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Proteolysis and Flash Photolysis of Bacteriorhodopsin in Purple Membrane Fragments

K. Rosenheck, M. Brith-Lindner, P. Lindner, A. Zakaria, and S. R. Caplan

Department of Membrane Research, The Weizmann Institute of Science, Rehovot, Israel

Abstract. Pronase treatment of aqueous suspensions of purple membrane fragments from *H. halobium* leads to the cleavage of bacteriorhodopsin. The protein fragments remaining in the membrane after treatment with relatively small concentrations of enzyme (2% w/w) in normal daylight range in molecular weight from 20,000–21,000 daltons, indicating that cleavage occurs mainly near the extremities of the protein chain. At higher enzyme concentrations the relative amounts of protein fragments having smaller molecular weight increase. Generally, the relative loss of retinal chromophore is larger than that of protein and thus the retinal binding site seems to be located near one of the chain ends that is cleaved off by enzyme.

Irradiation with white light during the time of proteolysis (at both low and high enzyme concentrations) results in extensive cleavage, so that under certain conditions no high molecular weight components can be detected in SDS-polyacrylamide gels. It, therefore, appears that parts of the bacteriorhodopsin chain become more exposed to enzyme digestion when the purple membrane is illuminated.

Enzyme treated aqueous purple membrane fragment suspensions still show photocycle activity. The main consequence of proteolysis is a pronounced appearance of biphasicity in the decay of M_{412} and the regeneration of bR_{570}. Simultaneously the yield of O_{660} is reduced. As with untreated purple membrane, the correlation between the rates of decay of M_{412} and regeneration of bR_{570} is greatest when the yield of O_{660} is lowest.

Key words: *Halobacterium halobium* – Pronase – Gel electrophoresis – Photocycle.

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Introduction

Bacteriorhodopsin, the protein of the purple membrane (PM) consists of a single polypeptide chain having a molecular weight of ~25,000 daltons (Oesterhelt and Stoeckenius, 1971; Bridgen and Walker, 1976). Electron diffraction studies have shown that ~75% of the amino acid residues in the chain are accounted for by seven helical segments of about equal length, running across the entire width of the membrane, with probably, little exposure to the outside (Henderson and Unwin, 1975). The remainder of the residues may be thought to form non-helical links between these helical segments, however, the degrees of exposure of these links, as well as of the N-terminal and COOH-terminal ends of the chain, are unknown. The protonated Schiff base linkage of retinal to the protein has been found to be situated within a hydrophilic sequence of seven amino acid residues, that can be split off the PM by digestion with thermolysin (Bridgen and Walker, 1976). The central residue of this sequence is proline, an imino acid that cannot be fitted into an α-helical structure. Furthermore, the sequence Asp-Pro is frequently found to be located at β-turns (Venkatachalam, 1968; Lewis et al., 1971; Chou and Fasman, 1974) in the secondary structure of proteins. This fact, and the hydrophilic character of the retinal containing sequence, suggest that the chromophore containing site may be situated at one of the non-helical links at which the protein chain turns back on itself[1]. Whether this particular link is intra- or extramembranous, and whether it is centrally or peripherally situated in the amino acid sequence, is of relevance to the still unclarified mechanism by which light energy absorbed by the chromophore is utilized to transfer protons across the PM (Oesterhelt and Stoeckenius, 1973). Furthermore, in the same context, it is of interest to determine whether the degree of exposure of the pigment-containing site, as well as of other parts of the protein chain, would be influenced by irradiation, such as that used to activate the proton pump. Limited proteolysis studies of PM under "dark' and "light' conditions were expected to give some information on these questions. Such studies form a part of the object of the present work, in which the molecular weight distributions of the fragments obtained after pronase treatment under various conditions are reported, and the degrees of proteolysis and bleaching of the chromophore compared.

