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# PROTEINS EXPOSED AT THE SURFACE OF CHROMATOPHORES OF *RHODOSPIRILLUM RUBRUM*

## THE ORIENTATION OF ISOLATED CHROMATOPHORES

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## Summary

The exposure of proteins at the surface of isolated chromatophores (i.e., the cytoplasmic face of intracytoplasmic membranes) of *Rhodospirillum rubrum* was studied by proteolysis as well as by enzymatic iodination with $^{125}I$. Analyses were performed after polyacrylamide gel electrophoresis of chromatophore proteins solubilized with sodium dodecyl sulfate. Reversible light induced proton uptake by partially digested chromatophores was used as a criterion for the integrity of the permeability barrier and thus, as evidence for proteolysis only of proteins outside of this barrier. Trypsin or α-chymotrypsin completely cleaved four proteins which were identified as the heavy subunit of succinate dehydrogenase ($M_r$ = 64 000), the α- and β-subunits of coupling factor ATPase ($M_r$ = 55 000 and 51 000), and the heavy (H) subunit of photochemical reaction centers ($M_r$ = 31 000). α-Chymotrypsin, in addition, attacked the protein ($M_r$ = 9000) of light harvesting bacteriochlorophyll preparations. By enzymatic iodination, the same proteins were labeled as were digested with trypsin or α-chymotrypsin except for the protein of $M_r$ = 9000. In addition, significant label was incorporated into three more proteins, one of which ($M_r$ = 41 000) could be identified as a major protein of the cell wall. The complete cleavage with trypsin of four proteins exposed at the surface indicated that isolated chromatophores were homogeneously oriented regardless of the method employed for cell breakage, i.e., passage through a French pressure cell at different forces or osmotic shock of sphaeroplasts.

## Introduction

The photosynthetic apparatus of phototrophic bacteria is largely localized in intracytoplasmic membranes. Upon cell homogenization, the continuity of the

intracytoplasmic membrane system is broken down into vesicles known as chromatophores [1]. Studies on the light-dependent formation of proton gradients have indicated that the orientation of the membrane system of intact cells is opposite to that of isolated chromatophores [2,3]. In other words, chromatophores which are responsible for the formation of net proton gradients represent inside-out vesicles. Thus, localization of proteins at the outer face of chromatophores provides a means of localizing proteins at the inner or cytoplasmic face of the intracytoplasmic membrane system of whole cells.

While it is known for many organisms that the majority of membrane proteins are exposed at the inner or cytoplasmic face of cellular membrane systems [4—6] little is known about the arrangement of proteins in the chromatophore vesicles of phototrophic bacteria. The knowledge that is available focuses primarily on complete functional units rather than on the individual membrane proteins which are detectable after polyacrylamide gel electrophoresis of membranes in the presence of sodium dodecyl sulfate. Reed and Raveed were the first to conclude that ATPase was exposed at the outside of chromatophores of *Rhodopseudomonas sphaeroides* [7]. Subsequently, photochemical reaction centers were also localized at the outside of chromatophores [8—11]. However, investigations to identify which of the three subunits, designated heavy (H), intermediate (M) and light (L), of reaction centers were exposed led to somewhat conflicting results. Incubation of chromatophores of *Rps. sphaeroides* with antisera against either the H subunit or the L · M complex indicated that both entities were exposed at the outside [8—10]. On the other hand, enzymatic iodination of chromatophores of *Rhodospirillum rubrum* revealed that only the H subunit was accessible from the outside [11].

One complication which must be kept in mind when doing research on the molecular architecture of isolated membrane fractions is the possibility that different methods of cell breakage will create differently oriented membrane vesicles [4,12]. Accordingly, membrane preparations may contain right-side-out and/or inside-out vesicles as well as vesicles which have lost asymmetry after translocation of proteins from one side to the other [12].

With this background, the present paper describes the localization of membrane proteins at the outer face of chromatophores, that is to say, the cytoplasmic face of the intracytoplasmic membranes of cells of *R. rubrum*. Since enzymatic digestion of membrane proteins was employed as the principal method of investigation, the results also present information on the orientation of isolated chromatophore vesicles.

