Lipid-Induced Conformational Changes of an Integral Membrane Protein: An Infrared Spectroscopic Study of the Effects of Triton X-100 Treatment on the Purple Membrane of *Halobacterium halobium* ET1001

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ABSTRACT: Exposure of purple membrane from *Halobacterium halobium* to sublytic concentrations of Triton X-100 results in significant changes in the bacteriorhodopsin (BR) photocycle (Mukhopadhyay et al., 1994). Infrared spectra of purple membrane samples exposed briefly to Triton indicate that this change in protein function accompanies the preferential release of purple membrane glycolipids and squalenes, an association of Triton with purple membrane, and a perturbation of specific lipid headgroup interactions within the membrane. Specifically, the bilayer alterations induced by Triton entail a disruption of lipid headgroup hydrogen bonding in addition to protein conformational changes involving a loss in β-turn and α_{II}-helical structures in BR. We propose that the purple membrane glycolipids and squalenes are critical for the normal functioning of the BR photocycle and that perturbations of these lipids cause the profound photocycle changes induced by exposure to Triton. Lipid reconstitution studies demonstrated that although several of the infrared spectral parameters characteristic of the structural changes induced by Triton were reversed, the photocycle characteristics of BR in native purple membrane were not regained. The observed changes in the vibrational spectra induced by lipid-mediated bilayer perturbations suggest a useful approach for clarifying structure−function relationships of intrinsic membrane proteins exhibiting transmembrane helices.

Integral membrane proteins are critical to a wide variety of cellular activities, such as cytochrome oxidase in mediating electron transport (Winkler et al., 1995) and the activation of intracellular signals by transmembrane receptors coupled to G proteins (Simon et al., 1991). In these and many other membrane activities, the mechanisms by which protein behavior is modulated by the surrounding lipid matrix are unclear. Bacteriorhodopsin (BR),[1] a heptahelical, integral transmembrane protein which functions as a proton pump in the purple membrane (PM) of *Halobacterium halobium*, has provided a tractable system for exploring the effects of lipid perturbations on the function of an integral membrane protein (Oesterhelt & Stoeckenius, 1971; Henderson et al., 1990). Since the BR photocycle is composed of many intermediates, the precise nature of the relaxation pathway depends upon a variety of factors, including incident light intensity, pH, temperature, and pressure, as well as lipid environment (Rothschild, 1992; Ebrey, 1993; Tokaji, 1995). In particular, the dependence of the decay pathway on the lipid components of the membrane bilayer and the intensity of the incident light has been related to both the functional cooperativity between BR monomers within each trimer unit and the heterogeneity of the bilayer lipids [for a general discussion, see Shrager et al. (1995)].

Lipids constitute 25% of the purple membrane from *Halobacterium cutirubrum*, which is closely related to *Halobacterium halobium*, and comprise approximately 60% phospholipid, 30% glycolipid, and 10% neutral lipids, primarily squalenes (Kates, 1988). The polar lipids are unique in that their phosphatidyl or triglycosyl headgroups are attached by ether linkages to two phytanyl chains, moieties consisting of saturated palmityl chains with branched methyl groups every four carbons (Kates, 1993). Phytanyl chain lipids result in larger headgroup spacings than lipids with saturated, unbranched alkyl chains, such as dipalmitoylphosphatidylcholine (Lindsey et al., 1979). The major phospholipid in purple membrane is a methyl ester derivative of phosphatidylglycerophosphate (PGP) with phosphatidylglycerosulfate (PGS) and phosphatidylglycerol (PG) appearing as minor membrane components. The glycolipids are predominantly composed of glycolipid sulfate (GLS) with some triglycosyl diether forming during the lipid purification process (Kates et al., 1982). Although the exact percentages of each lipid constituent vary from species to species of the *Halobacterium* genus and within each strain, approximately 10 lipid molecules are associated with each BR monomer (Glaeser et al., 1985). Furthermore, the glycolipids are located on the extracellular surface, as typically observed in cell membranes (Henderson et al., 1978). Based upon these observations, a model for the purple membrane lipid bilayer which describes the distribution of lipids between the cytoplasmic and exterior sides of the bilayer has been proposed (Jonas et al., 1990).

Studies on the effects of lipid perturbation on purple membrane function have focused primarily on changes in the BR photocycle. A decrease in the photocycle's M

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[1] Abbreviations: BR, bacteriorhodopsin; FWHH, full width at half-height; GLS, glycolipid sulfate; PM, purple membrane; PG, phosphatidylglycerol; PGP, phosphatidylglycerophosphate; PGS, phosphatidylglycerosulfate.

intermediate lifetime is observed when either ethanol (Fukuda & Kouyama, 1992b) or anesthetics (Nishimura et al., 1985; Henry et al., 1988) are added to the membrane; in contrast, the lifetime is increased either by exposure to Tween-20 (Fukuda et al., 1990) or by reconstitution of monomeric BR into detergent micelles (Milder et al., 1991). Triton X-100, a common surfactant used to solubilize integral membrane proteins, also causes an increase in the lifetime of the M intermediate (Drachev et al., 1986). Exposure of purple membrane to mild Triton solutions induces a number of alterations in the BR photocycle, including a loss in the ability to modify the proportion of fast and slow M intermediates (M_f and M_s, respectively) through intensity variations of the incident light, the elimination of the decay path of M_f through O, and the production of new, longer-lived M intermediates without the loss of the BR trimer structure (Mukhopadhyay et al., 1994).

In view of the considerable integral membrane protein effects that originate from mild bilayer lipid perturbations, we used infrared spectroscopic techniques to detail the structural alterations within the lipid bilayer and the transmembrane protein of purple membrane dispersions from both limited and extended Triton exposures. We also indicate the relative sensitivities of specific lipid classes to Triton treatment with respect to membrane extraction. By emphasizing the structural reorganizations induced by exposure of surfactant solutions to purple membrane assemblies, we demonstrate the direct effects of specific lipid bilayer perturbations on subtle conformational rearrangements of intrinsic membrane proteins.

EXPERIMENTAL PROCEDURES

Experiments were performed with the ET1001 strain of *Halobacterium halobium*. Purple membrane suspensions (8.2 mg mL^{-1}) were incubated at room temperature with an equal volume of 0.01−0.10% Triton X-100 for ∼2 min to 24 h and were then centrifuged at 200000*g* in a Beckman TL-100 ultracentrifuge at 4 °C for either 7 or 20 min. The infrared spectra and chemical analyses of samples incubated with Triton for 5 min at room temperature and centrifuged for either 7 or 20 min were identical, indicating that little change in lipid composition and BR structure occurs after the first 7 min of centrifugation. Lipid extraction of the pellet and supernatant fractions was performed as described by Kates et al. (1982). Delipidation of BR was performed by the method of Huang et al. (1980). For reconstitution studies, purple membrane samples which had been exposed to Triton were immediately centrifuged and washed to remove any residual Triton or extracted lipids. Lipids that had been previously extracted from native purple membrane were sonicated and then incubated with the Triton-exposed purple membrane for 24 h, centrifuged, and finally washed. For spectroscopic analysis, reference samples of phytanic acid, squalene, glucose 6-sulfate, and galactose 6-sulfate were obtained from the Sigma Chemical Co.