Another purpose of this study was to enquire whether structural integrity of PM was required for the functioning of the photocycle. As is well-known, light absorbed by bacteriorhodopsin induces a reaction cycle that can be described as consisting of two main parts. In the first part a proton is lost from the Schiff base linkage between the chromophore and the protein (Lewis et al., 1974) and a relatively stable intermediate (M_{412}) is formed. The half-life of this intermediate is several ms at room temperature. This first part of the photocycle is a sequence of very fast reactions. Several short-life intermediates have been identified (Kaufman et al., 1976; Lozier et al., 1975; Dencher and Wilms, 1975; Chukung et al., 1975). In the second part of the cycle a proton is taken up and bR_{570} is regenerated. The mechanism of the second part of the cycle, the regeneration process, is less well understood. One

[1] As regards the possibility that the site is located at one of the chain ends, a recent study (Gerber et al., 1977) has given the sequence for the COOH-terminal. This sequence is different from that containing the retinal moiety (Bridgen and Walker, 1976)

intermediate in this part of the cycle, first observed by modulation excitation spectroscopy (Slifkin and Caplan, 1975), is the O_{660} transient, however, its role and chronological relationship in the regeneration process are still disputed (Lozier et al., 1975; Sherman et al., 1976). Data on the decay of M_{412} and O_{660}, and the regeneration of bR_{570}, as studied by flash photolysis of PM suspensions that had been subjected to varying degrees of proteolysis, are reported in the present work. The data are compared to the results of Sherman et al. (1976a, b), obtained by flash photolysis of untreated PM. A study of reconstituted PM vesicles subjected to selective proteolysis was recently carried out by Gerber et al. (1977). These authors reached the conclusion that the proteolytic treatment did not interfere with the activity of the proton pump.

Materials and Methods

H. halobium strain M_1 was grown as described by Danon and Stoeckenius (1974). The purple membrane fragments were isolated according to a procedure described in earlier publications (Brith-Lindner and Rosenheck, 1977; Sherman et al., 1976a). The purified membranes were resuspended in distilled water and stored at 4° C. All studies were carried out with light-adapted membrane.

Proteolysis: PM suspensions were incubated with pronase (Calbiochem – B grade) for 18 h at 37° C. Typically, the mixture contained PM (1 mg/ml bacteriorhodopsin), pronase (in amounts varying from 2–16% w/w with respect to bacteriorhodopsin) and 20 mM phosphate or tris-buffer (pH 7.4). For some samples, irradiation during the incubation was carried out with a slide projector having an iodine quartz lamp (24 V, 150 W). The unfiltered, total light output was used at a distance of about 10 cm from the sample. Light intensity, as measured by a Y. S. I. Kettering radiometer (model 65 A) was 700 W/m^2. After 18 h the incubation was stopped by cooling to 4° C, the PM were spun down at 30,000 rpm for 30 min, washed twice with distilled water and finally taken up in 3 ml distilled water. The UV and visible absorption spectra were measured on a Cary 15 recording spectrophotometer and the protein content was assayed by the method of Lowry et al. (1951), using bovine serum albumin as standard.

Gel Electrophoresis: Sodium dodecyl sulfate (SDS) – polyacrylamide gel electrophoresis was carried out in slabs using Tris-glycine buffer (pH 8.5). The acrylamide concentration was 15% and that of bis-acrylamide 0.4%. The samples were boiled with SDS for 5–7 min before application to the gel. Elctrophoresis was done at 30 mA for about 3.5 h. Staining was performed with 0.2% Coomassie brilliant blue in 50% methanol – 7% acetic acid and destaining with 20% methanol – 7% acetic acid.

Sucrose Gradient Centrifugation: Untreated and pronase treated PM were placed on a linear sucrose gradient (25–50% sucrose with a 60% sucrose solution at the bottom of the tube) and run at 25,000 rpm for 20 h in a Beckman SW 27 rotor.