## Materials and Methods

*R. rubrum*, strain FR1 (DSM-No 1068), was cultivated under phototrophic conditions on malate medium as described previously [13]. In order to radioactively label membranes U-$^{14}$C-labeled protein hydrolyzate (Radiochemical Centre, Amersham, U.K.) was added to the culture medium (50 $\mu$Ci/100 ml). Cells were harvested at the end of the logarithmic phase of growth, washed with Tris · HCl buffer (0.02 M; pH 7.6) containing EDTA (3 mM) and, unless

stated otherwise, homogenized in the same buffer by two passages through a precooled French pressure cell at 16 000 lb/inch$^2$. Particles in the 15 900 × *g* (20 min) supernatant were sedimented at 144 000 × *g* (60 min). The resultant crude membrane fraction was resuspended in Tris · HCl buffer (without EDTA) and 2.0-ml portions were layered on top of stepwise gradients (5 ml 1.5 M, 5 ml 1.2 M, 10 ml 1.0 M, 5 ml 0.6 M sucrose in Tris · HCl buffer). Gradients were run for 90 min at 113 000 × *g* [14]. Material banding in 1.0 M sucrose was collected and used as the chromatophore fraction. For sphaeroplast formation, cells of *R. rubrum* were suspended with Tris · HCl buffer supplemented with EDTA (3 mM), hen egg lysozyme (7 μM) and sucrose (0.3 M) and incubated for 30 min at 37°C. Following this the sphaeroplast suspension was diluted by addition of 1 vol. of ice-cold distilled water. After 10 min the osmotically shocked sphaeroplasts were sedimented and the resulting supernatant was centrifuged for 60 min at 144 000 × *g* in order to sediment the chromatophores. The chromatophore fraction was further purified by sucrose density gradient centrifugation as described above. Cell wall fractions were isolated as previously described [14]. For digestion of proteins chromatophores were incubated with trypsin (33 unit/mg) or α-chymotrypsin (75 unit/mg) at a constant pH of 7.5 in Tris · HCl buffer for 30 min at 37°C. Controls were incubated without enzymes. The reactions were stopped by addition of identical amounts of soybean trypsin inhibitor plus 2% aprotinin in Tris · HCl buffer [15]. The reaction system was incubated another 10 min at 37°C and washed once with buffer. Further details will be given in Results.

Light-dependent formation of proton gradients was followed in a reaction mixture according to Gromet-Elhanan and Briller [16] (200 mM NaCl/40 μM phenazine methosulfate/60 μM sodium ascorbate/1 μM 2-heptyl-4-hydroxyquinoline *N*-oxide). Intensity of incandescent light was $1 \cdot 10^5$ erg · cm$^{-2}$ · s$^{-1}$. Chromatophores for these experiments were digested with either trypsin or α-chymotrypsin in distilled water at a constant pH of 7.0. In this case reactions were not stopped with inhibitors. Enzymatic iodination of membrane protein (2 mg) was performed in the presence of 1 unit of lactoperoxidase (160 unit/mg) 1 unit of glucose oxidase (210 unit/mg)/0.003 mmol of glucose/0.01 μmol of NaI/250 μCi $^{125}$I (IMS. 30, Amersham, U.K.) per ml of 0.05 M sodium phosphate buffer (pH 7.6) [17]. The reaction mixture was incubated at 30°C and terminated after 30 min by addition of 0.1 M sodium azide [18]. Iodinated membranes were washed twice by centrifugation (113 000 × *g*, 60 min) with sodium phosphate buffer plus 0.01 mM NaI.

Coupling factor ATPase and photochemical reaction centers were isolated from membranes of the wild type and the blue-green mutant, strain VI, respectively, according to published methods [19—21]. For solubilization and purification of succinate dehydrogenase the method of Hatefi et al. [22] was modified as follows. Chromatophores were washed once with 0.02 M Tris · HCl buffer (pH 7.6) plus 1 M NaBr. The chromatophores were resuspended in Tris · HCl buffer plus 1 M NaI and incubated for 30 min at 4°C. The mixture was then diluted with 5 vols. of Tris · HCl buffer and centrifuged at 113 000 × *g* for 60 min. The resulting supernatant was fractionated as described [22]. Sodium dodecyl sulfate polyacrylamide gel electrophoresis on slab gels was carried out as described before [21,23]. Detection of labeled proteins on dried

polyacrylamide gels was most effective by means of autoradiography (Kodak, Kodirex-Xray-film).