Infrared spectra of the extracted lipids were obtained from dried films cast from $CHCl_3$ solutions onto BaF_2 or NaCl windows (Wilmad Glass). Films of purple membrane were typically obtained by drying 30 μL of an aqueous purple membrane suspension (with an absorbance of 1.2 units at 568 nm) onto a BaF_2 window at 30 °C to remove bulk water without changing the purple membrane surface structure; the spectra in Figure 3, however, were obtained from films dried at 40 °C. Infrared spectra were obtained at a spectral resolution of 0.5 cm^{-1} on a Bomem DA3.02 Fourier transform spectrometer evacuated to 2 torr and equipped with a mercury cadmium telluride (MCT) detector. Difference spectra were normalized to the intensity of the amide I modes from 1720−1600 cm^{-1}. Mole percentages of the purple membrane lipids were determined from the ratio of the integrated intensity of a distinct lipid marker band (*e.g.*, 1260 cm^{-1} for GLS and 1512 cm^{-1} for Triton) to the integrated intensity of the carbon−hydrogen (C−H) stretching mode region (3100−2800 cm^{-1}). Visible absorption measurements were obtained on an Aviv 14DS UV−Vis absorption spectrometer.

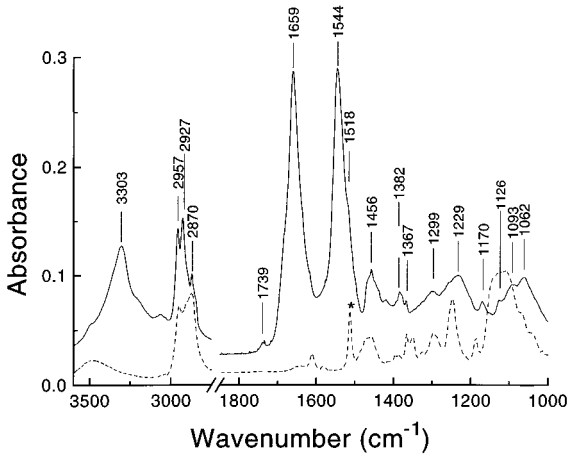

FIGURE 1: Infrared spectra in the 3600−2800 and 1800−1000 cm^{-1} regions of a native purple membrane film dried from a 4.1 mg mL^{-1} aqueous suspension at 30 °C (solid line) and a thin film of Triton X-100 pressed between two BaF_2 windows (dashed line). The asterisk denotes the vibrational mode at 1512 cm^{-1} in Triton used to quantify the amount of Triton in $CHCl_3$-extracted lipid fractions.

RESULTS AND DISCUSSION

Infrared Spectrum of Native Purple Membrane. The infrared spectra of partially dried purple membrane suspensions and of lipid extracts were examined to determine the changes occurring in membrane structure and in BR conformation upon exposure to Triton. Figure 1 displays the infrared spectrum of a thin film cast from an aqueous native purple membrane suspension with the infrared spectrum of a thin film of Triton X-100 which was used for the lipid perturbation studies. The infrared spectral assignments for purple membrane, summarized in Table 1, are based upon previously published infrared spectra of native purple membrane and related molecular systems (Rothschild & Clark, 1979; Braiman & Rothschild, 1988; Colthup et al., 1990).

The spectral region above 2800 cm^{-1} in Figure 1 is comprised of carbon−hydrogen (C−H), oxygen−hydrogen (O−H), and nitrogen−hydrogen (N−H) stretching modes. The feature at 3303 cm^{-1}, corresponding to the BR amide A mode, is composed primarily of N−H stretching modes (Dwivedi & Krimm, 1984). The broad feature centered at 3062 cm^{-1} corresponds to the BR amide B mode (Rothschild & Clark, 1979) and C−H stretching modes of aromatic rings in the Phe, Trp, and Tyr residues of BR. The bands in the C−H stretching mode region from 3000 to 2800 cm^{-1} arise primarily from the lipid phytanyl chains since the most

Table 1: Vibrational Spectra and Assignments of Native Purple Membrane in the 3600−1000 cm^{-1} Spectral Region[a]

frequency (cm^{-1})	assignment
3303	amide A
3062	amide B and aromatic CH stretch: Tyr, Trp, Phe
2957	CH_3 asym stretch
2927	CH_2 asym stretch
2901	aliphatic CH stretch
2870	CH_3 sym stretch
2854	CH_2 sym stretch
2844	CH_2 sym stretch: PG, Ser, Asp, Glu
1739	C═O stretch: Asp-96, Asp-115
1659	amide I
1544	amide II (E_1 species of α_{II}-helices)
1518	amide II (A species of α_{II}-helices) and Tyr
1464	CH_2 deformation, CH_3 asym deformation
1456	
1446	
1424	CH_2 deformation: Gly, Asp, Gln, Glu
1390	$(COO)^-$ sym stretch: Asp-85, Asp-212, Glu
1382	CH_3 sym deformation: phytanyl chains, Leu, Val
1380	CH_3 sym deformation
1367	CH_3 sym deformation: phytanyl chains, Leu, Val
1299	
1260	amide III
1229	$(PO_2)^-$ asym stretch
1203[b]	GLS sulfate stretch
1170	CH_3 rock + CH deformation: phyt, Leu, Val
1152	CH_3 rock: phyt, Ile, Ala
1126	
1093	CC stretch + CH_3 rock: BR
1062	C−O−C asym stretch: PGP, GLS

[a] Vibrational modes observed in purple membrane films dried at 30 °C. [b] Vibrational mode observed in purple membrane dried at 40 °C.

intense bands centered at 2957 cm^{-1} (asymmetric methyl stretching modes, $\nu_{as}[CH_3]$), 2927 cm^{-1} (asymmetric methylene stretching modes, $\nu_{as}[CH_2]$), and 2870 cm^{-1} (symmetric methyl stretching modes, $\nu_s[CH_3]$) correspond in frequency and intensity to the most intense features observed in the infrared spectra of phytanic acid and of lipids extracted from native purple membrane (spectra not shown). Two other weaker features, observed as shoulders of the 2870 cm^{-1} band, appear at 2854 and 2844 cm^{-1}. The 2854 cm^{-1} mode corresponds to symmetric methylene stretching modes ($\nu_s[CH_2]$) of carbons with no functional groups (*i.e.*, −C−CH_2−C−) (Hill & Levin, 1979; Senak & Mendelsohn, 1993), present on the lipid phytanyl chains and on certain BR amino acid residues (*e.g.*, Leu, Met, Glu, Lys, and Arg). This mode also corresponds to symmetric methyl stretching modes ($\nu_s[CH_3]$) attached directly to a N, O, or S atom (Hill & Meakins, 1958; Colthup et al., 1990). This structure is present on Met residues in BR and principally as part of the PGP headgroup since the terminal phosphatidyl region of the headgroup is composed of an *O*-methyl ester (Fredrickson et al., 1989; Tsujimoto et al., 1989; Kates et al., 1993). The 2854 cm^{-1} mode is as intense as the 2870 cm^{-1} mode in PGP purified from native purple membrane (spectrum not shown). The feature near 2844 cm^{-1} corresponds to symmetric methylene stretching modes ($\nu_s[CH_2]$) on carbons with OH, SH, or NH_2 functional groups (as, for example, in the phospholipid headgroups as well as in Ser, Gly, and Cys residues in BR) (Wright, 1959; Colthup et al., 1990). The shoulder of the asymmetric methylene stretching modes at 2900 cm^{-1} is assigned to the C−H stretching vibration involving trisubstituted carbon atoms.

