Vol. 120, No. 1, 1984 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
April 16, 1984 Pages 164-171

STRUCTURE OF A BACTERIAL PHOTOSYNTHETIC MEMBRANE: INTEGRITY OF REACTION CENTERS FOLLOWING PROTEOLYSIS AND DETERGENT SOLUBILIZATION

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Received March 2, 1984

The photosynthetic membranes of the purple bacterium **Rhodopseudomonas viridis** are composed of a semi-crystalline lattice of subunits. Proteolysis of isolated membranes with trypsin or pronase results in the degradation of polypeptides associated with the photosynthetic reaction center. However, two low molecular weight peptides which may form the light-harvesting complex survive the enzymatic treatment. The proteolysis does not affect the major absorbance peak (830nm) associated with the reaction center. However, treatment of proteolyzed membranes with detergents such as LDAO abolishes the 830 nm absorbance peak. The 830 nm peak is stable following LDAO solubilization of non-proteolyzed membranes. These results suggest that a combination of covalent and non-covalent interactions are important in maintaining the configuration of the reaction center, and are consistent with a model of membrane organization in which the light-harvesting componenents are buried in a lipid phase of the membrane and reaction center components form the large structures which electron microscope studies have shown to extend from either membrane surface.

The internal photosynthetic membranes of the purple, non-sulfur bacterium **Rhodopseudomonas viridis** exist as saccules composed of subunits arranged in a nearly crystalline order. The organization of the membrane lattice has been studied using image reconstruction techniques (1,2,3). A recent study from this laboratory has indicated that the membrane is composed of seven major polypeptides (4). Five of the polypeptides, with approximate molecular weights of 44,41,38 32, and 28 kd are thought to be subunits of the photosynthetic reaction center. The two remaining polypeptides with molecular weights of 16 and 11 kd may represent light-harvesting proteins associated with the reaction center.

We recently reported that extensive digestion of the membrane with either trypsin or pronase resulted in a complete abolition of the higher molecular weight membrane polypeptides with the result that only the light-harvesting polypeptides survived the enzymatic treatment. In this paper, we report that the spectral characteristics of the reaction center persist despite the enzymatic treatment. Further, we have discovered that the spectral signal for the reaction center disappears

Abbreviations: SDS: sodium dodecyl sulfate. LDAO: N,N,-dodecyldimethylamine N-oxide.

0006-291X/84 $1.50

when the proteolyzed membranes are treated with certain detergents capable of solubilizing the lipid matrix of the membrane.

MATERIALS AND METHODS

Culture of the organisms, membrane isolation, gel electrophoresis, and trypsin proteolysis were carried out as described previously (4). To analyze the effect of LDAO on trypsinized membranes, the membrane pellets were resuspended in 1.0 ml of LDAO as a 1% solution in sodium phosphate buffer, pH 7.2. The spectra of the membranes were then analyzed from the time of additon of the detergent (time zero) until 20 min had elapsed. The spectra were recorded in the range of 860 to 760 nm using a Cary 219 UV-VIS spectrophotometer with the repetitive scan feature at a rate of one complete scan per minute. Water was deionized and distilled.

RESULTS

In a previous paper, we found that extensive digestion of the photosynthetic membranes from **Rhodopseudomonas viridis** with concentrated solutions of either trypsin or pronase resulted in dramatic changes in the polypeptide composition of the membrane. Fig. 1, taken from Jacob & Miller (4), shows a typical SDS-polyacrylamide gel of a digestion experiment using trypsin. After 60 min of treatment in a full-strength trypsin solution at 23 degrees C. the only components of the membrane left apparently intact are light-harvesting polypeptides with molecular weights of 16 and 11 kd.