Flash Photolysis: Aqueous suspensions of light-adapted PM were studied using the conventional flash photolysis system described by Sherman et al. (1976a). Corning 3486 cut-off filters (0% T below 510 nm, 50% T at 530 nm) were always interposed between the flash tubes and the sample. To minimize photomultiplier fatigue and UV sample bleaching Schott GG4 (400 nm cut-off) filters were placed between cell and monochromator, as well as between cell and monitoring lamp. In addition Pyrex windows were positioned on both sides of the cell. The cell was 2 cm long and was placed in a Pyrex Dewar, the temperature of which could be controlled, above and below room temperature, with air passed through a hot tube and liquid nitrogen, respectively (Fisher, 1970).

Kinetic analyses were carried out by plotting logarithmically against time, the changes in absorbance at the wave-length maxima of bR_{570}, M_{410} and O_{660}. The biphasicity of the plots was analyzed by resolving into two first-order exponentials graphically, by the peel-off method.

Results

Protein digestion and chromophore bleaching: Although there was some variation in the extent of proteolysis from repeat experiments under identical conditions, the emerging picture was clear-cut. Pronase treatment results in loss of both chromophore and protein, the former effect being the more pronounced (Table 1). The magnitude of these losses depends on the concentration of pronase in the reaction mixture and on whether or not the sample was irradiated with white light during the incubation. Typically, a loss of ~10% in both protein and chromophore occurs after

Table 1. Normalized chromophore (OD_{570}) and protein (Lowry) concentrations, and apparent molecular weight of main bands on SDS-polyacrylamide gels, for pronase-treated "dark" and "light" PM. Conditions as quoted in the text

Sample	Pronase to protein ratio	Chromophore		Protein		Mol. weight (kdaltons)	
		dark	light	dark	light	dark	light
PM_0	0.00	1	1	1	1	23	23
PM_1	0.02	0.91	0.63	0.89	0.80	23 (wk)[a] 21 (str)[a] 20 (wk)	23 (wk) 21 (wk) 20 (str) 18, 17
PM_2	0.07	0.77	0.29	0.90	0.66	23 (wk) 21 20 (str) 18, 17 (wk)	18, 17 (wk)
PM_3	0.16	0.52	0.0	0.67	0.11	21, 20, 18 (all wk)	no bands

[a] The intensity of staining is classified as weak (wk) or strong (str)

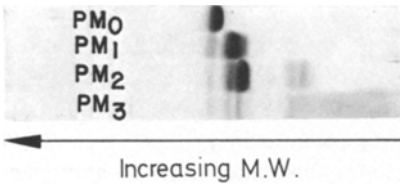

Fig. 1. SDS-polyacrylamide gel electrophoresis of pronase treated PM. Incubation time: 18 h, no irradiation, 37° C. For definition of PM_0, PM_1, PM_2 and PM_3, see Table 1

18 h incubation with 2% w/w pronase (without irradiation), while 30% protein and 50% chromophore are lost after incubation under similar conditions with 16% w/w pronase.

Irradiation during incubation, as described under Methods, results in more efficient digestion of protein and higher degree of chromophore bleaching. For example, in an irradiated sample containing 7% w/w pronase, 35% of the protein was digested and the degree of bleaching amounted to 70%.

Gel Electrophoresis: SDS-polyacrylamide gel electrophoresis patterns of bacteriorhodopsin before and after pronase treatment without irradiation, are shown in Figure 1. After digestion, the characteristic single band of the bacteriorhodopsin found in this study at 23,000 daltons is replaced by several discrete bands of 17,000–23,000 daltons. Treatment with low concentration pronase (2% w/w) results in high molecular weight bands (20,000–23,000 daltons). The relative amounts (as judged from intensity of staining) of the low molecular weight bands increase as the concentration of pronase in the reaction mixture increases. From samples irradiated during the incubation with pronase much weaker bands are generally obtained. In these samples, even at low concentrations of pronase (2% w/w) the most intense bands are in the 17,000–20,000 dalton range and only weak bands of higher molecular weight are observed. After treatment with 7% w/w pronase only very weak bands are obtained and no band pattern at all is observed after treatment with 16% w/w pronase (Table 1).

