Partial proteolytic digestion of isolated bacterial photoreaction centers without apparent alteration of pigment–protein interaction

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Photoreaction centers were purified from wild-type *Rhodospirillum rubrum* by a new procedure which reduced to undetectable levels the activities of contaminant endogenous proteinases. As shown by sodium dodecylsulfate–polyacrylamide gel electrophoresis, mild digestion of the centers with α-chymotrypsin degraded the 36 kDa (H) polypeptide but did not elicit significant alterations in the 29 (M) and 25 kDa (L) constituents. While short-term incubation with trypsin removed H and decreased the M_r of M to that of L, a prolonged treatment with trypsin yielded preparations with polypeptides of 19 and 5 kDa. Since the proteolyzed preparations retained the near infrared absorption spectrum of the native photoreaction center and exhibited photochemical activity, an apparent 3–4-fold increase of the primary donor : protein ratio was achieved.

Photosynthesis, bacterial *Pigment–protein complex* *Reaction center, bacterial*
Reaction center protein *Reaction center proteolysis* Rhodospirillum rubrum

1. INTRODUCTION

The photoreaction center is a membrane-bound complex of pigments, proteins and other constituents which performs the primary photochemical redox reaction of photosynthesis [1]. In purple bacteria, it contains 3 different polypeptides (H, M, L, in order of decreasing M_r) which seem to occur each in a 1 : 1 molecular ratio to the primary electron donor [2]. Two of the polypeptides could apparently be removed from the *Rhodospirillum rubrum* photoreaction center without loss of photochemical activity [3], which suggested that the removal might have involved selective degradation by contaminant proteinases. This could be the case because the photoreaction center preparations did contain proteinase activities (unpublished). Here, we describe a new preparative procedure which reduces to undetectable levels the endogenous proteinases and show also that, under controlled conditions, trypsin and/or α-chymotrypsin digest a large part of the photoreaction center protein but leave undisturbed the characteristic near infrared absorption spectrum and photochemical activity.

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2. METHODS

Photoreaction centers were prepared as follows from chromatophores which had been isolated from phototrophically grown *R. rubrum*, strain S1 [4]. The diluted chromatophore-free, detergent-solubilized extract [5] (200 ml, 1.6 μM) (centers) was brought to pH 8.5 with 5 M KOH and mixed with a thick paste of DEAE-cellulose in 10 mM Tris–HCl (pH 8.0) (buffer) to obtain a colorless supernatant solution. The mixture was filtered and the residue was washed twice with 200 ml buffer and twice again with 200 ml buffer containing

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0.3% Triton X-100 and 15 mM NaCl. The residue was suspended in this solution and placed in a chromatography column from which a photoreaction center-enriched fraction was eluted with buffer containing 0.3% Triton X-100 and 50 mM NaCl. Sometimes the late fractions contained antenna constituents and had to be discarded. After 3-fold dilution with buffer, the eluate was bleached with the DEAE-cellulose paste, the mixture was poured into a column and the photoreaction centers were eluted with buffer containing 0.1% Triton X-100 and 125 mM NaCl. The eluate was then loaded onto a Sephacryl S-400 column (70 × 2.5 cm) which had been pre-equilibrated with buffer containing 0.4% sodium deoxycholate. Elution with this solution yielded, after about 800 ml, photoreaction-center containing fractions which were pooled and stored at −70°C. Earlier fractions were enriched in antenna constituents and later fractions showed high levels of bacteriopheophytin. All steps were carried out at 0–4°C under dim light.

Proteolytic digestion of 3 μM photoreaction center samples (usually 25 ml) was carried out in the dark at 15°C. Trypsin type XI (EC 3.4.21.4), α-chymotrypsin type II (EC 3.4.21.1), and the soybean trypsin inhibitor type I-S, were obtained from Sigma Chemical Co. At the end of the incubation period, the mixtures were diluted 4-fold with buffer and mixed with the DEAE-cellulose paste until the supernatant was bleached. After filtration, the residue was sequentially washed on the filter with 100 ml buffer and 100 ml 125 mM NaCl in buffer to remove most of the proteases (photoreaction centers were not desorbed in the absence of added detergent). After suspending in buffer and packing in a chromatography column, DEAE-cellulose was washed with 100 ml 0.03% dodecyldimethylamine *N*-oxide in buffer and with 30 ml 0.1% Triton X-100 in buffer, to elute low M_r digestion products. Finally, photoreaction centers were recovered in buffer containing 0.1% Triton and 125 mM NaCl and dialyzed against detergent-free buffer for 24 h. The procedure was performed at 0–4°C in the dark.

Sodium dodecylsulfate–polyacrylamide gel electrophoresis was done as in [6], using 90 mg acrylamide/ml and 2.4 mg *N,N'*-methylenebis(acrylamide)/ml. Samples (10 μl) were applied to the gels after denaturation as in [3]. Absorption spectra and electrophoretograms were obtained and processed as in [3].

