EFFECTS OF BLEACHING AND REGENERATION ON THE PURPLE MEMBRANE STRUCTURE OF *HALOBACTERIUM HALOBIUM*

B. BECHER AND J. Y. CASSIM, *Department of Biophysics, The Ohio State University, Columbus, Ohio 43210 U.S.A.*

ABSTRACT Sequential bleaching in the presence of hydroxylamine and subsequent regeneration of the purple membrane of *Halobacterium halobium* was studied by concomitant monitoring of its absorption and circular dichroic spectra in order to ascertain its effects on protein interaction(s) (which may result in possible excitonic interaction between the retinal chromophores), chromophore-apoprotein interaction(s), and protein conformational stability in the membrane. It was concluded that (*a*) although experimental results are consistent with an exciton mechanism for the interaction between retinal $\pi - \pi^*$ (NV_1) transition movements in the purple membrane, no evidence for such a mechanism for interaction between retinaloxime transition moments is apparent in the case of the bleached membrane; (*b*) the bacteriorhodopsin molecules organized in clusters of three in the membrane appear to bleach simultaneously; (*c*) the retinaloxime produced on bleaching the purple membrane in the presence of hydroxylamine is strongly optically active, because of dissymmetry-inducing and/or -selecting constraints on the chromophore by a component of the membrane (most likely the apoprotein), and when the membrane is regenerated by the addition of retinal, these constraints are lost; and (*d*) evidence from ultraviolet absorption and circular dichroic spectra suggests that the membrane apoprotein undergoes appreciable conformational changes involving tertiary structure on bleaching with no significant secondary structure involvement. These results are compared with recently reported results from this laboratory on the effects of bleaching on the bovine rod outer segment disk membrane structure.

INTRODUCTION

The purple membrane of *Halobacterium halobium* consists of a single protein species to which retinal is bound by a protonated Schiff base linkage (1, 2). This chromoprotein has been termed bacteriorhodopsin by analogy to the chromoprotein rhodopsin found in the visual photoreceptors of vertebrates and invertebrates. However, the purple membrane has fundamental differences from these photoreceptor membranes both in function and structure. The chromophore of the purple membrane undergoes stereoisomerization from the 13-*cis* to the all-*trans* configuration without loss of the Schiff

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Dr. Becher's present address is: Department of Physiology and Biophysics, University of Illinois, Urbana, Ill. 61801. Reprint requests should be addressed to Dr. Cassim.

base bond between the chromophore and the apoprotein with light adaptation (3, 4). No physiological function has been attributed to this stereoisomerization and no significant alteration of the molecular organization and the protein structure of this membrane is indicated by circular dichroic (CD) and absorption studies (5). The light-adapted form of the membrane undergoes a photochemical reaction cycle, resulting in the transduction of hydrogen ions across the cell membrane and ATP synthesis (6, 7).

A structural model for this membrane has recently evolved from X-ray diffraction, electron microscopy, and electron spin resonance studies (8–11). Unique features of this model are the rigidity of the molecular organization and the equivalence of the local environments of the bacteriorhodopsins due to their threefold axis of symmetry. The membranes are organized with hexagonal symmetry with the chromoproteins, arranged in clusters of three, spanning the entire thickness of the membrane. Additional evidence in accord with this model has been obtained from absorption and CD studies, with the results consistent with an exciton mechanism for the interaction of the protein chromophores within the clusters (5, 12–14).

The bacteriorhodopsin molecule consists of a single polypeptide chain with approximately 12% aromatic amino acid residues and no cysteine (1, 15). The most recently reported molecular weight is 25,000 daltons (15). Recent CD studies indicate a secondary structure consisting of approximately 45% α-helical conformation (5). According to current X-ray diffraction analysis and electron microscopy, the tertiary structure of the apoprotein can be approximated by a polypeptide chain folded back and forth on itself, forming seven rodlike structural elements approximately perpendicular to the plane of the membrane (8–10).

In contrast to the purple membrane, the structural organization of the photoreceptor membranes of higher organisms, such as bovine rod outer segment membrane, allows considerable mobility of the rhodopsin molecules within the membrane (16). Conformational changes in the apoprotein, opsin, of the rhodopsin in bovine rod outer segment membrane resulting from the loss of the Schiff base bond between retinal and opsin during the bleaching process can be limited to very localized ones involving a few amino acid residues (17).