The region below 1800 cm^{-1} in Figure 1 is comprised of skeletal stretching and bending vibrations. The infrared spectral region near 1740 cm^{-1} has been used extensively in studying the proton transport pathway during the BR photocycle (Braiman et al., 1991; Sasaki et al., 1994); the weak band appearing at 1739 cm^{-1} corresponds to the carbonyl (C═O) stretching modes of the protonated carboxylic groups in Glu and the two protonated Asp residues in BR_{568} (*i.e.*, Asp-96 and Asp-115). More recent work has focused on the use of site-directed BR mutants to study the formation and decay of intermediates which have a very short lifetime in native purple membrane (Sonar et al., 1993; He et al., 1993).

The secondary structure of BR is composed primarily of α_I- and α_{II}-helices with smaller regions consisting of β-sheet and β-turn structures, as well as random coils (Vogel & Gärtner, 1987; Tuzi et al., 1994; Torres & Padrós, 1995). Each of these structures exhibits amide I vibrational modes which appear between 1710 and 1630 cm^{-1} (Krimm & Bandekar, 1986), and contribute to the broad, intense feature at 1659 cm^{-1} shown in Figure 1. The α_{II}-helical structure, which results in an abnormally high amide I infrared frequency compared to α_I-helices, consists of A, E_1, and E_2 symmetry species, the former two of which are infrared active and typically appear in the infrared spectrum of purple membrane (Krimm & Dwivedi, 1982; Dwivedi & Krimm, 1984). The band appearing at 1544 cm^{-1} corresponds to the E_1 species of the α_{II}-helical amide II mode in BR, while the weaker amide II A species overlaps with Tyr ring modes forming the shoulder at 1518 cm^{-1} (Rothschild & Clark, 1979; Dwivedi & Krimm, 1984).

The spectral region below 1500 cm^{-1} is comprised primarily of modes arising from amino acid residues in BR as well as stretching and bending modes of the purple membrane lipids. The broad feature centered at 1456 cm^{-1} is comprised of overlapping bands at 1464, 1456, and 1446 cm^{-1}. These modes arise from methylene deformation ($\delta[CH_2]$) and asymmetric methyl deformation ($\delta_{as}[CH_3]$) modes. The symmetric methyl deformation ($\delta_s[CH_3]$) modes of branched methyl groups (present primarily on the phytanyl lipid chains) appear at 1380 cm^{-1}; in isopropyl groups, present on the phytanyl lipid chains as well as Leu and Val residues in BR, this mode is split into two bands appearing at 1383 and 1367 cm^{-1} (McMurry & Thornton, 1952; Colthup, 1980), as seen in Figure 1. A weak shoulder of the 1380/1383 cm^{-1} feature appearing at 1390 cm^{-1} has been assigned to the symmetric COO^- stretching mode of Asp-85 and Asp-212, the unprotonated Asp residues in the ground state BR_{568} (Fahmy et al., 1993), although this mode had been previously assigned to a feature at 1417 cm^{-1} (Rothschild & Clark, 1979; Gerwert et al., 1989). The feature appearing at 1424 cm^{-1} is assigned to the methylene deformation ($\delta[CH_2]$) mode on carbons with OH, SH, and NH_2 groups (in, for example, Ser, Gly, and Cys residues in BR).

Lipid headgroup vibrational modes appear in the 1300−1200 cm^{-1} spectral region. The broad feature appearing at 1229 cm^{-1} is well characterized as the asymmetric phosphate stretching ($\nu_{as}[PO_2]^-$) mode of the hydrogen-bonded phospholipid headgroups (Mendelsohn & Mantsch, 1986). The phosphate symmetric stretching mode overlaps with C−O and C−C stretching modes in the 1100−1060 cm^{-1} spectral region. The bands appearing at 1170 and 1152 cm^{-1} are

assigned to CH_3 rocking modes of both protein and lipid. Isopropyl groups, present on both the phytanyl lipid chains as well as in Leu and Val residues in BR, typically have infrared absorbance bands near 1170 and 1150 cm^{-1} due to a mixing of methyl rocking and isopropyl C−H deformation modes (Lin-Vien et al., 1991; Sullivan et al., 1993). However, the rocking modes of the mid-chain methyl groups, primarily in the lipid phytanyl chains, have been assigned near 1150 cm^{-1} as well, so the 1152 cm^{-1} band most likely represents features from both mid-chain methyl rocking modes and mixed rocking−deformation modes in isopropyl groups.

The structural changes which occur upon perturbation of the purple membrane lipid environment are determined from variations in the spectral parameters reflecting the infrared band assignments. To determine the changes which occur upon exposure of purple membrane to Triton, infrared spectra were measured for dried purple membrane films and for lipid isolated from purple membrane both before and after exposure to Triton.

Infrared Spectra of Purple Membrane Supernatant and Bilayer Lipid Fractions. Purple membrane suspensions are typically centrifuged to isolate intact purple membrane trimers from the Triton solution. Surfactant-induced perturbations in lipid composition may be elucidated from the infrared spectra of lipids extracted from the supernatant fraction (representative of the composition of lipids released from purple membrane after exposure to Triton) and the spectra of lipids extracted from intact purple membrane trimers (representative of the lipid composition within the purple membrane bilayer after exposure to Triton). The infrared spectra of lipids extracted from intact purple membrane bilayers were compared to spectra obtained from the lipids extracted from native purple membrane (before Triton exposure) in order to determine changes in the purple membrane lipid composition after exposure. Films of the supernatant and pelleted lipids for spectroscopic analysis were cast from $CHCl_3$ solutions.

Infrared spectra of the lipids in the supernatant and bilayer fractions were obtained for samples which were exposed to Triton solutions of 0.01, 0.02, 0.03, 0.05, 0.075, and 0.10% Triton. The spectra of lipids present in the supernatant fraction indicated that a relatively small fraction of purple membrane lipids were released upon exposure to a very mild Triton solution at room temperature (0.01%, 5 min, spectrum not shown). Based upon the integrated relative band intensities, the composition of the released lipids was different from their proportion in native purple membrane. At concentrations above 0.01% Triton, the supernatant fraction was composed primarily of Triton, and little information on released lipids could be determined from the infrared spectra. Direct analysis of the released lipids by chemical methods is provided by Dracheva et al. (1996).