Determination of protein and bacteriochlorophyll was done as described before [24]. Aprotinin and 2-heptyl-4-hydroxy-quinoline *N*-oxide was obtained from Sigma Chemical Co., St. Louis, Missouri. Trypsin, α-chymotrypsin, soybean trypsin inhibitor, lactoperoxidase and glucose oxidase were obtained from Boehringer, Mannheim, G.F.R. Lysozyme and chemicals for polyacrylamide gel electrophoresis were from Serva Entwicklungslabor, Heidelberg, G.F.R. Activities of the purchased enzymes and conditions of the assays are given by the respective producers. Molecular weights ($M_r$) of proteins were determined on 10% polyacrylamide gels (*N*,*N*'-methylenebisacrylamide, 0.27%) by comparison with standards.

## Results

*Proteolytic digestion of chromatophore proteins.* Chromatophores of *R. rubrum* were incubated with different amounts of trypsin or α-chymotrypsin and the digestion of proteins was estimated after polyacrylamide gel electrophoresis of membranes solubilized in the presence of sodium dodecyl sulfate [21,23]. Fig. 1 shows membrane protein patterns obtained after trypsin treat-

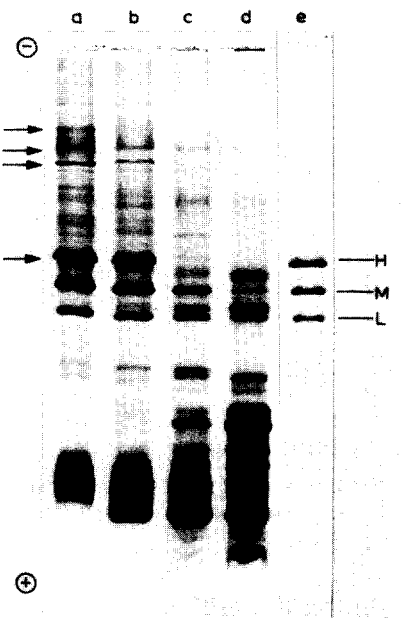



Fig. 1. Concentration dependence of trypsin digestion of chromatophores. Chromatophores of *R. rubrum* were incubated with trypsin at 37°C for 30 min. The reactions were terminated by addition of soybean trypsin inhibitor (1 mg per mg of trypsin) and aprotinin to a final concentration of 2%. After the incubation at 37°C had been extended for 10 min, chromatophores were sedimented by centrifugation and washed once with buffer. Chromatophores were then solubilized with sodium dodecyl sulfate and subjected in quantities of 50 μg to electrophoresis on 15% polyacrylamide (*N*,*N*'-methylenebisacrylamide, 0.41%) gels. Proteins were stained with Coomassie Blue. Trypsin concentrations per mg of membrane protein were: a, untreated control; b, 0.1 mg; c, 1.0 mg; d, 5.0 mg; e, untreated preparation of photochemical reaction center.

ment. As compared with the control (Fig. 1a) a concentration of at least 1 mg of trypsin per mg of membrane protein was required to lyse the proteins denoted with an arrow (Fig. 1c). Coelectrophoresis of a photochemical reaction center preparations (Fig. 1e) revealed that one of the digestable membrane proteins migrated exactly at the position of the heavy (H) subunit of reaction centers. At higher concentrations trypsin also was able to attack the intermediate (M) and light (L) subunits. The intensities of both bands decreased and since identical amounts of membrane protein originally incubated with trypsin were applied on top of the gels for electrophoretic separation, this can be taken as a roughly quantitative estimation. Identically the same membrane proteins were digested with α-chymotrypsin as with trypsin (Fig. 2). Better separation of slow migrating proteins allowed the identification of one more digestible protein. Unlike the results obtained with trypsin, a protein exhibiting the highest motility was also digestable with α-chymotrypsin. This protein, with a molecular weight of 9000, was shown before to be associated with the light harvesting bacteriochlorophyll moiety in *R. rubrum* chromatophores [21,25,26]. While this protein vanished with increasing amounts of chymo-