In this communication we address ourselves to the problem of structural stability of purple membrane during the bleaching process, which results in the loss of the Schiff base bond between the retinal and the apoprotein. We report the sequential bleaching in the presence of hydroxylamine and subsequent regeneration of the purple membrane with concomitant monitoring of its CD and absorption spectra. These spectral studies indicate that the loss of the Schiff base bond results in a significant change in the retinal-apoprotein interaction(s) and relatively more drastic conformational changes in the apoprotein than the one reported for bovine rhodopsin. Results accord with tertiary structure involvement only and not secondary structure.

METHODS

Cultures of *Halobacterium halobium* R_1 were grown and purple membrane was isolated according to the procedures of Becher and Cassim (18).

The procedure utilized for bleaching the purple membrane was as follows: The membrane was suspended in a 0.3 M hydroxylamine solution and titrated to pH 7.0 with 1 N NaOH. The final concentration of the bacteriorhodopsin in the membrane suspension was such that the light-adapted form had an absorbance of 1.0 at 568 nm. 3 ml of this suspension were bleached at once in a cylindrical optical cell with a 1-cm path length with constant stirring. A constant temperature of 20°C was maintained by circulating water through a channeled aluminum block serving as a cell holder from a constant temperature circulator. Bleaching was achieved by exposing the sample to light focused from a 500-W DAK projector lamp (O. A. Kadan Co., Inc., Mount Vernon, N.Y.) and filtered through 3.2 cm of $CuSO_4$ solution and a number 12 (yellow) Wratten filter. The filter combination passed light from 500 to 700 nm, the wavelength region of maximum absorption of bacteriorhodopsin (λ_{max} = 568 nm). The samples were illuminated for periods of 90, 210, and 300 min, washed free of hydroxylamine by repeated pelleting and suspension in water, and finally suspended in 0.02 M potassium phosphate buffer (pH 7.0). Samples were considered to be completely bleached after 300 min of illumination when the induced optical activity of the retinal was completely eliminated.

Regeneration of bleached samples were performed by 1-μl additions of 3 mM all-*trans* or 13-*cis* retinal in ethanol to 3-ml aliquots of membrane suspensions bleached according to the procedure described above. The retinal solution was added to the bleached membrane suspension in the dark with constant stirring and incubated for 24 h at 10°C. Approximately 17 μl of the retinal solution were required to completely regenerate 3 ml of 17 μM bleached bacteriorhodopsin to an approximate absorbance of 1.0 at 568 nm.

0.03 M retinaloxime was prepared by combining equal volumes of 0.06 M hydroxylamine in water and 0.06 M all-*trans* retinal in ethanol. Complete conversion of retinal to retinaloxime occurred within several minutes at room temperature, as determined by the formation of a single absorption band at 360 nm. The retinaloxime was then added to bleached membrane preparations from which unreacted hydroxylamine had been eliminated by repeat pelleting and washing.

Absorption and CD spectra of the membrane preparations were recorded as previously described in detail (5).

RESULTS AND DISCUSSION

Visible Spectra

The absorption and CD spectra from 625 to 300 nm of the bleaching sequences in purple membrane are shown in Figs. 1*a* and 1*b*. Light-adapted membrane has a major absorption band at about 567 nm, attributable to the $\pi - \pi^*$ (NV_1) transition of the chromopore (5). The CD spectrum includes a positive and negative band at about 535 and 602 nm with crossover near the wavelength of the absorption band maximum. There is a relatively intense CD band centered at about 317 nm that corresponds to absorption band(s) with very weak transition dipole strength(s). This intense CD band may arise from higher $\pi - \pi^*$ (NV) transition(s) of the retinal that are magnetic dipole-allowed but electric dipole-forbidden. There are indications that this band is very sensitive to the rigidity of the retinal-apoprotein interaction(s), possibly because of secondary noncovalent linkage(s) between the retinal and the apoprotein (5).

In this wavelength region CD is due to the induced optical activity of the bound retinal. There are a variety of mechanisms by which optically inactive chromophores, such as retinal, can become optical active when bound to optically active apoproteins. In the past, two different mechanisms have been suggested to account for the induced

