Importance of Specific Native Lipids in Controlling the Photocycle of Bacteriorhodopsin

Manoj K. Joshi, Swetlana Dracheva, Anup K. Mukhopadhyay, Salil Bose, and Richard W. Hendler*

Laboratory of Cell Biology, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, Maryland 20892-0510

Received April 28, 1998; Revised Manuscript Received July 20, 1998

ABSTRACT: Brief treatment of purple membrane (PM) with dilute detergent can cause major disruption of the BR photocycle without disrupting the trimer structure of BR [Mukhopadhyay et al. (1996) *Biochemistry 35*, 9245−9252]. Normal photocyle behavior can be recovered by incubating the damaged membranes with a total extract of the five types of native lipids present in PM. It is shown here that full restoration can also be obtained with combinations of squalene (SQ) and phosphatidyl glycerophosphate (PGP) which act synergistically. The addition of SQ to suboptimal levels of PGP induces complete reconstitution, principally by restoring the characteristics of the fast M intermediate, M_f (as defined in Mukhopadhyay et al. (1996) *Biochemistry 35*, 9245−9252). The addition of small amounts of PGP to SQ, which alone is ineffective, also induces full reconstituion. At very high levels, full reconstitution can be obtained with PGP alone. These results, in combination with earlier studies which implicate an acidic amino acid residue [Bose et al. (1997) *J. Phys. Chem. B 101*, 10584−10587], suggest that a crucial interaction between a particular amino acid residue and a SQ−PGP lipid complex may be essential for normal BR photocycle activity.

There are many published reports in which the purple membrane (PM)[1] of *Halobacteria* has been disrupted using detergents, and the resulting effects on the structure and/or proton-pumping ability have been studied. For the most part, these reports can be grouped in three categories. In the first category, either the secondary structure of bacteriorhodopsin (BR) or the 2-D hexagonal array structure after partial delipidation and after reconstitution with endogenous or exogenous lipids has been studied (*1*−*7*). No photocycle characteristics were examined in these studies. In the second category, single-wavelength photocycle kinetics and, in some cases, the structure have been studied in the delipidated membranes; however no reconstitution has been attempted (*8*−*13*). In the third category, extensively or completely delipidated BR was incorporated into liposomes made of exogenous or endogenous lipids, and then it was demonstrated that these BR-liposomes are capable of pumping protons (ref *14* and references therein, refs *15*−*17*). This functionality, however, requires neither the presence of endogenous lipids nor the normal trimer structure of BR.

The emphasis in the current studies and those published recently from this laboratory is on the importance of specific, intimate interactions between lipid components of the purple membrane (PM) and BR, which change the photocycle decay pathways and kinetics and the ability of light intensity to influence the photocycle. It was demonstrated that exposure of PM to 0.05% Triton X-100 for 1−2 min removed the influence of actinic light on the regulation of the relative proportion of fast and slow forms of the photocycle M intermediate, destroyed the $M_f \rightarrow O$ decay pathway, and created new very slow forms of M intermediate (*18*). The trimer structure of BR remained intact after the Triton treatment. By using a slightly higher Triton concentration (0.1%), it was shown that 25% of SQ, 20% of GLS, and 6% of PL were removed from the membrane (*19*). It was also shown that normal photocycle behavior could be restored by treating the Triton-damaged PM with an extract of PM lipids in the presence of 4 M NaCl. The role of lipids was further defined in Mukhopadhyay et al. (*20*) and Barnett et al. (*21*). Mukhopadhyay et al. described a set of quantifiable parameters that measured the degree of Triton-induced damage and restoration by lipids. It was shown that the degree of reconstitution was correlated with both NaCl concentration and lipid concentration, that the requirement for lipid was specific, and that profound changes in protein α-helical structure occurred during the Triton treatment. At the higher level of Triton (0.1% instead of 0.05%), ∼20% decrease in trimer structure occurred. The loss of α-helical structure was reversed by treatment of lipids alone, but recovery of trimer structure required high salt. Barnett et al. (1996), using FTIR, further refined the nature of perturbation to protein structure and reversal by lipids alone. In later studies, Barnett et al. showed that a high-salt environment was specifically required to restore α-helical conformational flexibility of BR (manuscript in preparation). The current work was designed to establish the importance of individual native lipids of PM in re-establishing normal photocycle behavior in Triton-damaged membranes.

[1] Abbreviations: BR, bacteriorhodopsin; PM, purple membrane; SVD, singular value decomposition; PL, phospholipid; PG, phosphatidyl glycerol; PGS, phosphatidyl glycerosulfate; PGP, phosphatydyl glycerophosphate (methyl ester); GLS, glycolipid sulfate; SQ, squalene; M_f, fast M photocycle intermediate; M_s, slow M photocycle intermediate.