3. RESULTS

The method in section 2 yielded photoreaction center preparations which did not contain endogenous proteinases as assayed by standard procedures (unpublished). However, it was observed that prolonged storage at 4°C caused some degradation of the H protein. This seems to be the reason for the decreased levels of this constituent in some of the preparations used in this work. The method allowed recovery of about 45% of the centers present in the starting chromatophore material, twice as much as that obtained in [5].

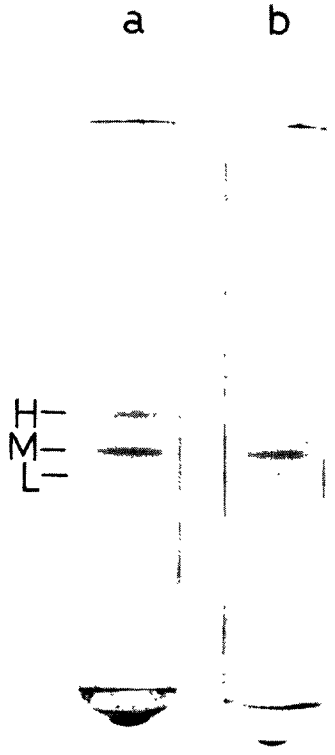

Fig.1. Gel electrophoretograms of photoreaction centers: (a) undigested; (b) after 10 min incubation with 2 μg α-chymotrypsin/ml. Each gel contained 9 pg equiv. primary donor. Relative areas of the bands in the electrophoretograms are (a) 156, 140 and 100 and (b) 142 and 109, in order of decreasing M_r. An arbitrary value of 100 was assigned to the area of undigested L.

As indicated by sodium dodecylsulfate–polyacrylamide gel electrophoresis, incubation of photoreaction centers with either or both trypsin and α-chymotrypsin has led to a differential degradation of their characteristic protein constituents. It was found that some of these proteolytic treatments evoked no significant alteration in the near infrared absorption spectrum of the centers, a sensitive indicator of the native pigment–protein interaction. Additional, non-destructive treatments will be described below.

After 10 min, in the presence of 2 μg α-chymotrypsin/ml, the preparation exhibited only two major electrophoretic bands that had the same areas and positions as those corresponding to M and L in untreated centers (fig.1). A similar pattern was obtained after α-chymotrypsin treatment when the starting centers lacked H, completely or in part, as the result of endogenous proteinase activity during prolonged storage at 4°C (not shown). Thus, it seems that, under the conditions used, α-chymotrypsin selectively degraded H.

Trypsin at 70 μg/ml caused in 1 h the disappearance of both the H and the M bands and a large increase in the area of the L band (fig.2). Since the same results were obtained when centers lacking H were similarly treated, it appears that the enlarged band which resulted from tryptic digestion corresponded to both the product of M degradation and the essentially unaltered L protein. One of these constituents seemed to be removed by posterior treatment with 2 μg α-chymotrypsin/ml, because the area of the band was decreased to a value similar to that of the

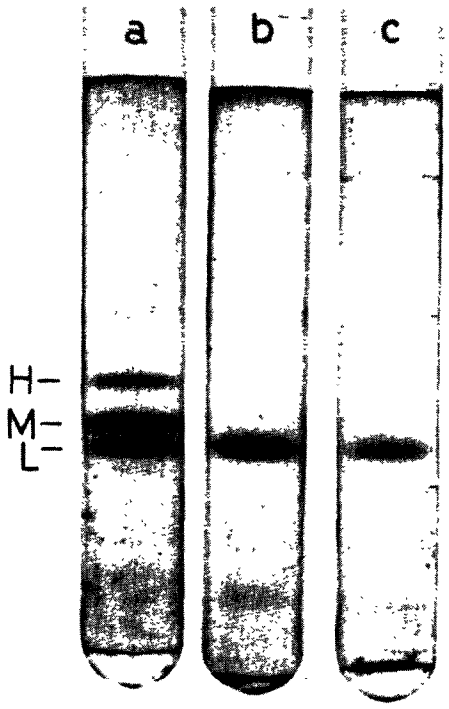

Fig.2. Gel electrophoretograms of photoreaction centers: (a) undigested; (b) after 1 h incubation with 7 μg trypsin/ml; (c) first treated as (b), then 14 μg trypsin inhibitor/ml and 2 μg α-chymotrypsin/ml were added and the incubation was continued for 20 min more. Each gel contained 15 pg equiv. primary donor. Relative areas: (a) 98, 137 and 100; (b) 178; and (c) 104.