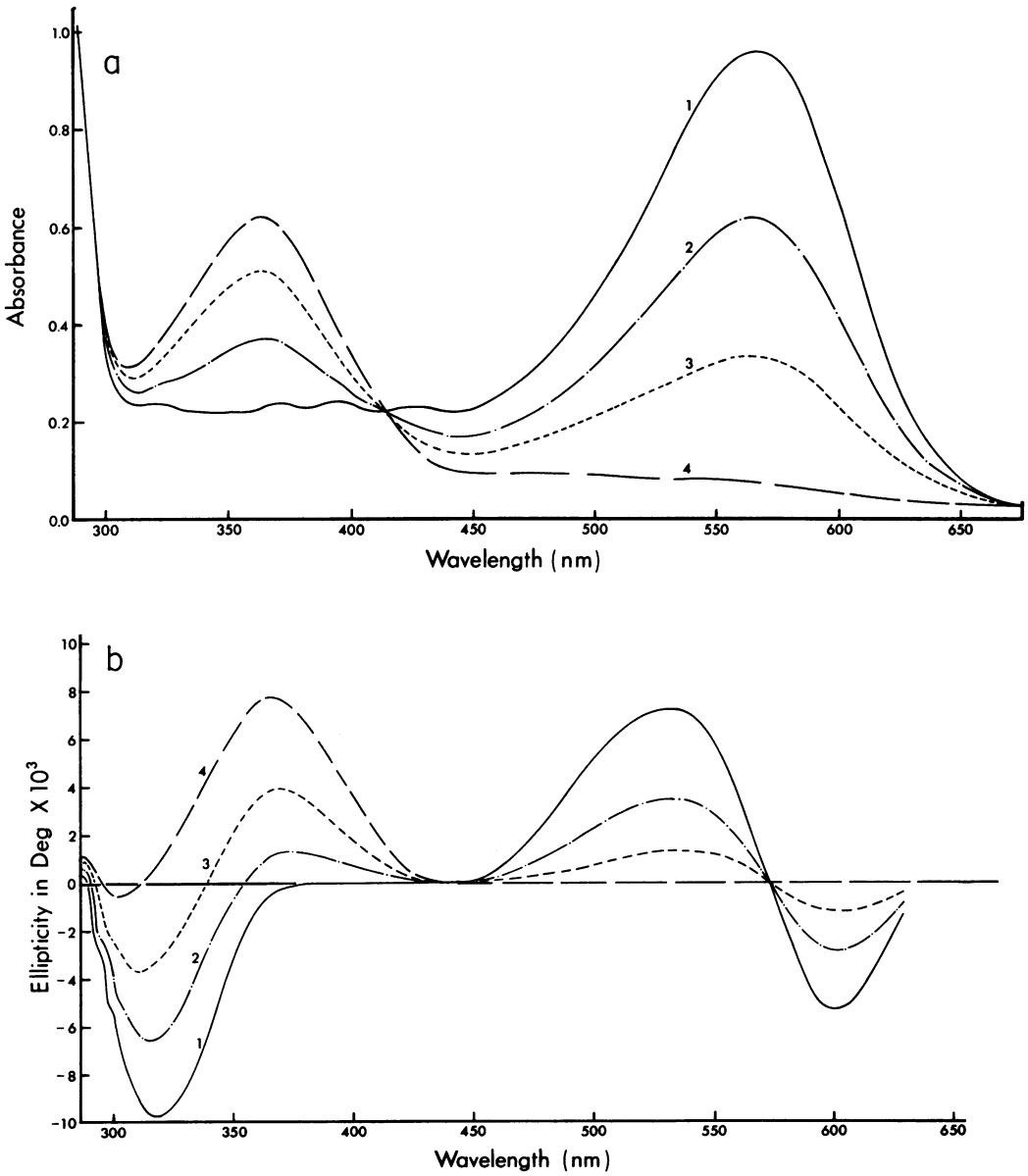

FIGURE 1 Bleaching sequence of purple membrane in 0.3 M hydroxylamine. Curve 1, light-adapted membrane before bleaching; curve 2, after 90 min of filtered light exposure; curve 3, after 210 min; curve 4, after 300 min (complete bleach). *a*. Visible absorption spectra; *b*. Visible CD spectra.

optical activity of the bound retinal of rhodopsin: the spatially distorted chromophore mechanism (intrinsic mechanism) and the coupled oscillation mechanism (extrinsic mechanism) (19–28). According to the intrinsic mechanism, optical activity in the visible spectral region is achieved by the isolation of optically active enantiomers of the prosthetic group, retinal, by preferential binding to the apoprotein, opsin. The extrinsic mechanism achieves induced optical activity through dipole-dipole resonance coupling of the retinal transitions with the optically active transitions of the apoprotein aromatic amino acid residues or the apoprotein amide bond. However, experimental support for either mechanism has not been forthcoming (17, 26, 27). Other dissymmetry-selecting and dissymmetry-inducing mechanisms are also possible.