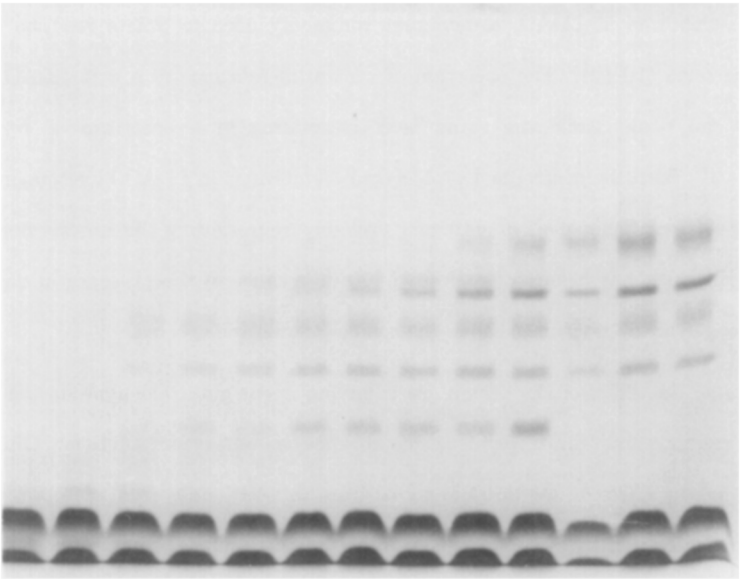


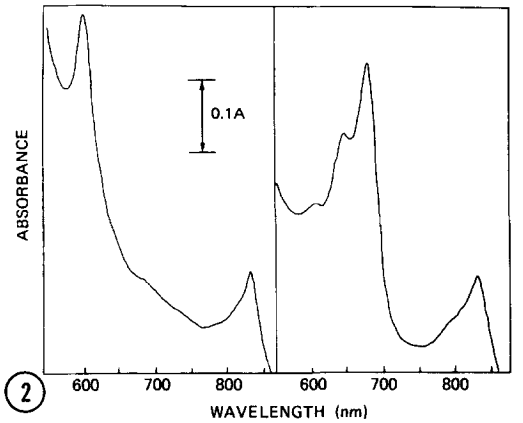
Figure 1: SDS-polyacrylamide gel of **Rh. viridis** membrane components during trypsin digestion. Wells 1-10 (numbered from left) were treated for 35 minutes at 25 degrees C. with a series of dilutions obtained from a 10mg/ml trypsin stock solution. Wells 1-10 contained 3, 6, 12, 18, 24, 36, 54, 72, 108, and 216-fold dilutions of the trypsin stock solution. Well 11 contained membranes treated with 100 mM sodium carbonate (pH 12) for 35 minutes at 4 degrees C to remove non-integral membrane proteins (22). Wells 12 and 13 contained untreated photosynthetic membranes. Gel stained with Coomassie blue. Taken from reference (4).

The polypeptide composition of the digested membrane samples indicates that the high molecular weight bands (44, 41, 38, 32 and 28 kd) have been cleaved by the proteolytic treatment. Does this mean that the reaction center of the membrane has also been destroyed? Spectral studies of isolated reaction center preparations (5-14) and of the reaction center **in situ** (13, 15, 16) indicate that a major peak at 830 nm is due to bacteriochlorophyll molecules specifically associated with the reaction center. The 830 nm absorbance is not appreciably affected by light- or chemical-induced reduction-oxidation changes, unlike another reaction center absorbance peak in the near-infrared at 960 nm (5, 7-13, 15, 16). Therefore, an absorbance peak at 830 nm may be taken as a signal indicating the presence of a reaction center.

Spectra taken of control and trypsin-digested membrane samples showed that the 830 nm signal persists even after proteolysis (Figure 2). Therefore, a recognizable reaction center signal is seen in these membranes, even though the high molecular weight polypeptides have apparently been cleaved by trypsin. Changes were observed in the 600-700 nm region of the proteolyzed membranes, suggesting that trypsin treatment may affect the light-harvesting components of the membrane in ways not detected by gel electrophoresis.

Does the trypsin treatment affect the stability of the 830 nm reaction center signal when membranes are solubilized in LDAO, a detergent commonly used to isolate reaction centers from photosynthetic bacteria (10-13)? The spectrum of control membranes in a 3 % LDAO solution has the typical 830 nm peak, while the same peak is missing in a spectrum of trypsin-digested membranes (Fig. 3). Both the control and proteolyzed membranes in LDAO have major absorbances at 673 nm with a lesser "shoulder" at 640 nm, probably representing the absorbance of degraded bacteriochlorophyll which has been shifted from 680 nm because of the presence of detergent. The 673 signal is stronger in the trypsinized than the control membrane spectra.