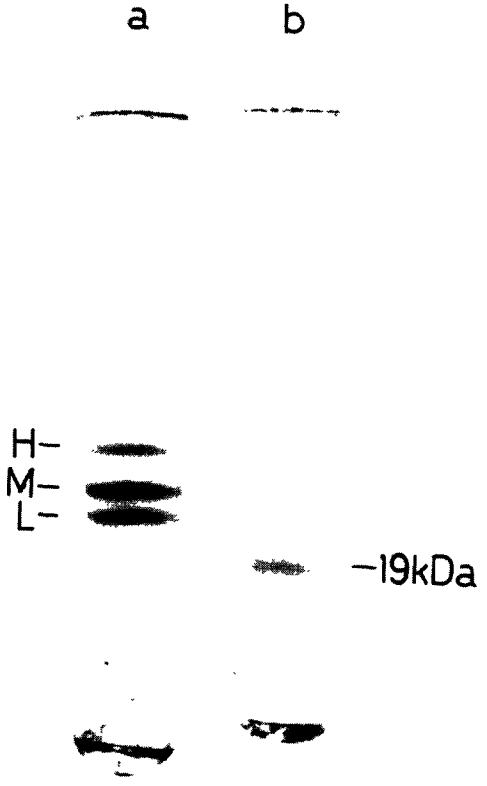

Fig.3. Gel electrophoretograms of photoreaction centers: (a) undigested; (b) after incubation for 24 h with 120 μg trypsin/ml. Each gel contained 15 pg equiv. primary donor. Relative areas: (a) 77, 147 and 100; (b) 70.

native L band (fig.2). Therefore, successive proteolysis by trypsin and α-chymotrypsin yielded photoreaction centers apparently similar to those in [3] by a prolonged dialysis treatment which presumably involved degradation by the endogenous proteases.

Raising trypsin concentration and extending the treatment beyond 1 h caused first a faster migration of the enhanced band and then a reduction of its area. Fig.3 shows that, after 24 h in the presence of 0.12 mg trypsin/ml, the major remaining band was located at the position corresponding to M_r ~19000 and had an area within the value expected for a polypeptide of that size being in a 1 : 1 ratio to the primary electron donor.

Up to here the electrophoresis conditions were chosen to obtain good resolution in the M_r range of the undigested photoreaction center proteins.

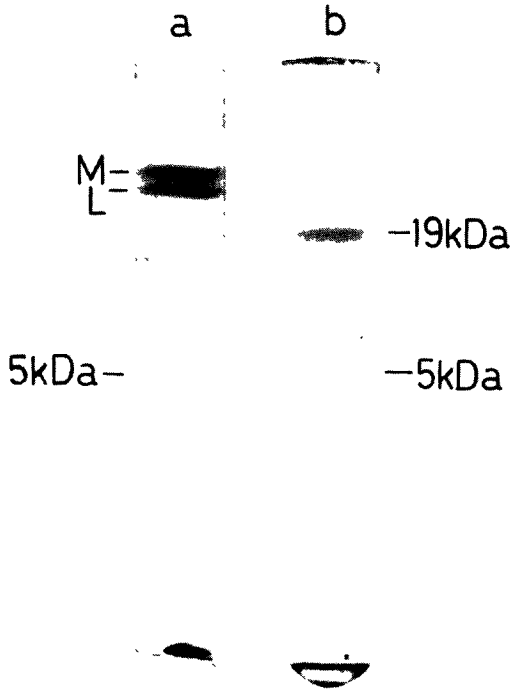

Fig.4. Gel electrophoretograms of photoreaction centers: (a) digested as in fig.1b; (b) fig.3b. Each gel contained 30 pg equiv. primary donor. The concentrations of acrylamide and *N,N'*-methylenebis-(acrylamide) were higher by a factor of 1.6 than those in other experiments. Relative areas: (a) 100 (M + L) and 25; (b) 100 and 60.

Since it seemed possible that low M_r degradation products could also be present, even after DEAE-cellulose chromatography, the digested preparations were re-examined in gels prepared at higher acrylamide concentrations. Fig.4 shows that, in both tryptic and chymotryptic digests, a diffuse band, located at the position of M_r 5000, could be observed. Although the band is barely detectable in the photograph (and in the gels), its area in the electrophoretogram was compatible with the existence of a 5 kDa polypeptide in a 1 : 1 ratio to the other constituents.

4. DISCUSSION

The differential digestion of the photoreaction center proteins by trypsin and α-chymotrypsin may be understood in terms of both enzyme specificity and peptide bond accessibility. The observation that H is digested first by both proteinases suggests that such a constituent protects the lighter polypeptides from proteolysis. A similar situation seems to occur in the intact photosynthetic membrane because, in investigations of proteins exposed at the chromatophore surface, H was observed to be degraded more easily [7,8]. However, digestion of the M and L proteins went along with clear changes in the near infrared absorption spectrum [7].

This work shows that a considerable fraction (65–75%) of the photoreaction center protein can be removed by controlled proteolysis without any apparent modification of the functional prosthetic group of the complex. Although it is not yet known whether both the 5 and the 19 kDa products of digestion are required to maintain native pigment–protein interaction, and the original constituents from which those products derived are not yet identified, these results are a step towards elucidation of the primary protein structure in the active site of the complex.

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