In the case of the visible CD of the purple membrane, there is evidence consistent with an exciton mechanism resulting from interactions between the retinals within the clusters (5, 12–14). Therefore, much of the rotatory strengths of the 535 and 602 CD bands can be attributed to this mechanism. However, the highly fluid state of the sites that individual rhodopsin molecules occupy in the visual receptor membrane would exclude the possibility of exciton interaction in this membrane, since the geometric integrity of the interacting molecules is an essential requirement of the molecular exciton theory (16, 29). In fact, no spectroscopic evidence for intermolecular excitonic interaction in such photoreceptor membranes has been found (17). Since the 317-nm CD band is due to transition(s) with extremely low oscillatory strength(s), the resonance coupling mechanism previously suggested for the induced optical activity of rhodopsin is not reasonable in this case. The induced optical activity is due to some kind of constraint imposed on the retinal by the dissymmetric apoprotein. Elucidation of the specific mechanism requires further investigation.

Bleaching the purple membrane in the presence of hydroxylamine involves the loss of the Schiff base bond between retinal and the apoprotein and the conversion of retinal to retinaloxime due to the reaction between retinal and hydroxylamine. This process results in a decrease in the 567-nm absorption band and the simultaneous appearance of a band at 366 nm (Fig. 1 *a*, curves 1–4). An isosbestic point at 414 nm suggests that only two molecular species are present in measurable quantities during bleaching. Furthermore, the 317-nm CD band of purple membrane decreases and a CD band at 366 nm arises simultaneously with the decrease in the absorption band at 567 nm during sequential bleaching (Fig. 1*b*, curves 1–4). These spectral results are associated with the conversion of retinal to retinaloxime, which remains bound to the membrane. The intense CD band at 366 nm in the bleached membrane is due to the induced optical activity of the retinaloxime resulting from dissymmetric interaction(s) between the retinaloxime and the apoprotein or the lipid component of the membrane by a mechanism yet to be elucidated. By comparison, the bleaching of bovine rhodopsin *in situ* and in the presence of hydroxylamine results in the essential loss of ellipticity in the 300–600 nm region (17). Therefore, it is apparent that in the bleaching of this rhodopsin no dissymmetric binding of the retinaloxime (formed during the bleaching process) to any component of the membrane is evident. It is noteworthy that the absorption and CD bands at 366 nm in the bleached purple membrane are

at about the same wavelength as the absorption band of the unbound retinaloxime, whereas the absorption and CD bands centered at about 567 nm in the unbleached membrane are strongly red-shifted from the position of the absorption band of the unbound retinal. This indicates that the mechanism(s) by which retinaloxime is bound to some molecular component of the bleached membrane must be different than the mechanism(s) by which the retinal is bound to the apoprotein of the membrane.

The interpretation of the effects of bleaching on the positive and negative CD bands at 535 and 602 nm is more complex. If one assumes excitonic interaction between the retinals in the clusters of three bacteriorhodopsins in the purple membrane, and if the chromophores within a cluster bleached simultaneously, then the CD bands would be expected to decrease proportionally to the absorption decrease at 567 nm. However, if the chromophores bleached randomly, then the CD bands would be expected to decrease faster than the absorption band since, at a given percent of bleached chromophores, a greater percentage of exciton-interacting clusters would be disrupted. From Fig. 1*a* and *b* it is clear that the loss of the ellipticity does approximately follow the loss of absorbance, indicating a tendency for the chromophores in clusters to bleach simultaneously. In addition, the isosbestic point in the absorption spectra and the isoelliptic point in the CD of the bleaching sequence (Fig. 1*a* and *b*) imply the presence of only two species, bleached and unbleached bacteriorhodopsin. Consequently, the presence of membrane with partially bleached clusters of bacteriorhodopsins is not indicated, since such a membrane would have spectra different from the unbleached membrane and would prevent the appearance of an isosbestic and an isoelliptic point in the spectra of the bleaching sequence. These results are interesting comparisons to previous CD and absorption results (12, 14), which indicate that regeneration of bleached membrane occurs randomly.