We investigated the effect of the protein cross-linking agent glutaraldehyde on the survivability of the reaction center signal in trypsinized membranes in detergent solution. Glutaraldehydge fixation by itself has no effect on the 830 nm signal from control (undigested) membranes (Fig.6, A). Also, glutaraldehyde fixation preceeding trypsinization did not inhibit the destruction of the reaction center absorbance (Fig.6, C). However, cross-linking with glutaraldehyde following proteolysis did protect against the loss of the reaction center absorbance (Fig.6, B). The cross-linking agent provided effective protection even in the case where 3% LDAO was used to solubilize the membranes - a condition where the loss of 830 nm activity is normally very rapid.



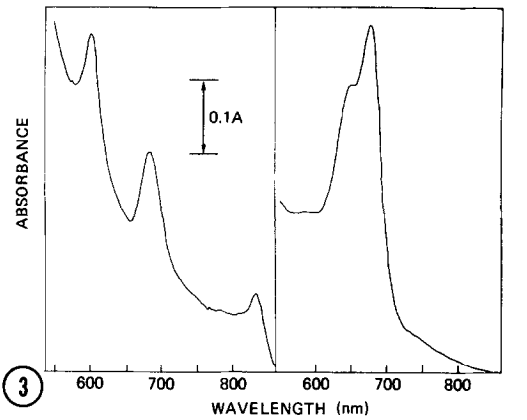


Figure 2: At left is an absorption spectrum of isolated **Rh. viridis** photosynthetic membranes. Although other peaks are visible at shorter wavelengths (at 600 nm, for example), the major peak associated with the photosynthetic reaction center is visible at 830 nm. The tracing at the right is a similar spectrum taken from membranes treated with trypsin as described in the text and in Figure 1. There are some changes associated with shorter wavelength peaks, but the reaction center signal at 830 nm is not altered, even after extensive trypsinization.

Figure 3: Absorption speectra of membranes solubilized in LDAO detergent. At left is the spectrum of membrane material after dissolution in 3% LDAO. At right is the spectrum of trypsinized membrane material following its dissolution in 3% LDAO. Several changes are seen in the shorter wavelength peaks between these two spectra and also when compared to spectra taken in the absence of detergent (Figure 3). However, the loss of the 830 nm signal in the trypsinized sample shown in the right-hand tracing indicates the loss of the reaction center. This 830 nm signal is resistant to treatment with trypsin (see Figure 3) and to LDAO treatment (left-hand spectrum, this Figure), but is lost when proteolytic and detergent treatments are combined.

DISCUSSION

These results suggest that enzymatic cleavage leaves the reaction center structure generally intact. These suspicions are confirmed by the presence of the 830 nm peak following trypsin digestion (see Fig. 3).

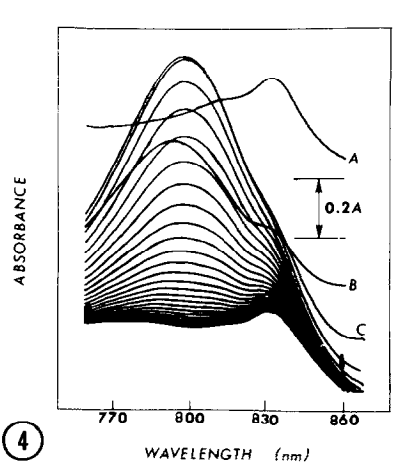
The experiments with LDAO solubilization were performed to test whether reaction centers that were intact following proteolysis would break apart during detergent solubilization. Solubilization of control (undigested) membranes with the detergent LDAO had no effect on the 830 nm reaction center signal, but a major new absorbance peak did result from the detergent treatment. The new peak, absorbing at 673 nm may be due to the dissociation of antenna bacteriochlorophyll b absorbing at 1015 nm (9,18). However, LDAO treatment completely abolished the 830 nm peak in trypsinized membranes, suggesting that the environment of the reaction center, although capable of withstanding either proteolysis or detergent -solubilization singly, cannot withstand the two in concert.