The infrared spectra obtained from the purple membrane bilayer lipid fractions show two major changes after exposure to Triton; namely, an increasing degree of GLS being released from purple membrane, in addition to Triton association with purple membrane. Relative infrared band intensities were used to monitor these effects, as shown in Figure 2. The degree of GLS release is noted by the decrease in the mode at 1260 cm^{-1}. This mode appeared at 1265 cm^{-1} in GLS extracts from *Halobacterium cutirubrum* and was assigned to a stretching mode of the anionic sulfate moiety (Kates & Deroo, 1973); the assignment was verified from the spectra of model carbohydrates similar to GLS (*e.g.*, glucose 6-sulfate and galactose 6-sulfate). This vibrational mode was not observed in the infrared spectra of dried purple membrane films (*cf.* Figure 1), indicating that the sulfate group is hydrogen bonded with other lipid headgroups, with BR, or with water bound to the surface, causing it to appear at approximately the same frequency as the asymmetric stretching mode of the hydrogen-bonded phospholipid headgroups at 1229 cm^{-1}. Figure 2 illustrates the progressive release of GLS from purple membrane with treatments of 0, 0.01, 0.03, 0.075, and 0.10% Triton for 5 min, while Table 2 lists the relative percentages of GLS, based upon the integrated relative band intensities, in the lipids of native purple membrane under different conditions of Triton exposure. Although there was extensive removal of GLS, a fraction of GLS remains very difficult to extract, as observed previously in purple membrane heavily delipidated by either Tween-20 (Fukuda et al., 1990) or deoxycholate (Glaeser et

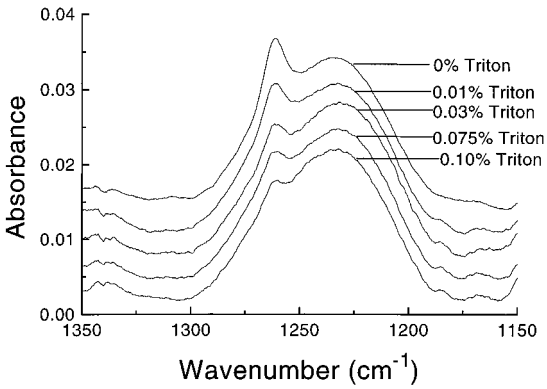

FIGURE 2: Infrared spectra in the 1350−1150 cm^{-1} region of lipids extracted from purple membrane pellets after exposure (from top to bottom) to 0, 0.01, 0.03, 0.075, and 0.10% Triton for 5 min. Samples were prepared as a film dried from a $CHCl_3$ solution. The decrease in the 1260 cm^{-1} transition is indicative of GLS release (*cf.* Table 2).

Table 2: Determination of Triton and Glycolipid Sulfate in Purple Membrane

Triton (%)[a]	Triton in PM[b] (mol %)	GLS in PM[b] (mol %)	$\nu_{as}([PO_2]^-)$[c] (cm^{-1})
0	0.0	4.5	1229
	$t = 5$ min		
0.01	1.1	3.8	1233
0.02	2.8	3.0	1232
0.03	11.5	3.0	1234
0.05	17.6	2.9	1234
0.075	32.1	1.17	1239
0.100	32.3	1.10	1238
	$t = 24$ h		
0.01	4.1	3.5	1234
0.02	6.4	2.1	1234
0.03	25.8	1.49	1235
0.05	28.7	1.24	1234
0.075	31.2	1.28	1237
0.100	30.8	1.31	1237

[a] Concentration of Triton for exposure of purple membrane sample. [b] mol % of Triton and GLS expressed as a percentage of the total purple membrane lipids released after exposure to Triton as determined from the ratio of the integrated band intensities of the modes at 1512 (Triton) and 1260 (GLS) cm^{-1} to the integrated band intensity of the C−H stretching region (3100−2800 cm^{-1}). [c] Frequency of the PO_2^- asymmetric stretching mode of the phospholipid headgroups as a function of Triton exposure.

al., 1985). This effect was also observed by chemical analysis of Triton-exposed purple membrane (Dracheva et al., 1996).

The association of Triton with purple membrane, as observed previously at sublytic and marginally lytic concentrations (González-Mañas et al., 1990; Uruga et al., 1990), may be determined from the infrared spectra of the lipids extracted from intact purple membrane trimers using the 1512 cm^{-1} feature of Triton (noted by an asterisk in Figure 1), a medium intensity, sharp band assigned to a coupling of aromatic ring C−C stretching and C−H deformation modes in Triton (Matsuura et al., 1991). There are other more intense spectral features in the infrared spectra of Triton due to the oligomeric poly(oxyethylene) chain, especially near 1100 cm^{-1} (Yoshihara et al., 1964); however, the sharp 1512 cm^{-1} feature was chosen since there is no significant interference from any modes of the purple membrane lipids (*cf.* Figure 1). The data for Triton association with purple membrane, shown in Table 2, indicate that at low concentrations and short exposure times of Triton, the degree of surfactant association was minimal (there was one PM-associated Triton per three BR trimers after 5 min with a 0.01% Triton exposure), but with harsher and more extended exposures, a greater degree of association was noticed. These observations correlate well with earlier observations that Triton can affect the bacteriorhodopsin photocycle in detergent-solubilized micelles (Milder et al., 1991).

Spectra of Partially Dried Purple Membrane Films. Infrared studies of partially dried purple membrane films provide a method for observing changes in BR and the lipid matrix. Spectra were obtained from Triton-exposed samples, as dried purple membrane films, for observing changes in BR conformation and lipid composition within intact purple membrane bilayers. These spectra were compared directly to spectra obtained from native purple membrane, in addition to being examined as difference spectra. Although infrared spectral changes could be expected as a result of molecular reorientation effects arising from the mild exposure of the films to Triton, we do not consider this likely since the Triton concentrations used in this study are known not to disrupt the bacteriorhodopsin trimer structure within the purple membrane (Mukhopadhyay et al., 1994). Additionally, bacteriorhodopsin solubilization induced by greater concentrations of Triton than used in this study occurs primarily at the edges of each purple membrane sheet (Viguera et al., 1994).

Figure 3 shows infrared spectra in the 1400−1000 cm^{-1} region for native purple membrane and for purple membrane exposed to 0.05% Triton for 5 min in films cast at 40 °C. The feature at 1229 cm^{-1} is characteristic of hydrogen-bonded phospholipid asymmetric PO_2^- stretching modes. Upon exposure to Triton, the frequency of this mode increases from 1229 to 1234 cm^{-1}, consistent with partial dehydration of the membrane surface (Flach et al., 1993; López-García et al., 1993) or some other disruption of lipid headgroup hydrogen bonding. Table 2 lists the frequency of this mode for different conditions of Triton exposure. The frequency of this vibration increases about 4 cm^{-1} under very mild Triton treatment (0.01−0.03% Triton) and another 3−4 cm^{-1} upon harsher treatment (>0.05% Triton), indicating a greater disruption of lipid headgroup hydrogen bonding.

The mode appearing at 1203 cm^{-1} shows a marked decrease in intensity after exposure to Triton (*cf.* Figure 3).