Sucrose Gradient: On a sucrose gradient pronase treated membranes yielded only a single purple band. Compared to the band from untreated membranes, this band was somewhat more diffuse and of slightly lower density. The density tended to decrease as more protein was digested. Enzymatic cleavage thus seems to occur fairly uniformly and to leave a homogeneous membrane population.

Flash Photolysis: In both untreated and enzyme-treated samples, the flash produces a temporary bleaching of the bacteriorhodopsin chromophore, the absorption band of which is centered at 570 nm, while transients with absorption maxima at 412 and 660 nm are formed. Photo-transient spectra of a sample of PM treated with 7% w/w pronase were drawn at 0.5 and 10 ms after the flash (not shown). This was done by plotting the difference in absorbance point by point, for various wavelengths, before and after the flash. Maximum development of the 570 and 412 nm signals is obtained after 0.5 ms while the 660 nm transient is at maximum after 10 ms. The spectrum at 0.5 ms consists mainly of a very strong negative depletion band

at ~570 nm and a positive, relatively strong band at ~412 nm. In the spectrum corresponding to 10 ms the heights of the 570 and 412 nm bands are reduced and a weak positive band, centered at 660 nm, is obtained. In both spectra a rather pronounced negative shoulder is obtained at 490 nm. These results are comparable to those obtained by Sherman et al. (1976a) with untreated purple membrane. *Thus, it can be concluded that the same transients, with unshifted absorption maxima, are operative in the photocycle of the partially digested membrane.* In view of this we shall refer to the transients as M_{412} and O_{660} and to the ground state of bacteriorhodopsin as bR_{570}.

The flash-induced changes in absorbance, relative to the initial optical density at 570 nm, $\Delta A_\lambda/OD_{570}$, at times of maximum development of the transient signals, were measured as a function of the amount of pronase used. After digestion with 16% w/w pronase the depletion of bR_{570} and grow-in of M_{412} are slightly decreased, being reduced to 80 and 85% of their respective values in the untreated sample. On the other hand, the O_{660} absorption becomes almost undetectable. The results of a

Table 2. Depletion of bR_{570} and growth of M_{412} and O_{660} for PM treated with pronase in varying concentrations at 23° C. For definitions of PM_0, PM_1, PM_2 and PM_3, see Table 1

bR_{570} Depletion				
Sample	OD_{570}[a]	ΔA_{570}[b]	$\Delta A_{570}/OD_{570}$	Normalized $\Delta A_{570}/OD_{570}$[c]
PM_0	0.26	0.230	0.88	1
PM_1	0.20	0.182	0.90	1
PM_2	0.16	0.114	0.71	0.80
PM_3[e]	0.24	0.160	0.67	0.76
M_{412} Growth				
Sample	OD_{570}	ΔA_{412}	$\Delta A_{412}/\Delta A_{570}$	Normalized $\Delta A_{412}/\Delta A_{570}$[d]
PM_0	0.26	0.118	0.511	1
PM_1	0.20	0.092	0.507	0.99
PM_2	0.16	0.052	0.454	0.89
PM_3[e]	0.24	0.070	0.437	0.85
O_{660} Growth				
Sample	OD_{570}	ΔA_{660}	$\Delta A_{660}/\Delta A_{570}$	Normalized $\Delta A_{660}/\Delta A_{570}$[d]
PM_0	0.26	0.022	0.095	1
PM_1	0.20	0.015	0.082	0.86
PM_2	0.16	0.004	0.035	0.36
PM_3[e]	0.24	0.003	0.018	0.19

[a] Initial optical density at 570 nm
[b] Change in the absorption at the wavelength indicated
[c] Normalized to PM_0: $(\Delta A_\lambda/OD_{570})$ enzyme treated/$(\Delta A_\lambda/OD_{570})$ untreated
[d] Normalized to PM_0: $(\Delta A_\lambda/\Delta A_{570})$ enzyme treated/$(\Delta A_\lambda/\Delta A_{570})$ untreated
[e] The values for PM_3 are derived from an experiment separate from that of PM_0, PM_1 and PM_2