Our explanation of this phenomenon is that although the reaction center **in situ** has been cleaved into numerous tryptic fragments, the fragments are held oriented in the lipid environment of the membrane by non-covalent forces and that the orientation is sufficient to yield a strong reaction

center spectral signal. However, when detergents disrupt the lipid environment, the presence or absence of tryptic cuts in the polypeptide backbone of each band associated with the reaction center now becomes a factor. The fragmented polypeptides (in trypsinized membranes) fall apart and the reaction center signal disappears.

Time course studies of the solubilization process show some interesting characteristics. In the case of LDAO solubilization (of either control or proteolyzed membranes), a major absorbance at 795 nm appears within seconds of introduction of detergent and later disappears after 20 minutes (see Figs. 4, 5). This transient peak may represent the breakdown of unstable light -harvesting pigment-protein complexes (since the reaction center polypeptides are stable in LDAO). Stable light-harvesting complexes have not been isolated from **Rh. viridis** although the spectral characteristics of the intermediate breakdown products have been studied (8,9,18).

The experiments with glutaraldehyde-crosslinking generally support our explanation of reaction center stability **in situ** following detergent solubilization. Trypsin cleaves peptide (covalent) bonds located at lysine and arginine residues. Glutaraldehyde crosslinks free amino groups located at the same residues. Therefore, glutaraldehyde fixation may act to repair much of the damage incurred



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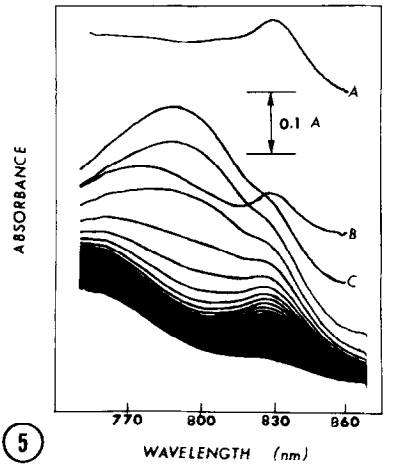

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Figure 4: Time course of absorbance changes associated with the solubilization of untrypsinized (control) membranes in 3% LDAO. Tracing A shows the initial spectrum in the 830 nm region. Tracing B shows the spectrum approximately 30 sec after the addition of 3% LDAO to the sample cuvette, while tracing C and those which follow document the absorbance changes associated with the solubilization process at approximate one minute intervals. Immediately following solubilization, a large component peaking at 790 nm appears, and then rapidly breaks down. This component may be associated with the breakdown of the long-wavelength absorbing light-harvesting complex by the detergent. The reaction center peak at 830 nm is momentarily obscured by this peak, but rapidly re-appears in the final tracings.

Figure 5: Dynamic recordings of the absorbance changes associated with 1.5% LDAO solubilization in trypsinized membranes. A transient component appears at 790 mn, and then is lost (as in Figure 5), however, the reaction center signal at 830 nm is also lost during the solubilization process.

during proteolysis and the reaction center signal persists following detergent solubilization. Reaction centers that have been "repaired" first and then enzymatically digested suffer the same fate as "unrepaired" reaction centers and the 830 nm signal is abolished (see Figure 6), suggesting that glutaraldehyde does not provide so much a protective function as a repair function.

The rapid degradation of reaction center components during proteolysis indicates that these components are exposed at the surface of the membrane. The relative inaccessibility of light-harvesting components suggests, in a similar fashion, that these components are not exposed at the membrane surface. In a recent study of the three-dimensional architecture of this membrane by electron microscopy, it was suggested that reaction center components might be the principal constituents of a large structure protruding from each surface of the membrane, and that light-harvesting components might be arranged around that central structure as spokes around a hub (1,