Addition of all-*trans* retinal to the bleached membrane results in nearly complete regeneration of the membrane as indicated by the reappearance of the 567 nm absorption band (Fig. 2*a*). The 366 nm absorption band, due to the retinaloxime bound to the bleached membrane, shifts to 362 nm on regeneration. The visible CD spectra is also nearly completely regenerated by the addition of retinal, indicating the reestablishment of the major structural features of the membrane (Fig. 2*b*). Interestingly, the CD band at 366 nm attributed to the induced optical activity of the retinaloxime is lost on regeneration, although retinaloxime remains bound to the membrane, as indicated by the persistence of the 362-nm absorption band after the membrane was pelleted and resuspended in buffer solution. The loss of the 366-nm CD band and the shifting of the 366-nm absorption band to 362 nm, near the absorption maximum of the unbound retinaloxime, indicates that the specific interactions that resulted in the induced optical activity of the bound retinaloxime are lost during regeneration of the membrane. By spectral criteria it would appear that the regenerated membrane is similar to the native membrane, with the exception that the regenerated membrane has weakly bound retinaloxime associated with it. Regeneration of the bleached membrane by 13-*cis* retinal in the dark gives absorption and CD spectra similar to those observed for dark-adapted purple membrane (5).

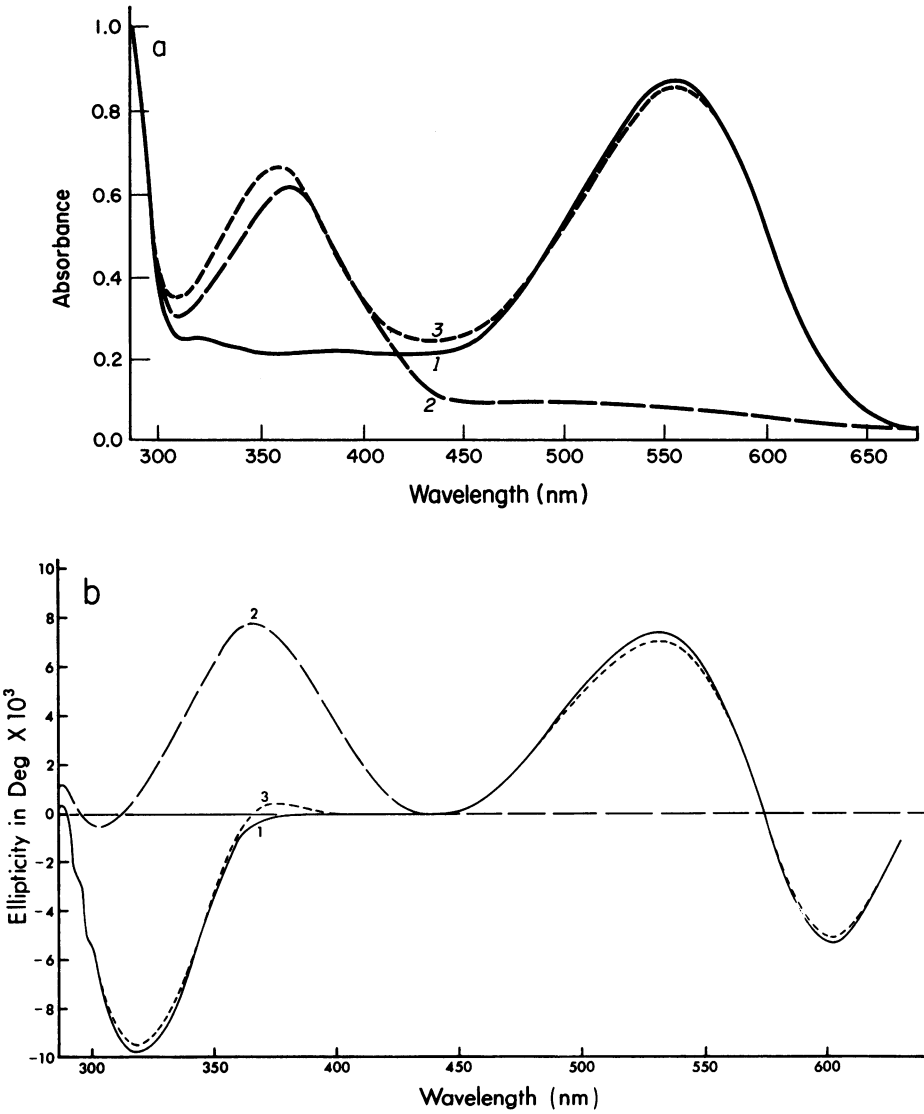

FIGURE 2 Regeneration of bleached purple membrane by addition of all-*trans* retinal. Curve 1, light-adapted membrane before bleaching; curve 2, bleached membrane; curve 3, regenerated membrane. *a*. Visible absorption spectra; *b*. Visible CD spectra.