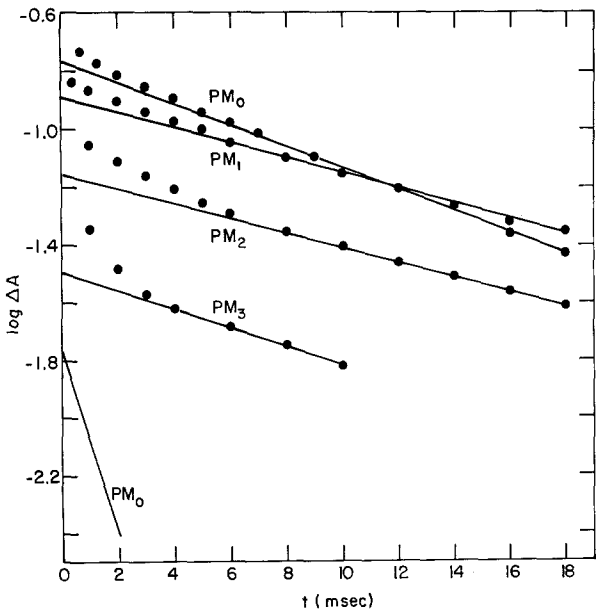

Fig. 2. First-order plots of the decay of M_{412} of pronase treated PM. For definition of PM_0, PM_1, PM_2 and PM_3, see Table 1. The half-lives (at 18° C) for the slower decay process are: $PM_0 = 8.1$ ms; $PM_1 = 10.6$ ms; $PM_2 = 12.2$ ms; $PM_3 = 9$ ms. The half-life of the rapid process (plot shown only for PM_0) is ~1 ms. Extent of fast process: $PM_0 = 8\%$; $PM_1 = 12\%$; $PM_2 = 25\%$; $PM_3 = 35\%$

representative set of samples are given in Table 2. No permanent bleaching of bR_{570}, as a consequence of the flash, was observed. The 570 nm absorption recovered to its initial value after each flash.

For untreated purple membranes flashed at room temperature (~20° C) the grow-in times of the bR_{570} depletion and M_{412} accumulation are about 0.5 ms, while the grow-in of the O_{660} transient is several ms. For pronase-treated purple membrane the grow-in time of the O_{660} signal increases with pronase concentration. Typically one finds 6, 8, and 10 ms grow-in times for 0, 2, and 7% w/w, respectively. No gross changes in the grow-in time of the bR_{570} depletion and M_{412} accumulation could be discerned (the limit of time resolution was ~0.1 ms).

The kinetics of the regeneration of the bR_{570} and the decay of M_{412} were found to be biphasic. Both could be resolved into two first-order processes having half times of about 1 ms and several ms for the first and second processes, respectively, at room temperature. The amplitude of the first process increases with the concentration of pronase. Thus, the amplitude of the first process (for both the bR_{570} regeneration and the M_{412} decay) amounts to 10, 20, and 30% of the total signal, for 2, 7, and 16% w/w pronase, respectively. The amplitude of the first process in untreated membranes is a few percent. A slow-down of the second process occurs, both for the M_{412} decay and the bR_{570} regeneration for samples treated with 2 and 7% w/w pronase. This tendency seems to be reversed in samples where the extent of digestion of the protein was relatively high. A representative set of results is shown in Figure