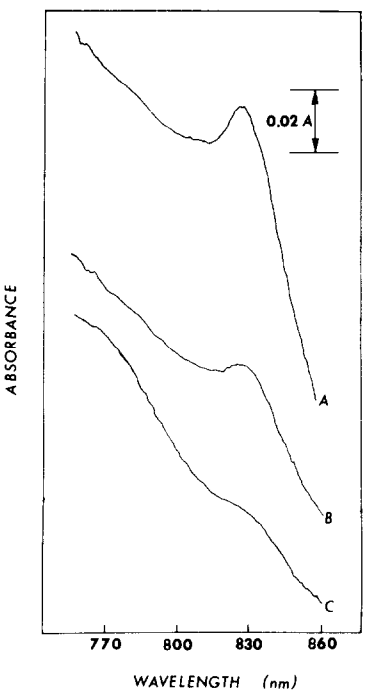

Figure 6: Glutaraldehyde crosslinking provides apparent protection for proteolyzed membranes and tends to preserve the 830 nm signal. Tracing A shows the spectrum of membranes following fixation with 1% glutaraldehyde for 15 minutes. Tracing B shows the spectrum a membranes extensively digested with trypsin, then fixed with glutaraldehyde for 15 minutes, then solubilized in 3% LDAO. Unfixed membranes would lose the 830 nm signal after this procedure. Instead, a significant portion of the 830 nm reaction center signal is preserved. Tracing C shows the effect of preceeding trypsin treatment with glutaraldehyde treatment. Some protection is still afforded and the result is a small shoulder at 830 nm following LDAO treatment. However, the most effective treatment is to follow trypsin with the crosslinking agent, suggesting that the formation of new covalent bond by glutaraldehyde can be a stabilizing factor for some fraction of trypsinized reaction centers when detergent is applied.

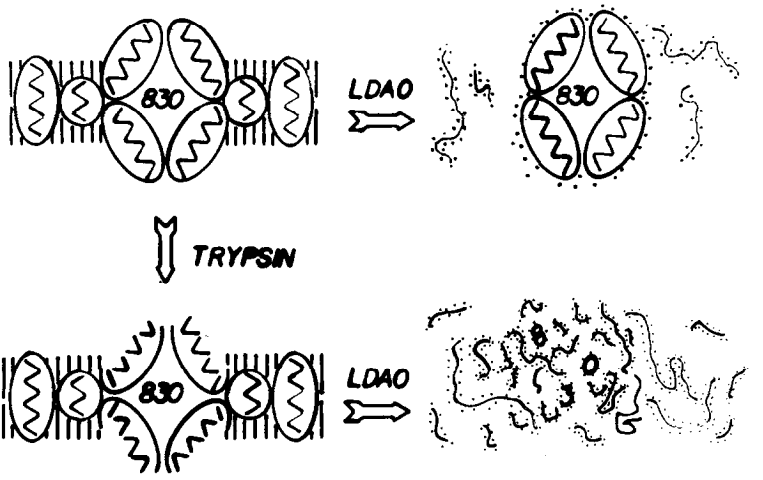

Figure 7: Highly diagrammatic interpretation of the effects of trypsin and detergent treatment on **Rh. viridis** photosynthetic membrane. The reaction center (830 nm absorbance) is shown in an environment created by several polypeptides which are exposed at the membrane surface and protrude to form a particle visible in electron microscope studies. At either side of the reaction center are two light-harvesting components, buried in the lipid phase of the membrane and protected from proteolysis. Although treatment with LDAO dissolves the membrane matrix, it does not disrupt the reaction center because of the protein-protein interactions which hold it together (upper right). Similarly, treatment with trypsin, although it cleaves exposed regions of the reaction center peptides, does not disrupt the reaction center signal due to non-covalent interactions which maintain the environment necessary for the 830 nm signal (lower left). However, when trypsin and detergent treatment are combined (lower right) all important interactions which are necessary to stabilize the 830 nm signal are lost, and the reaction center is broken up.

3). The work reported here tends to fit this general picture of membrane organization. Our studies on the presistence of the 830 nm signal following proteolysis also seem to fit this general picture. Solubilization of the membrane in detergents such as LDAO which do not disrupt reaction center organization does denature the light-harvesting components, suggesting that these structures are maintained by hydrophobic interactions which are disrupted by LDAO. This supports the notion that the light-harvesting components are closely associated with a phase of the membrane removed from the surface, presented diagrammatically in Figure 7.

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