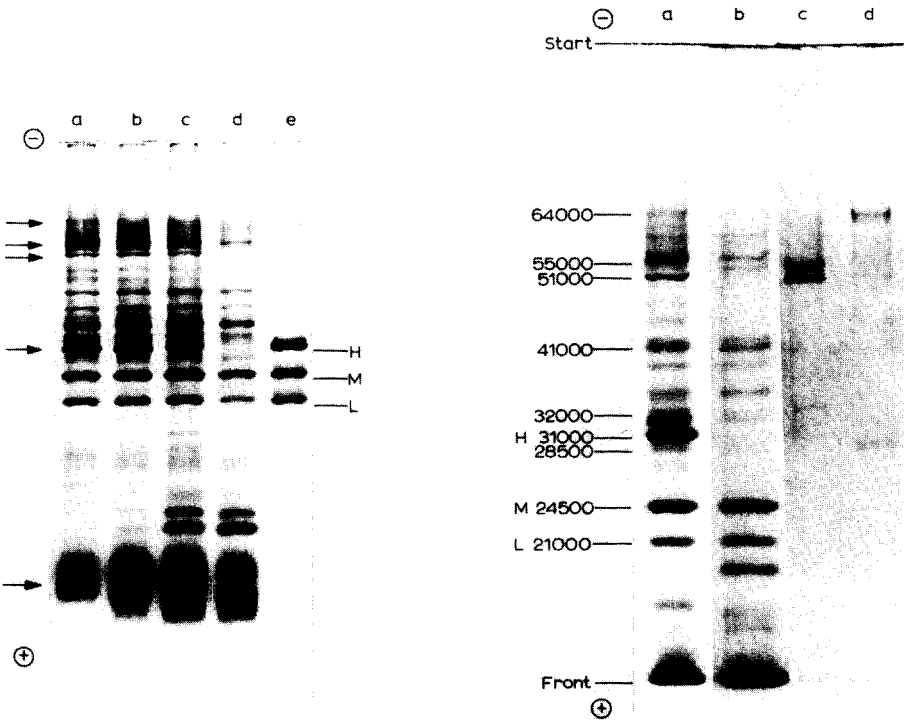


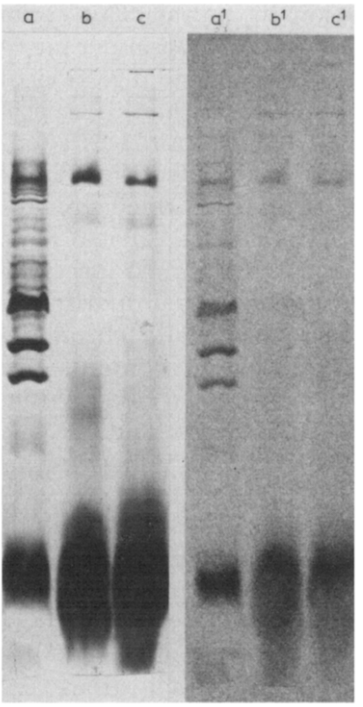

**Fig. 2. Concentration dependence of α-chymotrypsin digestion of chromatophores. Conditions were the same as in Fig. 1. α-Chymotrypsin concentrations per mg of chromatophore protein were: a, untreated control; b, 0.1 mg; c, 1.0 mg; d, 5.0 mg; e, untreated preparation of photochemical reaction center.**