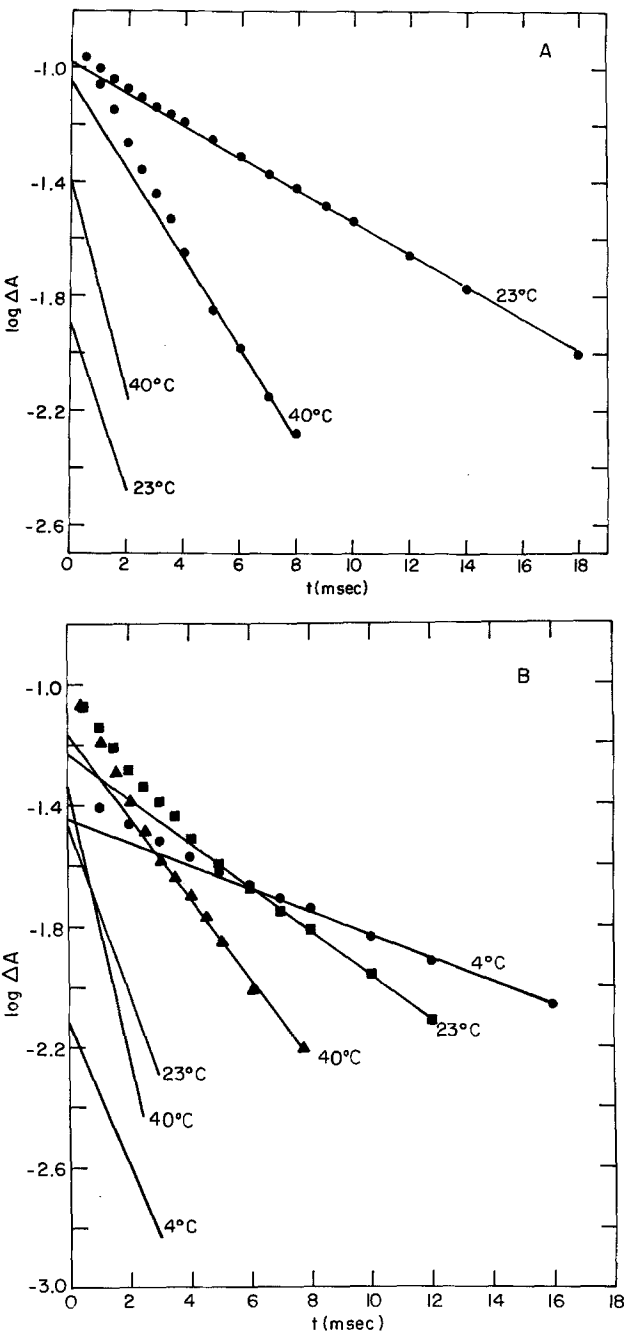

Fig. 3. First-order plots of the decay of M_{412} at various temperatures. (A) Untreated PM; half-life for *slow process:* 5.3 ms at 23° C, 2.0 ms at 40° C; for *fast process:* 1.0 ms at 23° C, 0.8 ms at 40° C. Extent of fast process: 8% at 23° C and 25% at 40° C. (B) PM treated with 13% w/w pronase; half-life for *slow process:* 7.4 ms at 4° C, 3.9 ms at 23° C, 2.2 ms at 40° C; for *fast process:* 1.4 ms at 4° C, 1.1 ms at 23° C, 0.67 ms at 40° C. Extent of fast process: 15% at 4° C, 30% at 23° C, 40% at 40° C

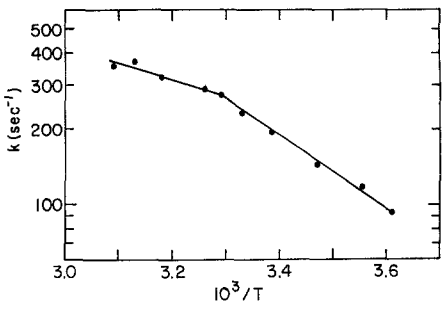

Fig. 4. Arrhenius plot of the slow decay process of M_{412} for PM treated with 13% w/w pronase

2, in which the logarithm of ΔA is plotted against time for samples treated with various concentrations of pronase.

The decay of M_{412} was analyzed over the temperature range 0–60° C. The amplitude of the first decay process is found to increase with temperature, rising from 15% of the total decay at 4° C to 40% of the total decay at 40° C (for 13% w/w pronase). The same trend is observed in undigested PM. In Figure 3, first order plots of the M_{412} decay at different temperatures are given, both before and after treatment with pronase. The rate constants of both decay processes increase with increasing temperature. Arrhenius plots of the rate constants of the two decay processes reveal breaks at ~ 30° C. This result is similar to that obtained for untreated PM (Sherman et al., 1976b)[2]. The energies of activation of the second process (see Fig. 4) are 6.7 and 3.2 kcal/mol in the low and high temperature ranges, respectively. These are smaller by a factor of three than those obtained for the untreated membrane. The energies of activation for the first decay process could not be determined because of the scatter in the data.

