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Transverse Topography of the Photochemical Reaction Center Polypeptides in the *Rhodopseudomonas capsulata* Membrane

JÜRGEN PETERS AND GERHART DREWS*

Institute of Biology II, Microbiology, Albert-Ludwigs-University, D-7800 Freiburg, Federal Republic of Germany

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The exposure of the three polypeptide subunits H, M, and L of the photochemical reaction center (RC) on both surfaces of the membrane of *Rhodopseudomonas capsulata* was studied by partial proteolysis with proteinase K and sodium dodecyl sulfate-polyacrylamide gel electrophoresis of degradation products. The possible association of RC subunits with bacteriochlorophyll *a* and bacteriopheophytin was investigated by spectroscopical measurements. Chromatophores (inside-out oriented) and spheroplasts (right-side-out oriented), as well as purified, detergent-solubilized RCs and RCs reconstituted into phosphatidyl choline liposomes, were used. Subunit H of the RC was degraded to fragments with apparent M_rs of 15,000 and 12,500, which were possibly derived from cleavage of a loop exposed on the cytoplasmic surface. Polypeptide M was digested at a comparable rate. The apparent M_r of M decreased by roughly 4,000 upon proteolytic cleavage. Subunit L was relatively insensitive to protease attack, except that a small peptide was clipped off. The primary donor P870 was also found to be only slightly affected by proteinase K. All three RC subunits appear to be exposed on the chromatophore surface.

Phototrophic bacteria harvest incident light energy by membrane-bound antenna complexes consisting of bacteriochlorophyll, carotenoid (crt), and integral membrane proteins (see references 5 and 7). Mobile excited singlet states migrate to the photochemical reaction center (RC) where they are trapped and converted into electrical potential by directed charge separation. The efficiency of primary photochemical events depends critically on the localization and orientation of pigments in the membrane lipid bilayer. As the arrangement and the native spectral properties of bacteriochlorophyll are determined by specific protein environments, knowledge of the topography of pigment-associated polypeptides is required to understand the mechanisms of energy migration and charge separation. Whereas data on the lateral topography of the RC have been reported recently (26, 32), this study is concerned with the transmembrane orientation and possible pigment association of RC polypeptides H, M, and L. The transmembrane arrangement of these polypeptides has been studied mainly with *Rhodopseudomonas sphaeroides* and *Rhodospirillum rubrum* by radioactive surface labeling (1, 7, 11, 12, 21, 25, 38, 42), proteolytic digestion (1, 16, 21, 40), and immunological methods (3, 37). However, no clear consensus has been reached concerning the exposure of RC subunits on either side of the membrane. The location of two molecules of bacteriopheophytin and four molecules of bacteriochlorophyll *a* (Bchl) within the RC is still unknown, except that they are not associated with H (9, 20). Proteolytic modification of polypeptides on the membrane surface has been demonstrated to be a sensitive and specific probe of pigment-protein association with the B800-850 antenna complex of *Rhodopseudomonas capsulata* (10). In this work we have used digestion with proteinase K of membrane-bound as well as detergent-solubilized RCs and RCs reconstituted into liposomes to investigate the RC topography. Proteinase K is known to unspecifically attack native proteins (8), and it has been the only protease reported to cleave not only subunit H but also M and L with membrane vesicles of *Rhodospirillum rubrum* (40). However, these authors have not identified digestion products. The degree of exposure of RC polypeptides on the membrane surface and possible associations with the three distinguishable pigment species bacteriopheophytin, P800, and the primary donor P870 have remained unclear.

MATERIALS AND METHODS

Reagents. All reagents employed were of analytical grade. Chemicals for sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis were of the highest purity available.

Acrylamide, methylenebisacrylamide, *N',N',N',N'*-tetramethylethylenediamine, glycine, SDS, and phenylmethylsulfonyl fluoride were purchased from Serva, Heidelberg, (FRG). Tris, anti-rabbit immunoglobulin (IgG), lysozyme, and phosphatidylcholine were from Sigma Chemical Co., Munich, FRG. Proteinase K, Triton X-100, and sodium peroxodisulfate were from Merck, Darmstadt, FRG. Lauryl dimethylamine oxide (LDAO) was a gift from Nordmann, Rassmann & Co., Hamburg, FRG, and ^{125}I was bought from Amersham Buchler, Braunschweig, FRG. 1,3,4,6-Tetrachloro-3α,6α-glycouracil (Iodogen) was from Pierce Eurochemie, Rotterdam, The Netherlands.

