicles with the more widely used lactoperoxidase method were not successful. With this latter method, the resultant radiospecific activities of the individual polypeptides were too low for peptide map analyses. The low level of labeling indicates that very little or no non-

enzymatic iodination occurred. Furthermore, attempts to radioiodinate membrane preparations without an oxidizing agent such as chloroglycoluril gave no detectable labeling of polypeptides as judged by autoradiography.

Recently, Valkirs et al. (1976) and Feher & Okamura (1976) reported that ferritin-conjugated rabbit antibodies prepared against H bound to the chromatophore surfaces and inner surfaces of punctured spheroplasts of strain R-26. These antibodies did not bind the outer surfaces of the spheroplasts. Possibly, portions of H which are exposed on this latter surface were not effective in eliciting antibody formation or have determinants with low antibody binding affinities. It is also possible that variations in the reaction center topography might occur between membranes of the wild-type strain (used in the present study) and the carotenoid-less strain.

The transmembrane orientation and exposure of M on both sides of the membrane seem consistent with recent observations that the primary electron acceptor (quinone) and donor (cytochrome c_2) of the photosynthetic RC are associated with this polypeptide (Marinetti et al., 1979; Rosen et al., 1979). Cytochrome c_2 is known to be located in the periplasmic space (Prince et al., 1975), and proton binding via the primary and secondary quinones is known to occur at the cytoplasmic surface (or chromatophore surface) (Wraight et al., 1978). M would thus serve as the RC component in direct physical association with the primary electron donor and acceptor to facilitate light-induced electron transfer across the membrane.

In contrast to M, it is difficult to speculate on the functional significance of the exposure of H on both sides of the photosynthetic membrane. Although tightly associated with M and L in the isolated RC complex, the role of H in RC function is unknown. Removal of H from the complex does not abolish characteristic light-induced absorbance changes of RCs (Okamura et al., 1974). Consequently, H does not appear to play a central role in the primary photochemical reactions. Nevertheless, our present findings suggest that the particular function of H involves some vectorial transmembrane phenomenon.

Added in Proof

Recently, Debus et al. (1981) have reported that RC polypeptide H may be associated with electron transfer between the primary and secondary quinones of the photosynthetic electron transport system. H may be required to provide a proper active site for secondary quinone function.

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