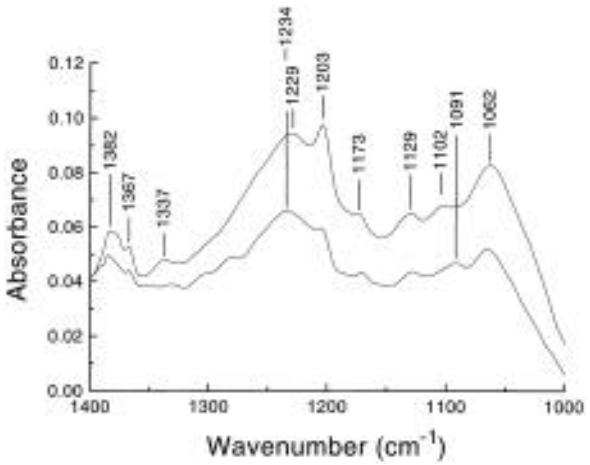

FIGURE 3: Infrared spectra in the 1400−1000 cm^{-1} region for native purple membrane (top) and a purple membrane sample exposed to 0.05% Triton for 5 min (bottom). Samples were prepared as films dried from aqueous suspensions at 40 °C.

This is a typical frequency for the symmetric stretching mode of alkyl-substituted sulfate groups (Lin-Vien et al., 1991) which form in GLS upon heating to 40 °C and appears as a weaker band in films dried at 30 °C. This feature reproducibly appears in the infrared spectra of films cast at 40 °C, and is weak or not observed in films cast at 30 °C. The intensity of this mode decreased by approximately 40% after exposure to Triton, which corresponds roughly to the quantity of GLS released under the conditions of Triton exposure used for Figure 3 (36%, Table 2). No protein or neutral lipid vibration can account for this transition. Neither PGP nor PGS can be attributed to these features since the band at 1229 cm^{-1} arises from the purple membrane phospholipid.

A new band appears at 1091 cm^{-1} after exposure to Triton and is characteristic of the Triton association observed in the spectra of the lipids extracted from purple membrane pellets. This feature, assigned to the asymmetric stretching mode of the C−O−C groups in the hydrophilic poly(oxyethylene) chain of Triton (Colthup et al., 1990), is the most intense mode of Triton below 1800 cm^{-1}. This band was easily observed in the infrared spectra of purple membrane upon exposure to Triton concentrations greater than 0.03%.

Difference Spectra of Purple Membrane Films. Difference spectra between native and Triton-treated purple membrane samples emphasize the changes occurring in the bilayer structure. Changes in lipid composition and BR conformation were observed upon brief exposure of purple membrane to mild Triton solutions. Figure 4 displays the difference spectrum between purple membrane and a sample treated with 0.05% Triton for 24 h. Positive bands depict vibrational modes present in native purple membrane which are diminished in the Triton-treated samples; negative features represent modes which are more intense in the Triton-exposed purple membrane. The release of lipids after 24 h is indicated by bands (with their assignment to lipid modes) at 2928 ($\nu_{as}[CH_2]$), 2872 ($\nu_s[CH_3]$), 1464 ($\delta[CH_2]$, $\delta_{as}[CH_3]$), 1378 ($\delta_s[CH_3]$), 1225 ($\nu_{as}[PO_2]^-$), 1205 (lipid headgroup; *cf.* Figure 3), 1179 (isopropyl CH_3 rock + CH deformation), and 1064 (glycolipid and phospholipid headgroup modes) cm^{-1}, as well as the broad region from 1200 to 1000 cm^{-1} arising from the release of glycolipid. The mode at 2966 cm^{-1} does not correspond to any protein vibrational mode

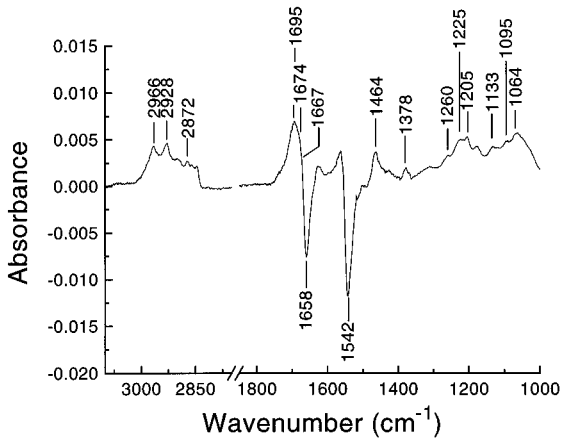

FIGURE 4: Infrared difference spectrum in the 3100−2800 and 1800−1000 cm^{-1} regions between native purple membrane and a purple membrane sample treated with 0.05% Triton for 24 h. Positive bands depict vibrational modes present in native purple membrane which are diminished in the Triton-treated samples; negative features represent modes which are more intense in the Triton-exposed purple membrane.

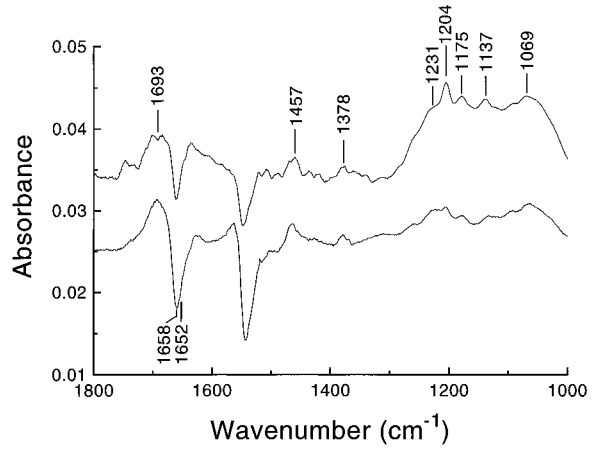

FIGURE 5: Infrared difference spectra in the 1800−1000 cm^{-1} region between native purple membrane and purple membrane centrifuged immediately after Triton addition (top) and after 24 h Triton exposure as in Figure 4 (bottom). Positive bands depict vibrational modes present in native purple membrane which are diminished in the Triton-treated samples; negative features represent modes which are more intense in the Triton-exposed purple membrane.

and is 9 cm^{-1} higher in frequency than the asymmetric methyl stretching modes of the phytanyl lipid chains. This band most likely corresponds to the asymmetric stretching mode of the methyl groups in squalene (present as approximately 10% of the purple membrane lipids; Kates, 1988), shifted higher in frequency by the double bonds in squalene and observed in the infrared spectrum of squalene (spectrum not shown). This is consistent with the finding of squalene release by Triton treatment of purple membrane (Dracheva et al., 1996).

Triton-induced protein conformational changes are also evident in Figure 4, particularly bands in the amide I region at 1695 cm^{-1} and in the region near 1674 cm^{-1}, as well as features in the amide I and amide II spectral regions near 1658 and 1542 cm^{-1}, respectively. There is also a change in the amide III mode at 1260 cm^{-1}. Figure 5 compares the difference spectrum between purple membrane and a sample exposed briefly to 0.05% Triton with immediate centrifugation (top trace), to that of Triton exposure for 24 h (bottom trace). The changes in the lipid headgroup region are similar in both spectra with greater changes in protein conformation observed after 24 h (Figure 5, bottom trace), as evidenced by the greater intensity of the infrared difference bands from 1710 to 1500 cm^{-1}.