Discussion

The data appearing in Table 1 show an enzyme concentration- and irradiation-dependent loss of chromophore and fragmentation of the bR chain. The fact that under mild conditions discrete bands are obtained in SDS-polyacrylamide gels indicates that the enzymatic cleavage occurs at a limited number of specific sites. Since no fragments smaller than 17,000 daltons were observed, it is likely that the protein is predominantly attacked at the chain ends and/or links between peripheral helical segments which therefore appear to be more exposed. Recent experiments, using in situ radioiodine-labelled bR (Tsuji and Rosenheck, in preparation), show that pronase treatment produces two fragments with molecular weights of ~17,000 and ~8,000 daltons, respectively, which are both located in the membrane, while the cleavage products released into the supernatant comprise no more than ~10% of the

[2] Note that the rate constants quoted by Sherman et al. (1976b) correspond to our second process

original protein chain. The sucrose gradient experiment described above (see Results) also shows that most of the bR chain remains in the membrane after proteolysis. The limited amount of proteolysis in the dark thus suggests that most of the protein is buried in the membrane, a conclusion similar to that reached in a preliminary note by Gerber et al. (1977).

When PM is subjected to irradiation with white light, the proteolytic action is much more effective, resulting in extensive digestion of the protein and drastic bleaching of the chromophore. It must be concluded that the protein or parts of it become more exposed to the outside under the action of light, so that it becomes more susceptible to the attack of the enzyme. Experiments showing an increased susceptibility of the chromophore to attack by small molecular weight reagents, when PM is irradiated, have been described by Oesterhelt et al. (1974) and by Peters et al. (1976). These experiments can be interpreted in two ways, indicating either increased exposure of the retinal site or facilitation of reagent migration through the membrane, while ours, using a macromolecular reagent – the enzyme, can be interpreted only in terms of an increased exposure of the protein chain.

The loss of chromophore outweighs that of protein (see Table 1) even at limited extent of cleavage, indicating that the retinal is located at, or near, one of the more susceptible sites to enzymatic cleavage. This suggests that one of the extreme terminal helix and/or turn segments is the region where retinal is bound. However, there remains a possibility that the chromophore site is situated in one of the middle sections of the protein. If so, the results of the present study would imply that the extremities of the protein chain are in interaction with the chromophore site. No new absorption bands, arising from retinal in either free or bound form, could be detected in spectra of pronase-treated and washed PM suspensions, in which the loss of the 570 nm chromophore amounted to as much as 50%. Similarly, the spectra of the original supernatants did not give any indication that retinal was present, neither free nor bound to a small molecular weight fragment of the polymer chain. Therefore, it can be assumed that retinal was split off the membrane, although its exact fate is not clear.

Flash photometric measurements of aqueous suspensions of PM were previously carried out by Sherman et al. (1976a, b), who found the half life of the decay of the M_{412} and O_{660} transients and the regeneration half life of bR_{570} to be in the range of several ms. Our more detailed study reveals that the half life of the bR_{570} regeneration at room temperature is at least 1 ms longer than that of the decay of M_{412}, and the decay half life of O_{660} is appreciably longer than that of M_{412}. Furthermore, the kinetics of the decay of M_{412} is found to be biphasic, presumably a sum of two first order processes. The first process has a half life of less than 1 ms. Its amplitude is very small at room temperature, consequently it was overlooked in the previous studies. It should be noted that biphasic behaviour was observed for the decay of M_{412} in aqueous PM suspensions containing NaCl (Eisenbach et al., 1976). Biphasic behaviour was also observed for the decay kinetics of PM suspensions containing high concentrations of valinomycin (Sherman et al., 1976a).