Additions of up to four molar equivalents of retinaloxime to the bleached membrane, from which unreacted hydroxylamine had been removed by repeated washing, results in linear increases in the intensity of the 366-nm absorption band. However, ellipticity of the 366 nm band increases 33% upon the addition of one molar equivalent and further additions have negligible effect. After the addition of approximately four molar equivalents of retinaloxime, additional retinaloxime could be washed out of the membrane preparations as indicated by absorption spectra. These results indicate that

approximately one molar equivalent of retinaloxime can interact dissymmetrically with the bleached membrane, in addition to the molar equivalent of retinaloxime formed and bound during the bleaching process. One interpretation of these results may be that the dissymmetric binding of the retinaloxime to the bleached membrane occurs at specific sites (in a ratio of approximately 2 mol of retinaloxime per 1 mol of bacteriorhodopsin). An alternate interpretation may be that the retinaloxime formed during the bleaching process is equilibrated between specific (dissymmetric binding) and nonspecific (nondissymmetric binding) sites and that additional retinaloxime fills the specific site.

Near and Far Ultraviolet Spectra

The near-ultraviolet (295–250 nm) absorption and CD spectra of the bleaching sequence in the purple membrane are shown in Figs. 3*a* and *b*. The absorption spectrum of light-adapted purple membrane (Fig. 3*a*, curve 1) includes distinct bands at 290,280, and 274 nm, primarily attributed to the $\pi - \pi^*$ transitions of the amino acids tryptophan, tyrosine, and phenylalanine (5). (No absorption is attributed to cysteine since this amino acid is not found in the purple membrane.) The near-ultraviolet CD spectrum of purple membrane (Fig. 3*b*, curve 1) includes several bands that can be correlated with bands found in the absorption spectrum (5). The distinct band at 290 nm in both the CD and absorption spectra indicates a significant tryptophan contribution.

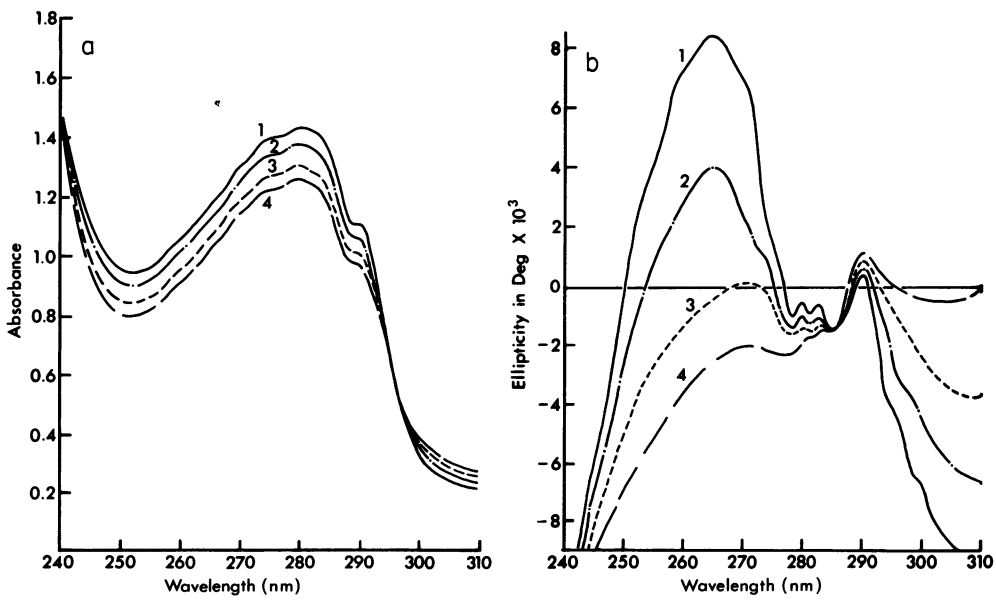

FIGURE 3 Bleaching sequence of purple membrane in 0.3 M hydroxylamine. Curve 1, light-adapted membrane after bleaching; curve 2, after 90 min of filtered light exposure; curve 3, after 210 min; curve 4, after 300 min (complete bleach). (Identical samples were used in recording Figs. 1 and 3.) *a*. Near-ultraviolet absorption spectra; *b*. Near-ultraviolet CD spectra.