Organisms and growth conditions. *Rhodopseudomonas capsulata*, wild-type strain St. Louis (ATCC 23782) and the mutant strains A1a$^+$ (B870$^+$, B800-850$^-$, Crt$^-$, Bchl$^+$) (6) and NK3 (B870$^+$, B800-850$^-$, Crt$^+$, Bchl$^+$) (N. Kaufmann, unpublished data) were grown anaerobically in the light (2,000 lx) at 30°C in screw-capped bottles (4). Cells were harvested in the late logarithmic phase.

Preparation of chromatophores and spheroplasts. Purified chromatophores were prepared as previously described (13). Spheroplasts were obtained as previously described (25). The integrity of chromatophores to proteinase K was controlled by measuring the content of cytochrome c_2, which is

* Corresponding author.

trapped inside the membrane vesicles (27). Specific staining of *c*-type cytochromes was performed with the method previously described (34). The green band at ca. M_r 12,500 on SDS-polyacrylamide gel electrophoresis was quantified with a Uvikon 810 Kontron spectrophotometer in combination with a gel-scanning unit. Vesicles containing at least 90% of the reference amount of cytochrome c_2 were considered intact with respect to protease penetration.

Spheroplast integrity was examined as previously described (25) by using iodination of the nucleic acid fraction with ^{125}I as a marker for burst particles. Again, 90% integrity was considered sufficient for the topographical studies.

Membranes were prepared from spheroplasts by ultrasonication and subsequent sucrose gradient centrifugation as previously described (13), followed by sedimentation of the chromatophore fraction at 144,000 × *g*.

RC and IgG preparation. RCs were isolated from the mutant strain A1a$^+$ of *Rhodopseudomonas capsulata*. Cells were disrupted by threefold passage through a French pressure cell (92 MPa) and layered on top of a sucrose step gradient (0.6, 0.9, 1.2, and 1.5 M sucrose in 10 mM Tris-hydrochloride, pH 7.6). After centrifugation at 144,000 × *g* for 16 h at 4°C, the chromatophore fraction was washed with the same Tris-hydrochloride buffer, sedimented and subjected to ultrasonication in the presence of 5 mM EDTA. The membranes were cooled in an ice-water bath and sonicated 10 times for 20 s at intermittent 1-min intervals. After another washing and sedimenting step, the purified membranes were adjusted to an optical density at 865 nm (1-cm light path) of 50 and treated dropwise with stirring with 12 mg of LDAO per ml for 45 min at room temperature. Solubilized membranes were layered on top of a sucrose step gradient (0.3, 0.6, and 0.9 M sucrose in 10 mM Tris-hydrochloride, pH 7.6; 4 mg of LDAO per ml). After centrifugation at 144,000 × *g* for 16 h at 4°C, the blue band near the middle of the gradient was collected and subjected to ammonium sulfate precipitation. The first step was precipitation with 50 g of solid $(NH_4)_2SO_4$ per 100 ml of solution containing RC. The second step was precipitation with an equal volume of 4.2 M $(NH_4)_2SO_4$ at 0°C. The third step was precipitation of impurities with one-half volume of 4.2 M $(NH_4)_2SO_4$. The fourth step was precipitation of RC with two volumes of 4.2 M $(NH_4)_2SO_4$. The ammonium sulfate solution was neutralized to pH 7.0, and precipitation was performed at room temperature. The redissolved precipitate was dialyzed against Tris buffer and purified by DEAE-column chromatography (10 mM Tris-hydrochloride, pH 7.6; 0.8 mg of LDAO per ml) by using an NaCl step gradient. Pure RC eluted at 0.1 to 0.12 M NaCl. After dialysis against 10 mM Tris-hydrochloride (pH 7.6), the RC was stored at −20°C in 50% glycerol. Purified IgG was prepared from rabbit antisera directed against RCs as previously described (39).

