EFFECT OF THE REMOVAL OF THE COOH-TERMINAL REGION OF BACTERIORHODOPSIN ON ITS LIGHT-INDUCED H^+ CHANGES

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ABSTRACT Removal of the COOH-terminal region of bacteriorhodopsin by digestion with trypsin or papain reduces the yield of light-induced H^+ release by 50–70%. The rate of H^+ release is not affected significantly, but the half time of H^+ uptake increases almost twofold. However, there is no effect on the photocycle of bacteriorhodopsin as judged by the yield and decay kinetics of the M412 photointermediate. The H^+:M ratio in enzyme-digested membranes is ~0.4–0.8, whereas untreated membranes have a H^+:M ratio of ~2. Purple membrane sheets stored in distilled water at 4°C for prolonged periods also have a low H^+:M ratio, probably due to protease activity associated with bacterial contamination. Electrophoresis on sodium dodecylsulfate-polyacrylamide gels showed that both the enzyme-treated and the stored purple membrane samples have a higher electrophoretic mobility compared to the fresh preparation. The reduction in molecular weight can be accounted for by the loss of several residues from the COOH-terminal portion of the bacteriorhodopsin. We propose that the COOH-terminal region is partially responsible for the high yield of H^+ release by the purple membrane.

INTRODUCTION

The purple membrane of *Halobacterium halobium* contains a single chromoprotein, bacteriorhodopsin (1), composed of 248 amino acid residues (2). The photocycle of bacteriorhodopsin consists of several spectroscopically distinct intermediates (3). The short wavelength absorbing intermediate M412 is of special interest because its retinal Schiff-base linkage is deprotonated (4, 5), whereas the Schiff base is protonated in the initial state of bacteriorhodopsin. During the photocycle of bacteriorhodopsin, approximately two protons are transported across the purple membrane (6–9). The mechanism by which the protons are pumped is not yet known.

Each bacteriorhodopsin molecule contains seven α-helical segments that span the membrane (10). The carboxyl end of the polypeptide consists of ~20 amino acid residues that do not seem to be part of any helix but are usually represented as projecting like a tail from the surface of the membrane into the interior of the cell (11, 12). Under certain reaction conditions, papain or trypsin can remove several amino acid residues from the COOH-terminal tail (11–13). The removal of part of the tail from bacteriorhodopsin has been reported not to affect the proton pumping activity when the digested membrane was incorporated into phospholipid vesicles (12, 13). However, we report here that in purple membrane sheets the number of protons released per photocycle (measured as the number of H^+ per M412) is reduced by 50–70%, and the rate of H^+ uptake becomes slower after digestion with trypsin or papain. This suggests that the COOH-terminal region of bacteriorhodopsin plays an important role in the release and uptake of at least one of the two protons normally released by light from the membrane.

EXPERIMENTAL PROCEDURE

H. halobium strain S-9 cells were grown for 5–7 d, and the purple membrane was prepared as described previously (14). The purple membrane sheets were incubated at 37°C with one of the following: bovine trypsin (Sigma Chemical Co., St. Louis, MO; No. T-1005) for 4 h, at a bacteriorhodopsin:enzyme ratio of 40; carboxypeptidase A (Sigma Chemical Co.; No. C-9762) for 2 h, at a bacteriorhodopsin: enzyme ratio of 60; or papain (Sigma Chemical Co.; No. P-4762) for 4 h, at a bacteriorhodopsin:enzyme ratio of 200. At the end of the treatment the purple membrane sheets were washed three times in distilled water, and finally suspended in 0.5 M KCl for spectroscopic measurements. Soluble peptide released into the supernatant after trypsin digestion was purified by high voltage paper electrophoresis, eluted, and hydrolyzed. The hydrolysate was analyzed for the amino acids. The number of bacteriorhodopsin molecules cycling, and the number of protons released, were determined as described earlier (8) by measuring the light-induced absorbance change at 400 nm in the absence and the presence of a pH-sensitive dye, *p*-nitrophenol; proton changes were calculated from light-induced absorbance change (ΔA) of the dye alone obtained by subtracting ΔA (with dye) from ΔA (without dye), after calibration with known amounts of HCl. These absorption changes were measured with a laboratory-assembled single-beam kinetic spectrophotometer; actinic

flashes were provided from a Xe-flash lamp (EG and G Electro-optics, 35 Congress Street, Salem, MA). Molecular weights of the digested and the untreated bacteriorhodopsin were determined by electrophoresis on 15% polyacrylamide gels containing 0.1% sodium dodecylsulfate (SDS) as described by Laemmli (15).