Bleaching of the purple membrane in the presence of hydroxylamine induces major changes in the near-ultraviolet absorption and CD spectra (Figs. 3*a* and *b*). Successive periods of light exposure decrease the intensity of all the absorption bands associated with aromatic amino acids (Fig. 3*a*). Increases in the intensity from 295 to 310 nm are attributed to the tail of the 366 nm band due to the bound retinaloxime. After complete bleaching, the 280 nm band complex is 13% less intense than in unbleached membrane. The CD spectrum of bleached membrane (Fig. 3*b*, curve 4) shows an increase in intensity at 290 nm, resulting at least partially from the loss of the negative band at 317 nm on bleaching. The rest of the spectrum is dominated by major losses in intensity resulting in negative ellipticity for the spectrum of the bleached membrane below 288 nm.

The near-ultraviolet absorption and CD spectra of bleached membrane regenerated by the addition of all-*trans* retinal are not measurably different from the spectra of unbleached membrane. (Due to a low signal-to-noise ratio inherent in CD measurements of purple membrane from 300 to 250 nm, it is estimated that changes of up to 5% in ellipticity at 260 nm could occur with detection.) Furthermore, the far-ultraviolet (250–185 nm) CD and absorption spectra, which suggest the presence of protein with about 45% α-helical content in the purple membrane (5), are not measurably changed on bleaching or regeneration. (An uncertainty of 1% in the CD measurement of purple membrane is estimated at 223 nm. That is, the net secondary structures of the unbleached, bleached, and regenerated membrane are identical within a high degree of certainty). This indicates that any differences in net secondary structures must not involve more than two amino acid residues (5). By comparison, both the near- and far-ultraviolet spectra of bovine rod outer segment disk membranes *in situ* are essentially invariant to the bleaching process (17). Additions of retinaloxime to bleached purple membrane preparations from which unreacted hydroxylamine has been eliminated result in no significant changes in the near- and far- ultraviolet spectra.

The large changes in the near-ultraviolet absorption and CD spectra on bleaching purple membrane could result from (*a*) a change in the secondary and/or tertiary structure of the bacteriorhodopsin, resulting in changes in the environment of the $\pi - \pi^*$ transitions of the aromatic amino acid side chains (30–32); (*b*) a loss of possible dipole coupling between the $\pi - \pi^*$ transitions of the retinal and the aromatic amino acids (23, 26, 28); or (*c*) a change in contributions from minor $\pi - \pi^*$ transitions of the chromophore in the near-ultraviolet wavelength region (33).

No one of these explanations of the changes in the near ultraviolet spectra on bleaching the purple membrane can be discounted and all may contribute to varying degrees. However, several observations minimize the likelihood that the last two explanations are a major factor in these spectral changes. (*a*) The loss of the Schiff base bond and the induced visible optical activity of the retinal of bovine rhodopsin *in situ* result in no significant near-ultraviolet spectral changes, in contrast to the purple membrane results (17). (*b*) The near-ultraviolet spectra of purple membrane are essentially invariant to light-adaptation of the purple membrane, which results in a 13-*cis*

to all-*trans* stereoisomerization of the retinal (5). (*c*) The intensity of the near-ultraviolet CD bands, considering the 12% aromatic amino acid composition of the purple membrane protein bacteriorhodopsin, is comparable to that of most proteins without prosthetic groups (30, 32) and not comparable to those with prosthetic groups, such as the heme proteins, the CD of which are believed to be enhanced by a coupled oscillator mechanism (34). (*d*) The near-ultraviolet difference absorption spectrum of the bleached membrane and the regenerated membrane closely resembles the profile of the spectrum of the membrane itself, with maxima at 290, 280, and 274 nm (B. Becher and T. G. Ebrey, unpublished results).

If there is a significant contribution to the near-ultraviolet spectrum of the purple membrane by a coupled oscillation mechanism and/or the short wavelength $\pi - \pi^*$ transitions of the retinal, it would be expected that the loss of the Schiff base bond or the configurational changes of the retinal would result in significant changes not only in the intensity of the bands but also in the profile of the spectrum. Since the observations given above are not in accord with such expectations, the most likely conclusion is that the major contribution to the change in the near-ultraviolet spectra on bleaching the purple membrane is the result of conformational changes involving the secondary and/or tertiary structures of the apoprotein. In general, significant changes in the conformation of proteins result in major spectral changes over the entire near-ultraviolet region. The change in conformation of the apoprotein could result from the loss of the Schiff base bond and secondary noncovalent interactions between the retinal and the apoprotein. However, it is not the result of the binding of retinaloxime to some molecular component of the bleached membrane, because the same differences in the near-ultraviolet absorption and CD spectra are found when bleached and retinaloxime-extracted membrane is regenerated (B. Becher and T. G. Ebrey, unpublished results). Furthermore, the spectra are also essentially invariant to the addition of retinaloxime.