Protease digestion. Digestion with proteinase K was conducted at 30°C in 20 mM Tris-hydrochloride (pH 7.6) at reaction times and concentrations specified below. The reaction was terminated by adding 5 μl of 100 mM phenylmethylsulfonyl fluoride in ethanol to 100 μl of sample. Isolated RCs were digested in the presence of 0.3 mg of LDAO per ml and 3 mM glutathione. Digested samples were stored on ice until treatment with an equal volume of 40 mg of SDS per ml–200 mM Tris-hydrochloride (pH 6.7)–200 μl of glycerol per ml and incubated at 37°C for 15 min to avoid, as much as possible, aggregation of RC subunits M and L.

Digested chromatophores were kept on ice and solubilized with Triton X-100 as previously described (26). Chromatophores prepared from spheroplasts were treated in the same way.

For spectral degradation kinetics, samples were placed in a half-microcuvette (1-cm light path), and absorption spectra were recorded on a Uvikon 810 Kontron spectrophotometer. With membranes, intactness of the primary donor P870 was followed by measuring reversible bleaching at 870 nm as previously described (28). With isolated reaction centers, the integrity of P870 was measured as absorbance at 865 nm.

Electrophoresis. SDS-polyacrylamide gel electrophoresis was carried out as previously described (29). Native Triton X-100–polyacrylamide gel electrophoresis and two-dimensional electrophoresis involving native SDS-polyacrylamide gel electrophoresis in the first dimension were conducted as described previously (26). Apparent relative molecular mass (M_r) calibration was performed by using phosphorylase *b* (96,000), bovine serum albumin (68,000), RC (28,000, 24,000, and 20,500) (20), and horse heart cytochrome *c* (12,500) as standards. Gels were stained with Coomassie brilliant blue R-250 (2 g/liter dissolved in 500 ml of 2-propanol–120 ml of acetic acid per liter of distilled water) for 1 h and destained with the same solvent mixture.

Immunological identification of polypeptides. For identification of fragments of RC subunit H, SDS-polyacrylamide gels of protease-treated and reference samples were subjected to immunoblotting as previously described (36). Polypeptide H and derived digestion products were detected by immunoadsorption of purified anti-RC IgG from rabbit, which reacted almost exclusively with H (see reference 26). Immunolabeled bands were detected by using anti-rabbit IgG labeled with ^{125}I by the method previously described (37). Nitrocellulose sheets were exposed to Kodak X-Omat AR film for 3 days with Kodak X-Omatic regular intensifying screens.

Preparation of liposomes containing RCs. RCs from the mutant NK3 were reconstituted into phosphatidylcholine vesicles by the method previously reported (19). The incorporated RCs were spectrally and functionally intact as judged by reversible bleaching.

RESULTS

Protease digestion of membrane-bound RCs. Protease-digested chromotophores of the wild-type strain of *Rhodopseudomonas capsulata* were fractionated by native Triton X-100–polyacrylamide gel electrophoresis as previously de-

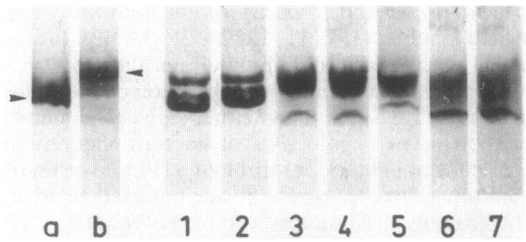

FIG. 1. Fractionation of proteinase K-digested chromatophores by Triton X-100–polyacrylamide gel electrophoresis (26). Only the parts of the gel containing pigmented bands are shown (R_f, ca. 0.3). Track a, Chromatophores of the RC-B870-defective mutant strain Y5. Track b, Chromatophores of the B800-850-defective mutant strain NK3. RC-B870 (top) and B800-850 complexes (bottom) are indicated by arrowheads. Tracks 1 through 7, Chromatophores of wild-type strain St. Louis (250 μg of Bchl per ml) incubated with proteinase K (500 μg/ml) for 0, 20, 40, 60, 80, 100, and 120 min, respectively.