RESULTS AND DISCUSSION

Fig. 1 shows the absorbance changes of M412 and those of the dye alone in the purple membrane sheets before and after proteolysis or prolonged storage (purple membrane sheets stored in distilled water for 1 yr at 4°C). The M412 decay is biphasic, and the release and uptake of protons, as measured from the decrease and the increase in the absorption of the dye, are monophasic (8). The yield and the half-decay time of M412 remain unaffected after proteolysis or prolonged storage of the purple membrane samples. In contrast, the amplitude of the dye absorption

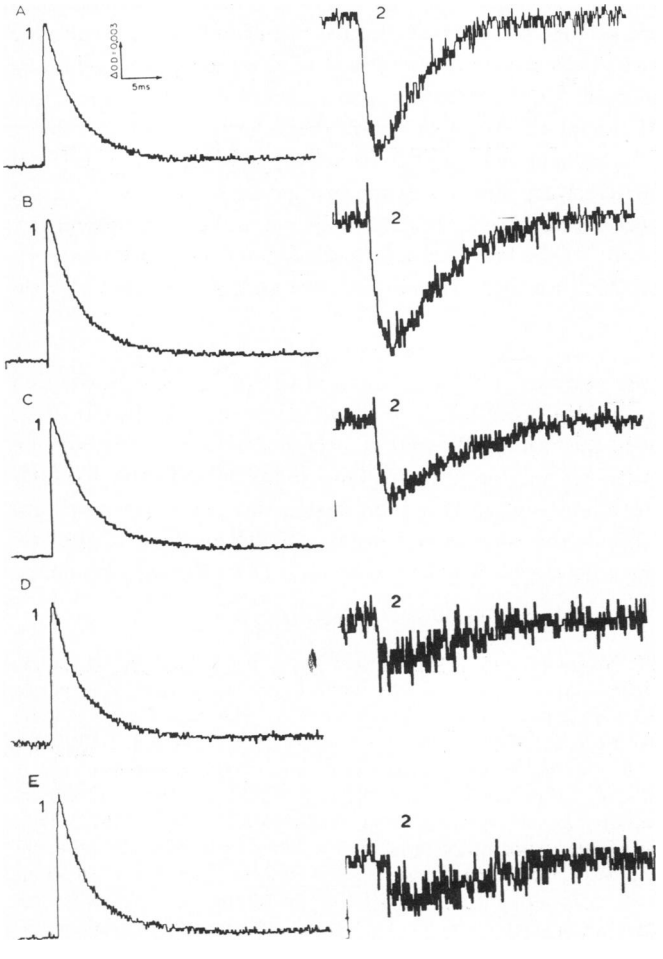

FIGURE 1 (*A–E*) Light-induced absorbance changes at 400 nm in purple membrane sheets suspended in 0.5 M KCl, 50 μM phosphate buffer, pH 6.68 ± 0.05. (*A*) Control purple membrane. (*B*) Carboxypeptidase A-treated purple membrane. (*C*) Trypsin-treated purple membrane. (*D*) Papain-treated purple membrane, and (*E*) 1-yr-old purple membrane kept at 4°C. Trace *1* is change in absorbance (ΔOD) at 400 nm for M412; trace *2* is change is absorbance of *p*-nitrophenol. Each trace is an average of four flashes, ~10 s apart; temperature, ~20°C.

TABLE I
HALF-DECAY TIME OF THE LIGHT-INDUCED ABSORBANCE CHANGES OF THE DYE (*p*-NITROPHENOL) BY PURPLE MEMBRANE AND THE H^+:M RATIO BEFORE AND AFTER PROTEOLYSIS

	$\tau_{1/2}$ decay (H^+ uptake)	H^+/M
Control	4–6	1.7
Trypsin-treated	10–15	0.8
Carboxypeptidase A-treated	4–6	1.7
Papain-treated	10–15	0.5
Old membrane	10–15	0.3

Absorption changes of the dye were measured at 400 nm. Number of H^+ was obtained by calibrating the ΔA dye with known amounts of HCl. Dye used, 38 μM *p*-nitrophenol; approximate concentration of bacteriorhodopsin, 2 μM; pH = 6.68 ± 0.05; temperature, ~20°C.

change is reduced by 50–70%, and the decay of the dye change (reflecting the rate of proton uptake) by the purple membrane is slowed down significantly except in the case of carboxypeptidase A (see Table I). Thus, the number of protons released per photocycle is greatly reduced after proteolysis with trypsin or papain. The result with prolonged storage of the samples was the same as with papain, but carboxypeptidase A treatment did not change the H^+:M412 ratio.