**Fig. 3. Co-electrophoresis of a, untreated chromatophores; b, trypsinized chromatophores (1 mg of trypsin per mg of chromatophore protein); c, coupling factor ATPase; d, succinate dehydrogenase. The concentration of acrylamide was 10%. The numbers denote molecular weights.**

trypsin a new, slightly faster-migrating protein began to appear. This could already be observed at a concentration of 0.1 mg of α-chymotrypsin per mg of membrane protein. Also, it should be noted that for a complete digestion of reaction center protein H, higher amounts of α-chymotrypsin than of trypsin were necessary, although the specific activity of α-chymotrypsin was about twice as high as that of trypsin. For a better separation and identification of proteins of higher molecular weight, electrophoresis was performed on 10% polyacrylamide gels (Fig. 3). In addition, chromatophores were isolated from cells with low specific bacteriochlorophyll contents (5—7 μg of bacteriochlorophyll per mg of cell protein). In such chromatophores the proportion of high molecular weight proteins was increased in comparison with chromatophores derived from cells of higher specific bacteriochlorophyll contents [1]. Results of coelectrophoresis of proteins derived from untreated and trypsin-digested chromatophores, together with coupling factor ATPase and succinic dehydrogenase indicated that in situ, major proteins of both functional systems could be completely digested with trypsin (Fig. 3).

Lack of proteolytic degradation of membrane proteins might be the result of either hindered accessibility or resistance to proteolytic enzymes. Steck et al. [27] reported that addition of sodium dodecyl sulfate to membranes stimulated proteolysis of otherwise undigested proteins. Accordingly, before electrophoresis, radioactively labeled chromatophores were incubated for 30 min at 35°C in sample buffer containing 1.4% sodium dodecyl sulfate and either α-chymotrypsin or trypsin (1 mg of enzyme per mg of membrane protein). The action of proteolytic enzymes was not stopped by addition of inhibitors or by washing of the membranes. Thus, the membrane protein patterns included proteins of trypsin or α-chymotrypsin as well. Nevertheless, an evaluation of the protein patterns was possible after autoradiographic detection of labeled membrane proteins. The results in Fig. 4 showed that all of the membrane proteins except for the protein associated with light harvesting bacteriochlorophyll ($M_r$ = 9000) were digestable with either enzyme. Whereas the latter membrane protein seemed not to be attacked with trypsin, treatment with α-chymotrypsin caused a speading of the protein band towards lower molecular weights.

*Reversible formation of light-induced proton gradients.* Chromatophores were treated for 30 min with trypsin or α-chymotrypsin. After this the membranes (still in the presence of proteolytic enzymes) were incubated in a reaction mixture elaborated for the determination of light-dependent proton gradient formation [16]. Proteolytic reactions were not terminated in order to assure that proton gradients could be built up even in the presence of active proteases. According to Fig. 5, digestion of chromatophores with increasing amounts of α-chymotrypsin (up to 5 mg of enzyme per mg of membrane protein) still allowed the formation of reversible proton gradients upon illumination. The slight drift of the base line in the presence of α-chymotrypsin presumably was a result of residual proteolysis which lowers the pH in unbuffered systems [27]. In all of the experiments shown in Fig. 5 the extent of proton uptake was about 600 nequiv. of protons per mg of bacteriochlorophyll. Incubation of chromatophores with increasing amounts of trypsin caused a decrease in the total proton uptake until, at a concentration of 5 mg of trypsin



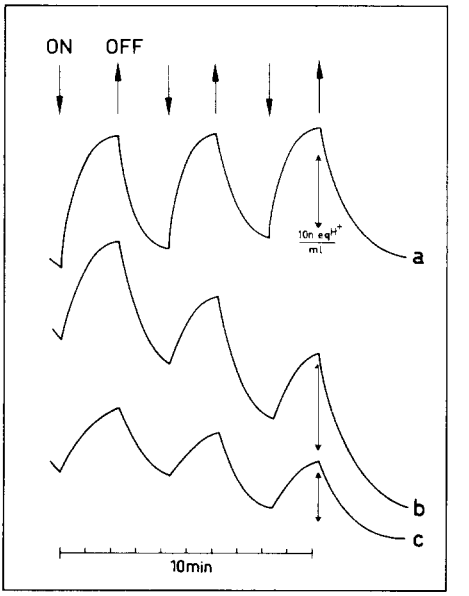




Fig. 4. Proteolytic digestion of chromatophore proteins in the presence of sodium dodecyl sulfate. $^{14}C$-labeled chromatophores were incubated with α-chymotrypsin or trypsin (1 mg per mg of membrane protein) and sample buffer containing 1.4% sodium dodecyl sulfate [23] at 37°C for 30 min followed directly by electrophoretic separation of the proteins on 15% polyacrylamide gels. After staining with Coomassie Blue, dried gels were exposed to X-ray film. a—c, Coomassie-stained gels; $a^1$—$c^1$, autoradiographs. a, $a^1$, Undigested control; b, $b^1$, chromatophores digested with α-chymotrypsin; or c, $c^1$, trypsin.