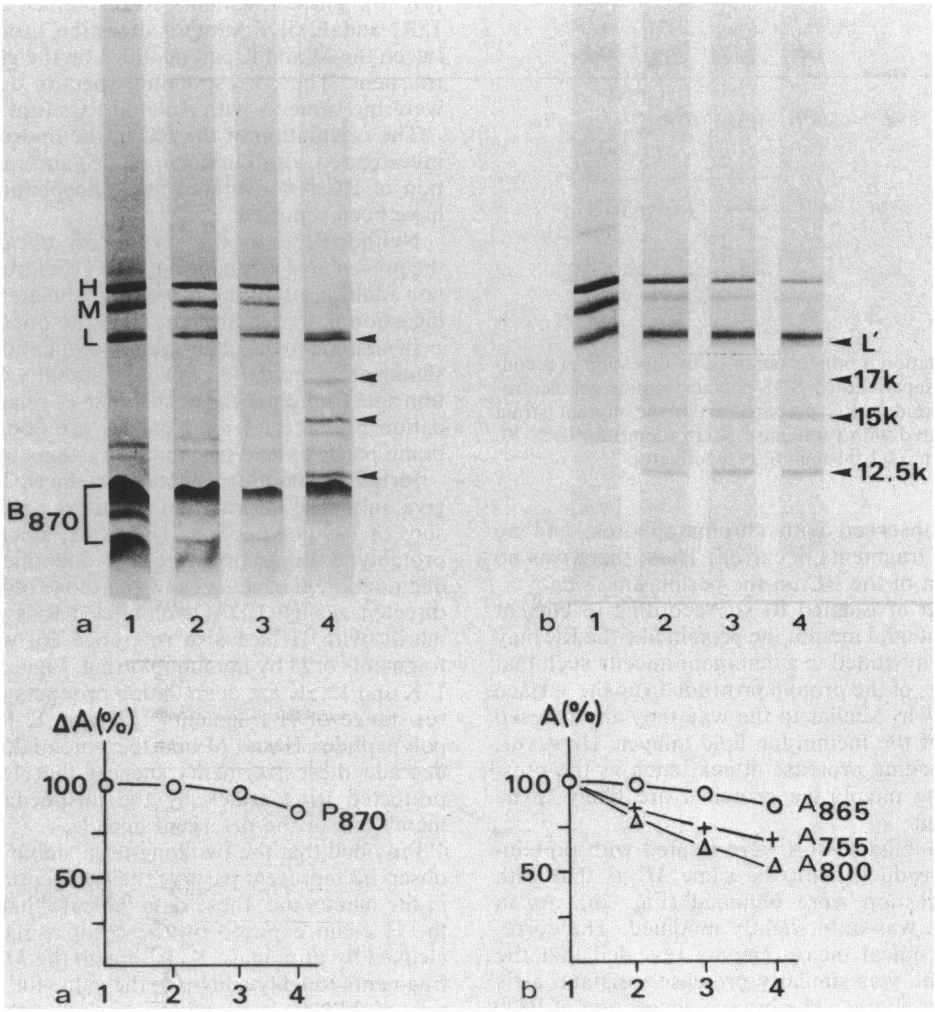

FIG. 2. RC degradation products obtained by digestion with proteinase K and corresponding spectral decay. Samples were separated on 11.5 to 18% SDS-polyacrylamide gradient gels. (a) RC-B870 fractions were obtained as described in the legend to Fig. 1. Tracks 1 through 4, Samples digested for 0, 40, 80, and 120 min, respectively. Arrowheads indicate RC degradation products. The plot below the gel shows the corresponding relative decrease (ΔA) of reversible bleaching of the RC (P870). (b) Tracks 1 through 4, Purified RCs (5 μM) digested with proteinase K (10 μg/ml) for 0, 10, 20, and 30 min, respectively. Arrowheads indicate degradation products with the same apparent (M_rs as those in (a). The plot below the gel shows the corresponding decrease of the absorbance (A) of P870 (865 nm), P800 (800 nm), and bacteriopheophytin (755 nm), respectively.

scribed (26). Results are shown in Fig. 1. The top band corresponding to the RC-B870 light-harvesting complex (26) was excised for analysis by SDS-polyacrylamide gel electrophoresis. RC polypeptides H and M were completely cleaved after 120 min of protease treatment, and two major degradation products with M_rs of 15,000 and 12,500 and a minor one with an M_r of 17,000 were formed (Fig. 2a). Polypeptide L is only slightly modified in size. Kinetics such as those shown in Fig. 3 demonstrate that the polypeptide designated L′ is formed after H and M have been completely degraded.