The decay of M_{412} and the recovery of bR_{570} can each be time resolved into two first order processes, according to:

$$Y = A_1 e^{-k_1 t} + A_2 e^{-k_2 t}, \tag{1}$$

where Y is the concentration of absorbing species, A_1 and A_2 are the extents of the two processes and k_1 and k_2 are the respective rate constants. The ratio of the two rate constants is at most 10, and we consider these two processes as being parallel. The extents of the two depend upon the temperature and degree of proteolysis (Figs. 2 and 3). The fact that the kinetics of the decay of M_{412} are similar to those of the regeneration of bR_{570} (apart from a temperature dependent deviation in half life of the second process which will be discussed below) implies that the two processes lead to a common product, i.e. bR_{570}. This biphasic kinetics can be attributed to the existence of two forms of M_{412} decaying to bR_{570} with different rate constants. Two different species of M_{412}, with absorption maxima at 405 and 435 nm were already claimed by Hess and Kuschmitz (1977) in order to explain the light-activated regeneration of bR_{570} from its fully bleached state in the temperature range of $-10°$ to $-40°$ C.

Experiments performed with untreated PM have shown that the appearance of O_{660} is temperature sensitive, becoming undetectable at 0° C, and can be completely inhibited by 10 μM valinomycin, in contrast to that of M_{412} which is only slightly sensitive to these agents (Sherman et al., 1976a, b). This has led to the supposition that O_{660} either lies on a route separate from that leading from M_{412} to bR_{570} (Sherman et al., 1976a) or on a competing minor pathway from M_{412} to bR_{570} (Sherman et al., 1976b). Our finding that partial proteolysis reduces the yield of O_{660} strongly, while influencing the bR_{570} depletion and M_{412} yield only slightly, is in accord with this assumption. Furthermore, if O_{660} and M_{412} decay in parallel to the ground state of bacteriorhodopsin this should be reflected in the regeneration kinetics of bR_{570}. Indeed an inverse correlation can be found between the *ratio* of the recovery rate of bR_{570} to the decay rate of the M_{412} and the *yield* of O_{660}. At low temperature where the yield of O_{660} is low (Sherman et al., 1976b) this ratio is equal to one and its value decreases at higher temperatures, as the yield of O_{660} increases. If, on the other hand, one considers the possibility that O_{660} is on the main route leading from M_{412} to bR_{570}, the above data can be explained only if the rate constants describing its appearance and decay would increase very much with decreasing temperature. From our preliminary findings, this does not seem to be the case, since even at temperatures at which the yields of O_{660} are extremely low the rate constants remain of the same order. Therefore, the latter possibility seems to us less probable, at present, than the former one.

One further feature of the photocycle that undergoes drastic changes due to the proteolytic cleavage of the bacteriorhodopsin molecule is the energies of activation in the decay of M_{412}, which are smaller by a factor of three than those of untreated PM. One way in which this effect, as well as the changes in rate constants and yields of some of the photochemical intermediates mentioned earlier, may be related to the state of the protein is via a conformational transition of the latter, that is normally coupled to the photocycle. Partial cleavage of the protein chain may be expected to influence both the kinetics and the energetics of such a transition, and is reflected also in the photochemical transformation, that triggers the proton pump mechanism. In fact, a conformational transition has been postulated to occur (Caplan et al., 1977) during the rapid phase in the light-induced pH changes observed with subbacterial particles and reconstituted proteoliposomes (Eisenbach et al., 1977), as well as with the overall light-induced pH changes which occur in purple membrane

fragment suspensions (Garty et al., 1977). Recently, it has also been shown that the amplitude of the slower phase of the light-induced pH changes decreases by ~80% in reconstituted proteoliposomes incorporating enzymatically degraded bacteriorhodopsin (Tsuji and Rosenheck, in preparation). It is our aim to probe in future studies into the mode in which conformational transitions of the protein are related to the coupling between the photochemical cycle of the retinal chromophore and the proton pump.

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