Fig. 5. Light-induced reversible pH changes in suspensions of chromatophores digested at different concentrations of α-chymotrypsin; a, untreated control; b, 1.0 mg; c, 5.0 mg of α-chymotrypsin per mg of chromatophore protein. Proton gradients were determined according to Gromet-Elhanan and Briller [16] at a bacteriochlorophyll concentration of 22 μg per ml reaction mixture. The vertical arrows on the right represent pH changes after addition of 10 nequiv. $H^+$ per ml.

per mg of membrane protein, no pH change was measurable. However, at a concentration of 1 mg of trypsin per mg of chromatophore protein proton uptake was completely reversible at an extent of 410 nequiv. of protons per mg of bacteriochlorophyll.

*Enzymatic iodination of chromatophore proteins.* Chromatophores were labeled enzymatically with $^{125}I$ according to the procedure described by Hubbard and Cohn [17]. Following electrophoretic separation of membrane proteins on either 10% or 15% polyacrylamide gels the slab gels were stained and dried. Labeled proteins were localized by autoradiography. Fig. 6 compares the stained membrane protein patterns with their respective autoradiographs. On 15% polyacrylamide gels the protein which is associated with light harvesting bacteriochlorophyll was separated from material migrating at the buffer front. No label could be detected at this protein's position (Fig. 6A).

As was previously demonstrated by Zürrer et al. [11], subunit H of photo-

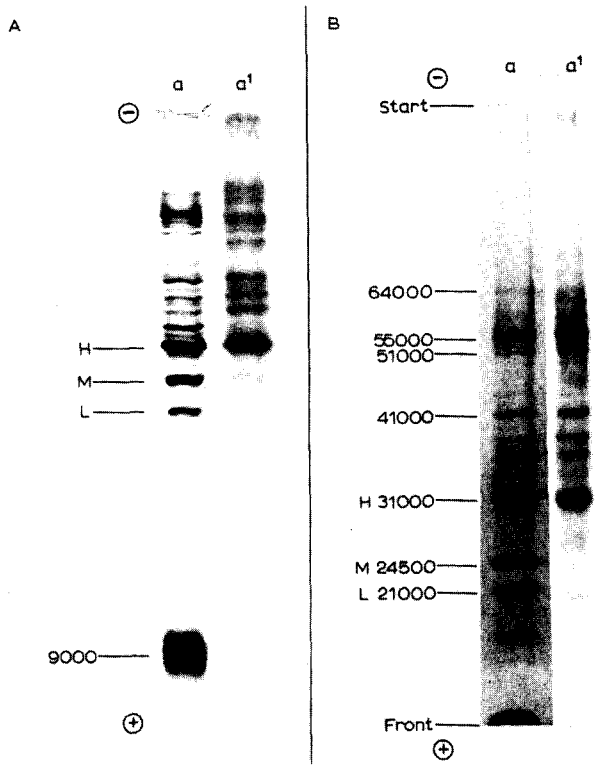



Fig. 6. Labeling patterns of chromatophore proteins with $^{125}I$. Protein patterns in A, 15% and B, 10% polyacrylamide gels. a, proteins stained with Coomassie Blue; $a^1$, autoradiographs of labeled proteins. Numbers denote molecular weights.

chemical reaction centers was heavily labeled while subunits M and L incorporated practically no label (Fig. 6 A, B).

A better separation of proteins of higher molecular weight was achievable on 10% polyacrylamide gels. Those proteins carrying major amounts of label were (a) proteins of $M_r$ = 55 000 and 51 000, characteristic of the $\alpha$ and $\beta$ subunits of ATPase [19,28]; (b) a protein of $M_r$ = 41 000, presumably representing cell wall contaminants [14]; and (c) two more proteins of molecular weights between 32 000 and 41 000 of so far unknown function and origin. Furthermore, proteins of $M_r$ = 55 000 also incorporated iodine, but from the autoradiographs no unequivocal relation to Coomassie Brilliant Blue stained protein bands could be drawn.