These results indicate that all three RC subunits are exposed on the chromatophore surface. The idea that L protrudes only slightly from this side of the membrane is in accordance with previous studies of different organisms that indicated little or no labeling of this polypeptide with antibodies (37) and chemical probes (1, 12, 21, 25, 38, 42). Also, the photochemical activity of the membrane-bound RC is remarkably resistant to proteolytic digestion (Fig. 2). The fragments of proteolytic digestion were not cleaved into smaller polypeptides during an additional 60 min of protease treatment, possibly because they are embedded in the membrane and thus relatively resistant to protease attack. After a very long incubation time (6 h), all polypeptides were digested to an extent that degradation products with M_rs larger than 6,000 were not detectable.

To investigate the exposure of RC subunits on the periplasmic side of the membrane, spheroplasts (25 μg of Bchl per ml) were treated with proteinase K (500 μg/ml). Within the 90-min lifetime of intact spheroplasts (90% integrity, see above, no degradation of RC polypeptides was observed, either by SDS-polyacrylamide gel electrophoresis or by measuring photochemical activity. To investigate whether the RC was attacked after prolonged incubation, spheroplasts were lysed by brief (5 s) ultrasonication (effective lysis controlled by phase-contrast microscopic observation) and digested for 180 min. This long-term proteolytic treatment of both faces of the membrane yielded only the digestion

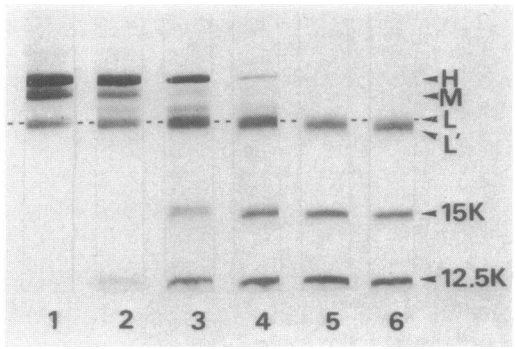

FIG. 3. RC degradation products obtained by digestion of reconstituted vesicles and separated by SDS-polyacrylamide gel electrophoresis (14% acrylamide). RC prepared from the mutant strain NK3 (5 μM) was treated with proteinase K (15 μg/ml) for 0, 5, 10, 20, 40, and 60 min (tracks 1 through 6, respectively).

products already observed with chromatophores, and no further cleavage of fragments occurred. Thus, there was no detectable digestion of the RC on the periplasmic side.

Protease digestion of isolated RCs. According to current views (17, 33), an integral membrane protein like the RC may be expected to be embedded in a detergent micelle such that hydrophilic domains of the protein protrude from the surface of the micelle in a way similar to the way they are exposed on either surface of the membrane lipid bilayer. However, sterical effects impeding protease attack, such as the presence of neighboring membrane proteins, are likely to be much less significant.

When LDAO-solubilized RCs were treated with proteinase K, digestion products with the same M_r as that with chromatophore digestion were obtained (Fig. 2b). As in chromatophores, L was only slightly modified. The corresponding spectroscopical measurements revealed that the primary donor, Bchl, was similarly protease resistant, as is the case in chromatophores. The in vivo absorption of P800 and bacteriopheophytin also declined slowly compared with polypeptides H and M on SDS-polyacrylamide gel patterns (Fig. 2b). At much longer digestion times (2 h) L is eventually degraded, with concomitant appearance of a new polypeptide at an M_r of 10,000 and enhancement of the 17,000-molecular-weight polypeptide (17K). Thus, 17K is likely to be derived from L (not shown). After 4 h these products are also digested. It should be noted that the intensities of the bands corresponding to degradation products cannot account for all of the RC polypeptides digested. Nevertheless, 15K and 12.5K are much more stable than the polypeptides from which they are derived.

The data obtained with isolated RCs are in agreement with the results of chromatophore and spheroplast digestion, which indicated that the RC is only attacked by proteinase K on the cytoplasmic side of the membrane. This does not contradict the established fact that cytochrome c_2 binds to the RC on the periplasmic side (2, 27), since the relatively small portion of the RC exposed (2) may be inaccessible to attack by proteinase K.