Conformational Changes in BR. Based upon normal coordinate analyses, the abnormally high infrared frequency of the BR amide I vibrational mode was assigned to transmembrane α_{II}-helices by Krimm and Dwivedi (1982) and confirmed empirically by Cladera et al. (1992). This assignment was subsequently confirmed by circular dichroism (Gibson & Cassim, 1989) and NMR (Tuzi et al., 1994) studies. An α_{II}-structure has the same rise per residue and residues per turn as α_I-helices, but the plane of the peptide group has a greater tilt in the α_{II}-helices (Dwivedi & Krimm, 1984). Since the N−H bonds point more toward the helical axis in the α_{II}-helix, weaker, bent hydrogen bond structures form with the carbonyl groups; the resulting stronger C═O bond accords a higher frequency for the amide I transition in α_{II}-helices. Conversion of the α_{II}-helical structure in BR to α_I-helices (which occurs upon delipidation of BR) results in a decrease of the amide I frequency from 1659 to 1653 cm^{-1} and a decrease in the intensity of the amide II mode, relative to the amide I mode.

Figures 4 and 5 illustrate many of the protein conformational changes which occur after exposure to Triton. With a very brief exposure to Triton (7 min centrifugation immediately after exposure), there is a significant loss of intensity in the feature centered at 1693 cm^{-1} (Figure 5, top trace), a characteristic amide I frequency for β-turn structures in BR (Krimm & Bandekar, 1986; Cladera et al., 1992). Since these secondary structures must be present in the BR N- and C-termini, or in the interhelical loops (Tuzi et al., 1994), the spectra suggest that Triton has a demonstrable effect on the BR surface structure, as well as an ability to dehydrate the membrane phospholipid headgroups (*cf.* Figure 3). There is also an increase in intensity for the modes at 1658 and 1542 cm^{-1} (Figure 4), which correspond to the E_1 species of the amide I and II modes, respectively, of the α_{II}-helices in BR (Dwivedi & Krimm, 1984). This symmetry species is polarized in the plane of the membrane, perpendicular to the α-helical axis (Dwivedi & Krimm, 1984). The A species of the amide I mode, which appears at 1667 cm^{-1} and whose transition moment is directed out of the membrane plane (Dwivedi & Krimm, 1984; Earnest et al., 1990), is not appreciably affected by this brief exposure to Triton. Previous studies of infrared difference spectra during the BR photocycle at −13 °C (where BR pumps protons) and at −33 °C (where BR does not pump protons) have correlated changes in the ratio of the two amide I bands near 1670 and 1658 cm^{-1} with distinct structural changes which occur during the photocycle (Ormos, 1991; Ormos et al., 1992; Perkins et al., 1992; Hessling et al., 1993; Vonck et al., 1994). Other studies have observed infrared bands in the 1675−1660 cm^{-1} region during the BR photocycle in difference spectra between BR and the M intermediate (Braiman et al., 1991; Ludlam et al., 1995), indicative of BR structural changes that occur during the photocycle. Therefore, the observed increase in the E_1 species of the α_{II}-helical amide modes probably reflects structural alterations which are related to the BR photocycle changes observed independently (Mukhopadhyay et al., 1994).

The infrared difference spectra also illustrate that although significant protein changes occur rapidly, other, more extensive structural changes occur after an extended exposure to Triton (*cf.* Figures 4 and 5). After 24 h, there was a greater loss in β-turn structure, noticeable in the loss in intensity of the amide II mode at 1563 cm^{-1} in addition to the broad amide I mode centered at 1695 cm^{-1} (Figure 4); the increases in intensity in the E_1 species of the amide I and II modes become more substantial. An intensity increase also at 1652 cm^{-1} (*cf.* Figure 5) results in a slight decrease in the frequency of the amide I mode toward typical frequencies calculated for α_I-helices (Krimm & Bandekar, 1986). These data suggest that extended Triton exposures convert some fraction of the α_{II}-helical structure to α_I-helices. For this conversion, the protein backbone dihedral angles would only have to change from $\Phi = -57.4°$, $\psi = -47.5°$ to $\Phi = -70.5°$, $\psi = -35.8°$ (Dwivedi & Krimm, 1984). Comparison of the relative intensities of the difference spectra in Figure 5 with the original (unsubtracted) data indicates that approximately 6% of the protein conformation is perturbed upon initial exposure to Triton and approximately 10% of BR conformation is perturbed upon prolonged (24 h) exposure to 0.05% Triton.

In summary, the protein conformational changes which occurred upon brief exposure to Triton are 2-fold. First, a significant degree of β-turn structure was lost, indicating disruption in the interhelical regions. Simultaneously, there was an increase in intensity in protein skeletal modes which have been shown to be polarized perpendicular to the α-helical axis (Dwivedi & Krimm, 1984; Earnest et al., 1990). With prolonged exposure, these changes were more extensive and lead to a loss in α_{II}-helical character with an increase in α_I-helical structure.

Role of Specific Purple Membrane Lipids in BR Function. BR photocycle changes can be induced by changes in the purple membrane lipid environment. Addition of small molecules (*e.g.*, alcohols or anesthetics) shortens the lifetime of the M intermediate (Nishimura et al., 1985; Henry et al., 1988; Fukuda & Kouyama, 1992b), while exposure to surfactants (*e.g.*, Tween-20 or Triton X-100) lengthens the M intermediate lifetime (Fukuda et al., 1990) and generates new, longer-lived M intermediates (Mukhopadhyay et al., 1994). From these observations, roles for the purple membrane lipids in the BR photocycle have been proposed (Milder et al., 1991; Fukuda & Kouyama, 1992a; Mukhopadhyay et al., 1994).

Partial retinal isomerization, a blue shift in the absorbance maximum, and some bleaching are observed when purple membrane is exposed to marginally lytic concentrations of Triton (González-Mañas et al., 1990). Fukuda et al. (1990) mentioned a greater degree of glycolipid release in studies with Tween-20, but they did not measure the effect of Tween-20 on the squalenes. Our results on sublytic Triton exposures, combined with the conclusions from the chemical analyses (Dracheva et al., 1996), indicate that while 0.10% Triton solutions immediately released approximately 7% of the phospholipids, a significantly greater fraction of the glycolipids and squalenes (which are present in near stoichiometric amounts to BR) was released. It is these changes in the purple membrane lipid environment that most likely are responsible for the changes in the BR photocycle, namely, the loss of sensitivity to incident light levels in the mediation of the M_f/M_s ratio, the generation of long-lived M intermediates, and the loss of the M_f decay pathway through the O intermediate, as observed by Mukhopadhyay et al. (1994). Our present study also demonstrates a disruption of lipid headgroup hydrogen bonding at the purple membrane surface (*cf.* Figure 3) as well as BR conformational changes arising from these alterations in lipid constitution (*cf.* Figures 4 and 5). These results clearly indicate the important role that the lipid environment and, in particular, the glycolipids and squalenes play in BR function.