*Proteolytic digestion of chromatophores liberated by different methods of cell breakage.* Trypsin treatment was applied in order to investigate whether different methods of cell breakage might produce chromatophores which exhibited different susceptibilities towards proteolytic digestion. Accordingly, cells were broken by one or two passages through a French pressure cell at different pressures. In addition, cells were transformed into sphaeroplasts and chromatophores were liberated by osmotic shock. The respective chromatophore preparations were incubated as described above in the presence

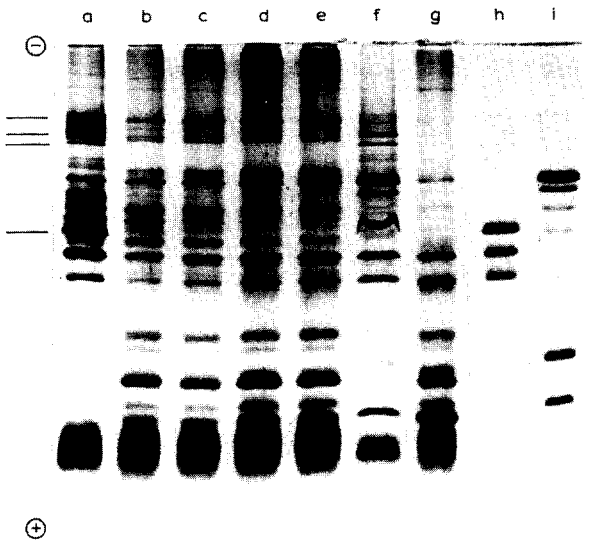



Fig. 7. The effect of cell breakage on the trypsinization of chromatophores. For cell breakage the following methods were employed: a—e, passages through a French pressure cell (a, b, once at 10 000 lb/inch$^2$; c, twice at 10 000 lb/inch$^2$; d, once at 18 000 lb/inch$^2$; e, twice at 18 000 lb/inch$^2$); f, g, osmotic shock of sphaeroplasts; a, f, untreated controls; b—e, g, chromatophores treated with 1 mg of trypsin per mg of chromatophore protein (for further details see Fig. 1); h, photochemical reaction center; i, cell wall. Acrylamide concentration was 15%.

of 1 mg of trypsin per mg of chromatophore protein. The reactions were terminated by addition of soybean trypsin inhibitor (1 mg per mg of trypsin) and 2% aprotinin. Untreated chromatophores liberated by either one of the two methods of cell breakage exhibited nearly identical protein patterns Fig. 7 a, f). Also, with all of the chromatophore preparations employed exactly the same protein bands as described above (Figs. 1 and 3) disappeared in the course of tryptic digestion. In the case of the chromatophores derived from sphaeroplasts some digestion of the major protein band typical of cell wall preparations could also be observed. Protein patterns shown in slots h and i of Fig. 7 are representative of photochemical reaction centers and cell wall preparations respectively. In the latter, however, the fastest migrating protein band represents lysozyme which can also be detected in patterns of chromatophores derived from sphaeroplasts.

## Discussion

Trypsin and α-chymotrypsin cleave peptide bonds with different specificities. Nevertheless, incubation of chromatophores of *R. rubrum* with either protease resulted in the complete digestion of the heavy subunit of succinate dehydrogenase ($M_r$ = 64 000), the α and β subunits of ATPase ($M_r$ = 55 000 and 51 000) and the H subunit of photochemical reaction centers ($M_r$ = 31 000). All of the molecular weights are largely in agreement with values reported by other authors for the respective preparations [19—22,28,29]. Only

α-chymotrypsin, however, attacked a protein ($M_r$ = 9000) which is known to be present in light harvesting bacteriochlorophyll preparations [21,25,26].