Studies with liposome-bound RCs. Reconstituted phosphatidylcholine vesicles have been used by several authors (2, 19, 22, 35) to study the RC. In the present study we investigated proteolytic digestion of the RC reconstituted into liposomes.

After treatment of liposome-incorporated RC with proteinase K, three stable digestion products were identified (Fig. 3). These fragments corresponded to polypeptides L', 15K, and 12.5K. Another digestion product appearing between the M and L polypeptides on the gel pattern was only transient. The corresponding spectral degradation kinetics were the same as with isolated RCs (not shown).

The orientation of the RC in the liposomes used was not investigated. Both uniform (2, 36) and random (22) orientation of RC reconstituted into phosphatidylcholine vesicles have been reported.

Neither rupture of liposomes by ultrasonic treatment in the presence of proteinase K at 0°C before incubation at 30°C nor addition of 10 mg of sodium cholate per ml resulted in digestion patterns different from the one shown in Fig. 3.

In summary, the cleavage of polypeptides H and M before significant degradation of L, the stability of the P870 absorption spectrum, and the occurrence of relatively stable degradation products of identical M_r are common to the membrane particles and two model systems investigated.

Identification of degradation products. The data presented give only little information about the actual degree of exposure of RC polypeptides H and M, since the fragments of proteolytic digestion were not identified. To distinguish degradation products of H from those of M, an IgG fraction directed against LDAO-solubilized RCs which reacted primarily with H (see also reference 26) was used to detect fragments of H by immunoblotting. Figure 4 shows that both 15K and 12.5K are degradation products of H. The protease resistance of H fragments 15K and 12.5K with respect to polypeptides H and M and the potential of proteinase K to degrade these fragments suggest that 15K and 12.5K are protected from attack by the unspecific protease by the membrane or the detergent micelle.

Provided that the two long-term stable digestion products observed represent parts of the polypeptide chain embedded in the membrane, these data indicate that there is a loop of the H chain exposed on the cytoplasmic surface which is cleaved by proteinase K. Although the M_r values of the two fragments roughly add up to the value for the intact polypeptide of 28,000, it cannot be excluded that there are other segments of H exposed, because M_r estimations of RC polypeptides by SDS-polyacrylamide gel electrophoresis are

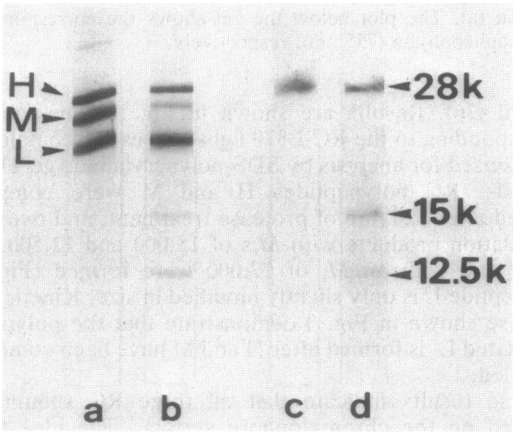

FIG. 4. Immunoblotting of RC digestion products separated by SDS-polyacrylamide gel electrophoresis (11.5 to 18% acrylamide). Tracks a and b were stained with Coomassie brilliant blue; c and d are autoradiograms of ^{125}I-labeled polypeptides. a and c are reference tracks; samples in b and c were digested with proteinase K as described for Fig. 2b, track 2.

known to be inaccurate (D. Rosen, G. Feher, and L. Steiner, Fed. Proc. **39**:1801, 1980). Since the 15K band is conspicuously faint while the transient 22K product is present (track 3 in Fig. 3), there might be another short segment of H exposed which is also clipped, resulting in the stable 15K fragment. Alternatively, there might also be a large loop which is eventually degraded to yield 15K and 12.5K. 22K is unlikely to have originated from RC subunit M, since a large initial decrease in the intensity of the M band (Fig. 3, track 2) is not paralleled by formation of the 22K product.