SDS-polyacrylamide gel electrophoresis of the samples is shown in Fig. 2. The papain-treated and the old purple membrane showed single bands and had a higher electrophoretic mobility on the gel compared with the untreated samples. The difference in molecular weight between control and either the papain-treated or the aged sample is ~2,000–3,000. This difference can be accounted for by the loss of ~20–25 amino acid residues. Results of trypsin digestion were somewhat variable. Sometimes there was no measurable difference in electrophoretic mobility between control and trypsin-digested purple membrane; analysis of the soluble peptide released into the supernatant after digestion gave the folowing ratio of amino acids: serine:threonine:aspartic acid:glycine:alanine (1:1:1:1:3). These residues can account for a loss in molecular weight of ~700, which may be too small to cause a detectable change in migration on the polyacrylamide gel. At other times trypsin digestion would cause a decrease in molecular weight of ~1,500. This would imply a loss of almost 15 amino acid residues.

The amino acid sequence of the tail of bacteriorhodopsin from the carboxyl end is ser-thr-ala-ala-ala-gly-asp-gly-ala-ser-pro-glu-pro-ala-glu-ala-glu-gly-phe-ile-ala (2, 11). As carboyxpeptidase has been reported to remove the last four residues (12) and has no effect on the H^+:M412 ratio, these residues are perhaps not involved in H^+ changes. However, removal of seven residues or more from the COOH-terminal region of bacteriorhodopsin by trypsin or papain treatment results in a twofold or greater reduction in the number of protons released per photo-

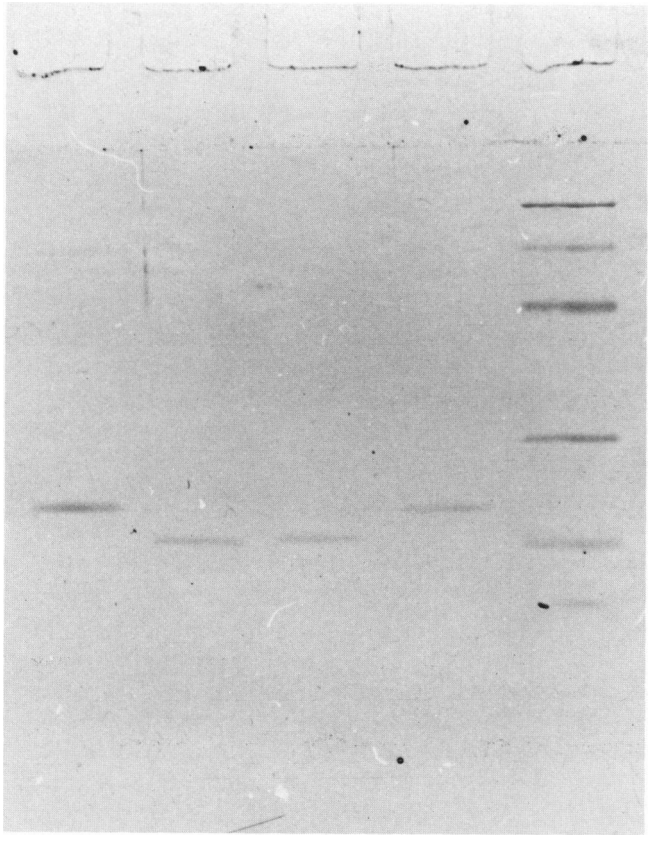

FIGURE 2 SDS/15% polyacrylamide gel electrophoresis of purple membrane before and after digestion with proteolytic enzymes. Samples from left to right are untreated purple membrane, 1-yr-old purple membrane kept at 4°C, papain-treated purple membrane, and trypsin-treated purple membrane. Standards from top to bottom are phosphorylase B (94,000), bovine serum albumin (68,000), ovalbumin (43,000), carbonic anhydrase (30,000), soybean trypsin inhibitor (21,000), and lysozyme (14,300).

cycle. It seems likely that the poor H^+:M412 ratio observed in purple membrane sheets stored under some conditions also results from a loss of the tail of bacteriorhodopsin, due perhaps to proteolysis by microorganisms. On the basis of all the results presented here, we conclude that the COOH-terminal region of bacteriorhodopsin plays an important role in light-driven proton release by the purple membrane.

We thank Dr. T. O. Baldwin for advice and assistance in the amino acid analysis and Burr Nelson for excellent technical assistance.
This work was supported by National Science Foundation grant PCM 79-11772.

Received for publication 17 September 1981 and in revised form 10 October 1981.

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