In view of the fact that the far-ultraviolet CD and absorption spectra (250–185 nm) of purple membrane are invariant to the bleaching process within a high degree of certainty, it appears that conformational changes involving the secondary structure of the apoprotein do not occur. Although a set of compensating secondary structure changes in the apoprotein may result on bleaching and therefore give no net change in the far-ultraviolet spectra, the possibility of such closely compensating changes is remote (35). Therefore, the changes in the near-ultraviolet spectra can best be interpreted as a consequence of a change in the tertiary structure of the apoprotein. Furthermore, considering the unusually large loss of intensity over the entire near-ultraviolet region on bleaching and the aromatic amino acid content of the apoprotein, a significant number of the aromatic amino acid residues of the protein are probably affected by the conformational change in the apoprotein. Therefore, the conformational change must be delocalized over a relatively large portion of the apoprotein chain (35).

Let us consider the fact that the retinaloxime, not intrinsically optically active, becomes optically active in the bleached membrane and loses this optical activity when

the membrane is regenerated by retinal. This implies that retinaloxime is strongly bound to the apoprotein or the lipid moiety of the bleached membrane. Furthermore, since the retinaloxime cannot be easily removed from regenerated membrane, the retinaloxime must still be bound to the membrane. Nevertheless this loss of the induced optical activity of the retinaloxime indicates that the mechanism by which retinaloxime is bound must be very different in the two membrane states, bleached and regenerated. This change in binding can be attributed to a conformational change in the membrane upon binding of retinal. If the retinaloxime is bound to the lipid moiety of the bleached membrane, then the conformational change resulting from the regeneration of the membrane, by rebinding of the retinal to the original retinal binding site in the apoprotein, must be transmitted over relatively large distances to the lipid molecules. For this type of transfer of distortion energy one would expect large delocalized conformational changes with possible significant secondary structure involvement in the apoprotein (35). On the other hand, if the retinaloxime is bound to the apoprotein, the transfer of distortion energy would most likely be over relatively shorter distances. Such a transfer of energy could be achieved by relatively less drastic changes in the conformation of the apoprotein. The spectral data seems to accord better with the latter possibility. Therefore, the apoprotein is perhaps the most likely candidate for the binding of the retinaloxime.

Presently, there is no direct information concerning the location of these binding sites on the apoprotein since very little is known about the structure of the apoprotein. However, one likely place may be the original retinal binding site, which has undergone structural modifications during the bleaching process. That the bleached membrane can be regenerated with all-*trans* retinal offers no obstacle to this possibility, since one would expect the binding constant of retinaloxime (which cannot form a Schiff base bond with the apoprotein) to be lower than the one for retinal. This possible binding site is attractive since retinaloxime formed during the bleaching process would not have to be dislocated over relatively large distances. Of course, further investigation is necessary to determine the merits of this suggestion.

To summarize, the bleaching of the purple membrane in the presence of hydroxylamine results in significant changes in the molecular organization and the conformation of the bacteriorhodopsin of the purple membrane. Bleaching results in the loss of the possible excitonic interaction between retinal chromophores within the clusters of three bacteriorhodopsins and there appears to be a tendency for the chromophores within the clusters to bleach simultaneously. This is in contrast to the regeneration of bleached membrane, in which retinal apparently regenerates the apoprotein randomly. Although no excitonic interaction between retinaloxime chromophores in the bleached membrane is indicated, an intense CD band is associated with retinaloxime that is not intrinsically optically active. This induced optical activity suggests some kind of dissymmetry-inducing and/or -selecting contraints imposed on the retinaloxime by the apoprotein by mechanisms yet to be explained. However, the most probable component to which the retinaloxime binds is the apoprotein. Large changes in the near-ultraviolet absorption and CD spectra occur on bleaching the purple membrane

with no discernible effect on the far-ultraviolet spectra. Evaluation of these results suggests that significant contributions to the bleach-induced changes originate from tertiary, possibly delocalized conformational changes in the apoprotein. This result contrasts with the bleaching of sonicated bovine rod outer segment membrane, in which no discernible change is seen in the far or near-ultraviolet CD and absorption spectra on bleaching in the presence of hydroxylamine.

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