To obtain clues about the exposure of subunit M on the cytoplasmic surface, RCs were treated with proteinase K for 10 min under the conditions described in the legend to Fig. 2b and subjected to two-dimensional electrophoresis as previously described (26). After mild SDS treatment LM migrates as a spectrally intact particle (see reference 26). Digestion with proteinase K caused the largely native blue band to shift from an M_r of 49,000 to one of 45,000. Although M_r estimations are not reliable with nondenaturing SDS-polyacrylamide gel electrophoresis (31), the observed value for the unmodified particle agrees fairly well with the figure expected from subunit M_r values. As L is not significantly affected under the conditions used, the M_r of subunit M is reduced by roughly 4,000 upon cleavage with proteinase K.

Figure 5 shows the result of two-dimensional mapping of the modified LM particle. Whereas polypeptide L may be identified as a constituent of modified LM, there is no cleavage product of M that may account for the M_r of 45,000 of the digested LM particle. The superimposition of a hypothetical 20,000-molecular-weight fragment with L, aggregation of the fragments upon denaturing SDS treatment, or the cleavage of M at several sites, resulting in small fragments, are three possible explanations. As expected, the two digestion products of H, which is dissociated from LM by SDS (see references 20 and 26), migrate separately.

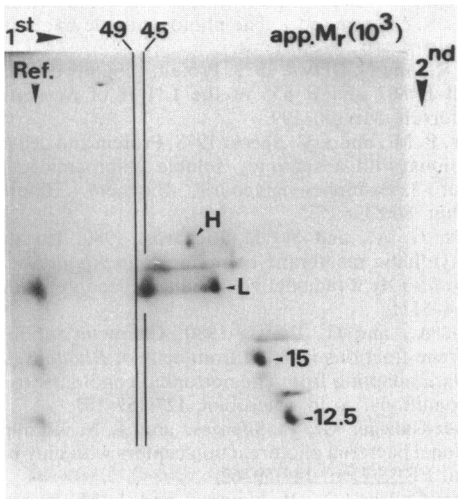

FIG. 5. Two-dimensional mapping of proteinase K-digested RC with mild detergent fractionation. RC was digested with proteinase K as described for Fig. 2b, track 2. For detergent fractionation, digested RC was treated with 1 mg of SDS per ml at 4°C. Two-dimensional electrophoresis (1st▶, 2nd▶) was conducted as previously described (26). Vertical lines at the M_rs 49,000 and 45,000 indicate the position of the undigested (49K) and digested (45K) LM particle in the first dimension. Constituents of the modified LM protein are expected to be found on the respective vertical line. Some H and L that migrated as a monomer in the first dimension, as well as the two fragments of H, 12.5K and 15K, are denoted. The digested sample was also separated only under denaturing conditions (Ref.▶). For dodecyl sulfate fractionation of intact RC in chromatophores see reference 26.

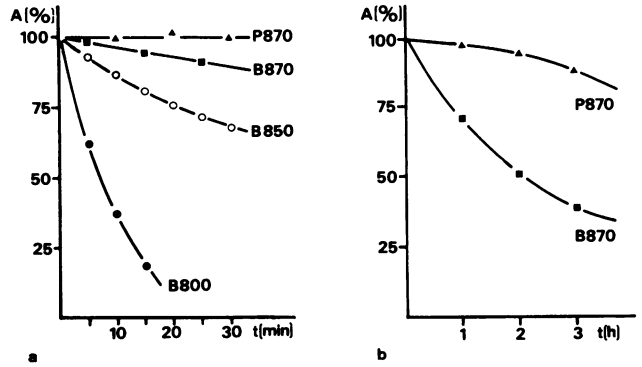

FIG. 6. Decay of in vivo Bchl absorbance during incubation of membranes with proteinase K. (a) Short-term data obtained with the wild-type strain from difference absorption spectra (B870, B850, B800), as well as reversible bleaching of the RC (P870). Membranes adjusted to 40 μg of Bchl per ml were treated with 80 μg of proteinase K per ml. (b) Long-term data obtained with membranes of the mutant strain A1a$^+$ of *Rhodopseudomonas capsulata*. The change of absorbance at 870 nm (B870), as well as reversible bleaching at 870 nm (P870), were recorded. Membranes adjusted to 10 μg of Bchl per ml were treated with 80 μg of proteinase K per ml.