Perturbation of BR and/or the lipid environment within each protein trimer aggregate has been shown to affect the BR photocycle. In particular, CH_2I_2 binding at the BR−lipid interface inside each trimer is accompanied by a decrease in the M intermediate decay time (Nakagawa et al., 1994). However, no description of the lipid distribution inside or outside of each trimer has been made. Most of the purple membrane lipids are in contact with at least one BR (Glaeser et al., 1985), with the glycolipids being present exclusively on the extracellular surface (Henderson et al., 1978; Jonas et al., 1990). The majority of the lipids on the exterior surface of each trimer must be composed of PGP and PGS, since they are the only lipids necessary for the growth of purple membrane sheets (Sternberg et al., 1992). Based upon models for lipid distribution proposed earlier (Glaeser et al., 1985; Jonas et al., 1990), the glycolipids (*i.e.*, GLS) are more prevalent within each BR trimer compared with the lipid composition between trimers. Since perturbations of the interior of each trimer can affect the BR photocycle (Nakagawa et al., 1994), and since the glycolipids, which are more prevalent within each trimer, are predominantly affected by purple membrane exposure to Triton, it is likely that the lipid environment *inside* each BR trimer is critical to normal BR function, and it is the perturbation of this environment that leads to profound photocycle changes observed upon exposure to sublytic concentrations of Triton.

Lipid Reconstitution Studies. Reconstitution of BR into purple membrane lipids has been used to study the thermodynamics of BR refolding (Popot et al., 1987). We performed preliminary studies on the reconstitution of these lipids into Triton-exposed purple membrane in an attempt to reverse the changes induced by Triton (*e.g.*, long-lived M intermediates, loss of the M_f decay pathway through the O intermediate, loss of α_{II}-helical and β-turn structure in BR, and disruption of lipid headgroup hydrogen bonding at the purple membrane surface). Reconstitution was performed with 0.5, 1.0, 2.0, 4.0, and 10.0 mol equiv/BR of purple membrane lipids where 1.0 mol equiv/BR is defined as the quantity of lipids associated with each BR [*i.e.*, 10 lipid molecules as defined by Glaeser et al. (1985)].

The effect of lipid reconstitution on Triton-exposed purple membrane is clarified by comparing the infrared spectra of purple membrane films before and after reconstitution. Figure 6 shows the infrared absorption spectra in the 1710−1400 cm^{-1} spectral regions for native purple membrane, purple membrane briefly exposed to Triton (0.10% Triton followed immediately by 20 min centrifugation), and purple membrane exposed to Triton after reconstitution with 1.0 and 10.0 mol equiv/BR of lipid, all normalized to the amide I peak height. After exposure to Triton, there was a 4.4 cm^{-1} decrease in the full width at half-height (FWHH) of the amide I mode, a slight decrease in intensity of the amide II mode, and a decrease in intensity of the broad band absorbing near 1460 cm^{-1}. The latter feature corresponds primarily to the

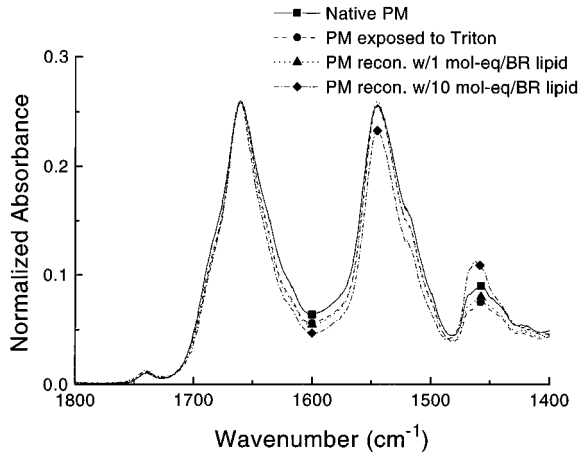

FIGURE 6: Infrared absorbance spectra from 1710 to 1400 cm^{-1} of native purple membrane (solid line), Triton-exposed purple membrane (dashed line), and Triton exposed purple membrane reconstituted with 1.0 (dotted line) and 10.0 mol equiv/BR of purple membrane lipids (dash−dot line). All spectra are normalized to the height of the amide I mode.

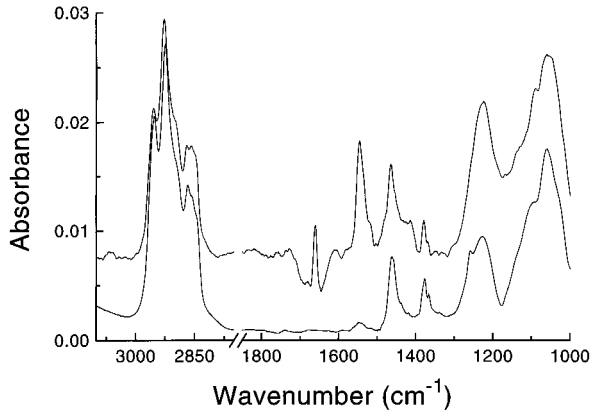

FIGURE 7: Infrared difference spectrum from 3100 to 2800 cm^{-1} and from 1800 to 1000 cm^{-1} of purple membrane exposed briefly to 0.10% Triton subtracted from purple membrane reconstituted with 1.0 mol equiv of purple membrane lipids after brief exposure to Triton (top) and infrared absorbance spectrum of purple membrane total lipids (bottom, film cast from $CHCl_3$ solution).

$\delta_{as}(CH_3)$ and $\delta(CH_2)$ modes of the purple membrane lipids and can be taken as an approximate measure of the lipid concentration within the bilayer. Reconstitution with 1.0 mol equiv/BR increased the lipid concentration to near the level in native purple membrane (as determined from the intensity of the modes at 1460 cm^{-1}); the spectra show a further decrease in the FWHH of the amide I mode and a slight decrease in intensity of the amide II mode. Upon reconstitution with 10.0 mol equiv/BR, the FWHH of the amide I mode became narrower with a greater decrease in intensity of the amide II mode and a significant increase in the $\delta_{as}(CH_3)$ and $\delta(CH_2)$ modes indicating extensive lipid reconstitution.

To identify the conformational changes due primarily to reconstitution, difference spectra were recorded by subtracting the infrared spectrum of a purple membrane sample exposed briefly to Triton from the spectrum of the same sample after reconstitution. The infrared difference spectrum from the sample reconstituted with 1.0 mol equiv/BR of lipid is shown in Figure 7 (top trace) with the infrared absorbance spectrum of the total lipids extracted from purple membrane ($CHCl_3$ extract, bottom trace). The two spectra are quite similar in the C−H stretching region (3100−2800 cm^{-1}) and in the region below 1500 cm^{-1} (composed primarily of lipid vibrational modes), indicating that the reinsertion of lipids into the bilayer was approximately stoichiometric with the purple membrane lipid concentration. However, the difference spectrum has slightly greater intensity in the symmetric methylene stretching ($\nu_s[CH_2]$) band at 2857 cm^{-1}, and the lipid modes at 2953 ($\nu_{as}[CH_3]$), 2926 ($\nu_{as}[CH_2]$), and 2868 ($\nu_s[CH_3]$) cm^{-1} are shifted 1−4 cm^{-1} from their frequencies in the spectrum of the purple membrane total lipids.