Trypsin and α-chymotrypsin are proteins too large to penetrate biological membranes. Accordingly, proteolysis of membrane proteins is normally interpreted as evidence for their exposure at the outer face of closed membrane vesicles [5,30,31], but it is also known that special types of membranes are quickly destroyed upon trypsinization [30,31]. This in turn would render normally unexposed proteins of the interior to proteolytic digestion. To make sure that trypsin and α-chymotrypsin did not enter the interior of chromatophores during the process of digestion the integrity of the permeability barrier was tested through the determination of proton gradients formed reversibly upon illumination. Lack of proton gradient formation, of course, does not necessarily mean that the permeability barrier was destroyed or that proteases could enter the interior of the chromatophore. On the other hand, the formation of a reversible proton gradient is clearly an indication for an operative permeability barrier. The results infer that trypsin (up to a concentration of 1 mg per mg of membrane protein) and α-chymotrypsin (up to a concentration of 5 mg per mg of membrane protein) did not demolish the membrane. On this basis the results in Figs. 1—3 indicate that the proteases digested only proteins which are exposed at the chromatophore surface. Results obtained after enzymatic iodinations support these findings. In addition to being found in proteins which were proteolytically digestable, label could also be detected at the position of the major cell wall protein ($M_r$ = 41 000) and of two more proteins migrating between $M_r$ = 32 000 and 41 000. One explanation for the discrepancy between the results of proteolysis and iodination might be that $^{125}I$ was incorporated unspecifically into proteins which were not exposed at the chromatophore surface. This argument can be ruled out on the basis of results obtained by Zürrer et al. [11] who reported that if made accessible, all three subunits of the reaction centers could be labeled equally with $^{125}I$. Another more likely explanation for the above discrepancy may be that certain surface proteins, all of the molecules of which were not completely digestable in situ, could also be labeled. That this was true at least for the cell wall protein ($M_r$ = 41 000) can be observed by comparison of f and g in Fig. 7.

There were other membrane proteins which were attacked only by one or the other of the methods applied. Reasons for this might be due to differences in the accessibility of reagent molecules of different sizes, or the inherent inertness of certain proteins towards special reactions because of the lack of specific sites of action. An example of hindered accessibility was given by observations that antisera against photochemical reaction centers bind to the chromatophore surface only after removal of ATPase [8—10]. Probes employed for the present study exhibit considerably lower molecular weights than γ-G-immunoglobulins. Therefore, removal of ATPase was neither necessary nor desirable.

Lack of specific sites of action is presumably a major reason for the fact that the protein of light harvesting bacteriochlorophyll complexes did not incorporate $^{125}I$. This presumption can be made on the basis of recent investigations which showed that the isolated protein contained only traces of tyrosine [26]. Nevertheless, very recently Cuendet et al. [32] reported that in their hands some iodination of the light harvesting bacteriochlorophyll complex was

observable. The successful action of α-chymotrypsin agrees well with the high contents of leucine and aromatic amino acids detectable in light harvesting bacteriochlorophyll protein complexes [26]. In principle, the presence of arginine and lysine should permit tryptic digestion of the protein. The relatively high resistance of the protein towards trypsinization even in the presence of sodium dodecyl sulfate is obviously the reason that Tonn et al. [25] used a method requiring a period of 15 h to digest the protein with trypsin.

During cell breakage, membranes are subjected to forces of different intensities. However, results obtained after trypsin digestion showed that regardless of the method of cell breakage the same identical chromatophore proteins could be removed completely from their original positions within the protein patterns. As trypsin removed proteins only from the outer face, the results indicated that regardless of the method of cell breakage, homogeneously oriented chromatophores were liberated from the cell. This is in contrast with findings reported for the orientation of vesicles derived from cytoplasmic membranes [4,6,12]. Homogenization of cells by passage through a French pressure cell resulted in the formation of largely inside-out cytoplasmic membrane vesicles, while osmotic shock of sphaeroplasts yielded predominantly right-side-out vesicles. The crucial difference between the generation of chromatophores and cytoplasmic membrane vesicles obviously resides in the fact that chromatophores derive from already vesicular intracytoplasmic membranes, while fragments of cytoplasmic membrane are formed and vesiculated only during cell breakage. This leads to the final conclusion that the original orientation of the vesicular intracytoplasmic membrane system of *R. rubrum* exclusively determines the orientation of the isolated chromatophore vesicles.

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