It is interesting to compare these results with most recent sequence data on subunit M (41): according to the hydropathy profile (18) of M previously presented (41), the most hydrophilic part of the M chain is situated ca. 50 amino acids away from the C terminus, following a hydrophobic stretch. The observed reduction in the M_r of M by 4,000 may be reconciled with the cleavage of this segment of the M chain by proteinase K. The possibility that this part of M is exposed on the cytoplasmic surface and cleaved, resulting in a 20,000-molecular-weight fragment, should therefore be investigated.

Comparison of protease sensitivity of the different protein-associated Bchl species. The spectral data of the digested RC have indicated a remarkable protease resistance of native pigment species. To investigate how these data compare to absorbance changes of light-harvesting complexes, absorption difference spectra of digested chromatophores of the wild-type strain were recorded. Thus, B870 and B850 could be resolved at the initial stage of digestion. To compare the degradation rates of B870 and P870 for a longer period of time, chromatophores of the B800-850-defective mutant strain A1a$^+$ were treated with proteinase K. Figure 6 shows that there is a clearcut hierarchy of protease sensitivity. B800-850 is degraded initially, followed by B870 at a much slower rate (see Fig. 1; Fig. 6a). Photochemical activity is only affected when the major fraction of B870 is degraded (Fig. 6b), After 5 h, photochemical activity of P870 has collapsed completely. However, at this stage of the digestion both surfaces of the membrane are accessible to proteolytic attack.

These data show that, with *Rhodopseudomonas capsulata*, the primary donor P870 is the protein-associated Bchl species most resistant to attack by the unspecific proteinase K.

DISCUSSION

Among the topographical tools used for studying the exposure of polypeptides on membrane surfaces, proteolytic digestion is particularly suited to investigate the degree of exposure and to obtain structural details not provided by methods such as surface labeling with antibodies or chemical probes.

The data presented confirm the reports of several authors (see reference 37) that indicate an asymmetrical orientation of the RC in the membrane. The idea that polypeptide H is cleaved at a loop protruding from the cytoplasmic surface of the membrane to give membrane-bound fragments accounting for at least the major part of the polypeptide is in agreement with X-ray and neutron diffraction data (2, 23, 24) that indicate little exposure of the RC particle on either membrane surface. Such a model would also fit with current concepts of how the RC polypeptides are arranged in the membrane: circular and linear dichroism studies of RCs from *Rhodopseudomonas sphaeroides* reconstituted into liposomes indicated a high α-helical content of RC polypeptides, with α-helical stretches being tilted by 35 degrees with respect to the normal axis of the membrane (19). Assuming a total of ca. 300 amino acids for the M subunit of *Rhodopseudomonas capsulata*, this polypeptide may span the membrane several times. A well-studied example of how these α-helical stretches may be connected is bacteriorhodopsin of *Halobacterium halobium*, which spans the membrane seven times, with transmembrane stretches being connected probably by short reverse turns exposed on the membrane surface (30).The hydropathy profile of M previously presented (41), which bears some resemblance to that of bacteriorhodopsin (18), lends support to this idea. Although the data presented indicate only one possible loop for the H chain, there might be more not sufficiently exposed to be cleaved by proteases. Considering that the unspecific enzyme pronase does not digest polypeptide M in membranes of *Rhodopseudomonas sphaeroides* (16), whereas this RC subunit is readily cleaved by proteinase K with *Rhodopseudomonas capsulata*-negative proteolytic data are certainly not conclusive by themselves.

A recent report of a photochemically active RC particle containing only subunit L as judged by SDS-polyacrylamide gel electrophoresis (14) has stimulated discussion on whether this subunit may bind all of the pigment and cofactors. These authors indicated that their findings might be attributable to the action of contaminant proteases. As suggested in a most recent report (15), the RC preparation described in reference 14 may contain a fragment of M which appears at the position of L in the gel, causing this band to become strongly enhanced. Our studies with *Rhodopseudomonas capsulata* seem to give different results, since the bulk of M degraded initially does not cause a concomitant increase in intensity of the L band (see Fig. 3, track 2). However, there is an increase in intensity of L in tracks 3 and 4. These tracks also show a strong decrease in intensity of H. Nevertheless, the failure to detect any possible degradation products of M in track 2 indicates that this problem must be considered unsolved in the present study.

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