Table 3: Infrared Spectral Parameters for Reconstitution of Purple Membrane

lipid addition[a]	integrated intensity ratio		full width at half-height amide I[c]
	CH/amide I[b,c]	amide I/amide II[c,d]	
native[e]	0.83±0.03	1.14 ± 0.01	48.4 ± 2.3
T[f]	0.64	1.12	44.0
0.5	0.77	1.10	43.2
1.0	0.78	1.11	43.4
2.0	0.98	1.11	43.4
4.0	1.10	1.16	42.3
10.0	1.93	1.33	39.2
1.0[g]	0.82	1.14	43.7

[a] Quantity of lipid added in mole equivalents per BR. [b] C−H stretching region: 3100−2800 cm^{-1}. [c] Amide I region: 1720−1600 cm^{-1}. [d] Amide II region: 1600−1480 cm^{-1}. [e] Values for native purple membrane with error limits. [f] Sample exposed to Triton but not reconstituted with purple membrane lipids. [g] Sample dialyzed after reconstitution.

Most of the changes in the infrared spectrum upon lipid reconstitution were related to the lipids although some changes in BR structure were observed (*cf.* Figure 7). Many of the important spectral parameters for reconstituted purple membrane samples, representative of structural features in purple membrane, are shown in Table 3. The ratio of the integrated intensity of the C−H stretching region (composed primarily of lipid modes) to the integrated intensity of the amide I mode is an indication of the amount of lipid present in the purple membrane bilayer, providing a measure of the degree of lipid reconstitution into the purple membrane bilayer. The loss of lipid immediately after exposure to Triton was evident since this ratio decreased from 0.83 to 0.64. This ratio increased to near 0.77 with the addition of 0.5 and 1.0 mol equiv/BR of lipid and became greater as the quantity of lipids used for reconstitution increases. The sample reconstituted with 10.0 mol equiv/BR, which showed a ratio 2.5 times greater than in native purple membrane, demonstrated extensive lipid reconstitution into the bilayer. Unfortunately, it was not possible to determine the exact nature of this reinsertion nor where in the bilayer these lipids reinsert. However, it is clear that the lipids added during reconstitution affect protein structure since BR did undergo some structural changes in the amide I and II regions (1720−1500 cm^{-1}; Figure 7, top trace).

The protein conformational changes resulting from reconstitution were examined by studying the integrated intensity ratios of the amide I region (1720−1600 cm^{-1}) to the amide II region (1600−1480 cm^{-1}) in Table 3; the peak height intensity ratio of the amide I and II modes generally showed the same pattern as the integrated intensity ratios. Upon exposure to Triton, there was an immediate decrease in this ratio (reflective of an increased intensity of the amide II mode relative to the amide I mode), and reconstitution with 1.0 mol equiv/BR further lowers this ratio. These alterations appear to be reflective of changes in both the amide I and

amide II intensities as the FWHH of the amide I mode decreases upon exposure to Triton and decreases further upon reconstitution. Accompanying this decrease, the height of the amide II mode decreases with a slight decrease in the FWHH. For all samples reconstituted with less than 4.0 mol equiv/BR of lipid, this ratio was approximately 4% lower than in native purple membrane. Reconstitution with excess lipid (*e.g.*, 10.0 mol equiv/BR) resulted in the least intense amide II mode and a slight increase in the frequency of the amide I mode (from 1659 cm^{-1} in native purple membrane to 1661 cm^{-1} in purple membrane reconstituted with 10.0 mol equiv/BR of lipid). These changes were observed to a lesser extent in the samples reconstituted with 4.0 mol equiv/BR of lipid, suggesting that in native purple membrane the abnormally high intensity of the amide II mode (relative to the amide I mode) and the abnormally high frequency of the amide I mode (1659 cm^{-1}) are not directly related. A recent study revealed that the relative dichroic ratios of the amide I and amide II modes may indicate the type of α-helix in membrane proteins, and that the dichroic ratio of these modes is highly dependent on α-helix length (Reisdorf & Krimm, 1995). Thus, the relative intensities of the amide I and II modes may prove important for assessing protein conformational changes, especially for integral membrane systems.

The FWHH of the amide I mode provides another description of BR conformation. This parameter decreased by approximately 9% after exposure to Triton. Part of this decrease was due to the loss of protein conformations that absorb at high frequency in the amide I region (*e.g.*, β-turns and α_{II}-helices), but there also appears to be a decreased FWHH in the remaining features which make up the amide I envelope, suggesting a decrease in the conformational flexibility of the transmembrane helices. Reconstitution with up to 2.0 mol equiv/BR resulted in a further narrowing of the amide I mode. The samples reconstituted with 4.0 and 10.0 mol equiv/BR of lipid showed progressively smaller FWHH values; the FWHH of the amide I mode for the sample reconstituted with 10 mol equiv/BR was 20% narrower than the amide I mode in native purple membrane.

Spectra of samples reconstituted with 1.0 mol equiv/BR of lipid, followed by dialysis against buffer for 18 h to remove any lipid or detergent loosely associated with the purple membrane bilayer, indicated partial recovery of native purple membrane structure. The values for the parameter relating the degree of lipid reconstitution (the ratio of the integrated intensity of the C−H stretching region to the integrated intensity of the amide I region) and the ratio of the integrated intensities of the amide I and II modes are approximately the same value as in purple membrane. However, there was no increase in the FWHH of the amide I mode.

Despite the reversals in most of the spectral parameters related to protein conformation, there were no observable changes in the BR photocycle kinetics after the reconstitution performed by incubating Triton-treated purple membrane with a mixture of lipids. This provides some insight into the important structural features of BR, and how they are reflected by the infrared spectrum of purple membrane. Changes in the parameters relating the degree of lipid reconstitution and the ratio of the amide I to amide II intensities, represented in Table 3, were recovered in one or more of the reconstituted samples. The only parameter which was not recovered (and which was made worse by reconstitution) was the FWHH of the amide I mode. This may be an important indication of a loss in conformational flexibility in the transmembrane α_{II}-helices of BR after exposure to Triton which was not recovered upon reconstitution with purple membrane lipids. Infrared and electron diffraction studies indicate changes occur in BR conformation during the photocycle (Dencher et al., 1989; Ormos, 1991); the loss of conformational flexibility could result in changes in the intermediate decay times and in the decay pathway during the photocycle.

CONCLUSIONS

The spectral data presented here demonstrate the critical role of the purple membrane lipid environment in mediating conformational changes in BR, an integral membrane protein. Exposure of purple membrane to sublytic concentrations of the surfactant Triton X-100, which leaves the BR protein trimer structure intact, resulted in several effects, namely, preferential release of purple membrane glycolipids and squalenes, an association of Triton with purple membrane, perturbations of specific headgroup interactions, and protein conformational changes within the purple membrane. These changes induced a disruption of lipid headgroup hydrogen bonding at the purple membrane surface, a loss in β-turn and α_{II}-helical structure in BR, and, as shown in other work, pronounced changes in the BR photocycle (Mukhopadhyay et al., 1994). The data indicate that the purple membrane glycolipids and squalenes appear to be crucial to the kinetics of the BR photocycle. Although preliminary reconstitution studies have shown that it is possible to reverse some of the infrared spectral parameters characteristic of structural changes induced by Triton exposure, no reversal of the photocycle kinetics has been attained at this point. From spectral data, it is clear that the lipids play an important role in mediating purple membrane structure and function and that bacteriorhodopsin provides an easily controlled system for investigating the effects of lipid perturbations on integral membrane protein reorganizations.

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