S0006-2960(98)00965-9
Published on Web 09/25/1998

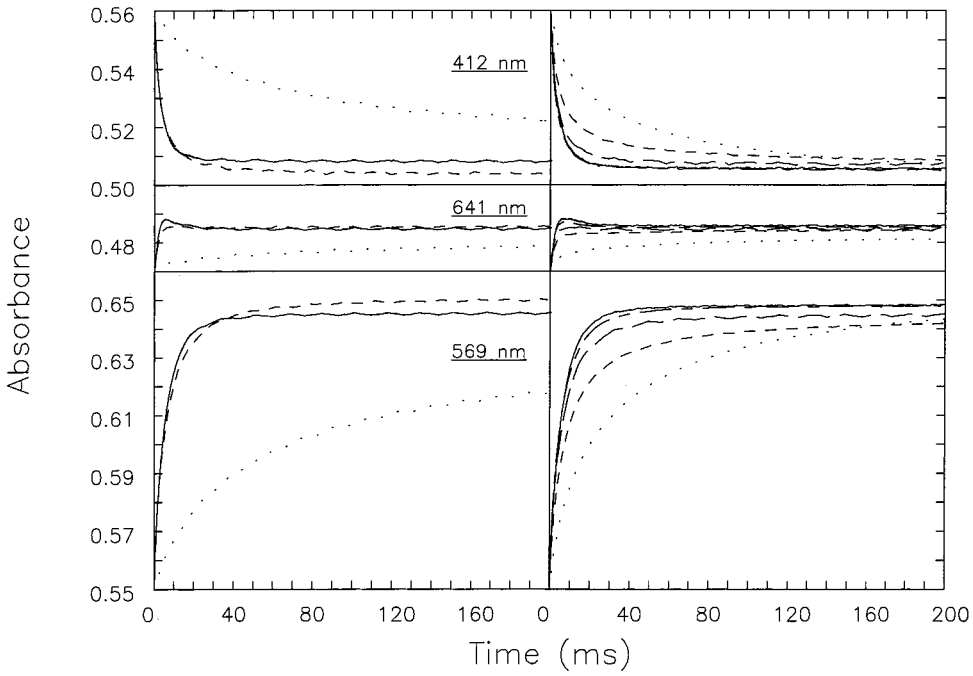

FIGURE 1: Kinetics for reconstitution of Triton-damaged PM measured at single wavelengths: 412 nm (M intermediate), 641 nm (O intermediate), and 569 nm (BR). (Left-side panels) Reconstitutions using unfractionated total PM lipid extract (solid lines), a mixture of all fractionated lipids (dashed line), and no added lipids (dotted line). (Right-side panels) Reconstitutions with mixtures lacking a single lipid type as follows: SQ (solid lines), GLS (alternating long and short dashed lines), PG (long dashed lines), PGS (short dashed lines), and PGP (dotted lines). The incubation medium was 3 mL of 50 mM potassium phosphate (pH 7.2) containing 500 μg of BR and amounts of lipid as described in Methods.

EXPERIMENTAL PROCEDURES

Isolation of PM and Extraction and Purification of Lipids. PM was isolated from *Halobacteria salinarium* ET100 by the procedure of Oesterhelt and Stockenius (*22*) modified as described in Mukhopadhyay et al. (*18*). Lipids were extracted from PM using the method of Bligh and Dyer (*23*) as described by Kates et al. (*24*). Fractionation of lipids was performed on silica columns and thin-layer chromatography as described by Kates (*25*). The identities of all lipids were based on chemical assays and Rf positions in comparison with those reported by Kates. Quantification of isolated lipid fractions was performed as follows: phospholipid was based on a molybdate assay using pyrolyzed samples (*26*); squalene was assayed using I_2-binding as described by Dracheva et al., (*19*); GLS was assayed using both a sulfate analysis based on chloranilate (*25*) and a carbohydrate analysis using phenol/sulfuric acid (*25*).

Triton Treatment of PM and Reconstitution with Isolated Lipids. The procedures of Mukhopadhyay et al. (*18*) are briefly described here. Two hundred microliters of 1% aqueous Triton X-100 were added to a suspension of 100 μL of PM containing 500 μg of BR and 1700 μL of 50 mM potassium phosphate (pH 7.2). The suspension was immediately centrifuged at 4 °C in a Beckman TL-100 ultracentrifuge using a TLA 100.3 rotor for 5 min at 200 000*g*. Allowing for formation of a vacuum, attaining speed, and stopping, the total time was 7 min. The pellet was washed by resuspending in 3 mL of H_2O and recentrifuging two times. Lipids used for reconstitution were dried under N_2 at room temperature and suspended in 500 μL of 50 mM potassium phosphate (pH 7.2) in 4 M NaCl. The preparation was then sonicated for three 10 s periods using a Tekmar Model TM-50 high-intensity ultrasonic processor with the output control set at ~35 and the power monitor at 25. The Triton-treated pellet (500 μg of BR) was suspended in the sonicated lipid suspension and incubated overnight in the dark at room temperature. The PM was then washed by two centrifugations in 50 mM phosphate (pH 7.2) and suspended to 3 mL of the same buffer for spectral kinetic measurements. The 1× level for lipids refers to the amount normally present in PM relative to BR. For 500 μg of BR, these are 168 μg of total lipids (including retinal), 7.9 μg of SQ, 7.9 μg of PG, 7.1 μg of PGS, 86.9 μg of PGP, and 49.2 μg of GLS.

Rapid Multichannel Spectral Kinetic Measurements and Data Analysis. The rapid-scan multichannel spectrometer and its operation were previously described (*27*, *28*). Data analysis using SVD was performed as previously described (*28*, *29*). The laser used for initiation of the photocycle was a Nd:YAG (Model Surelite-10, Continuum, Santa Clara, CA). Excitation employed the second harmonic at 532 nm in a 5 ns wide pulse. The high-intensity level of laser flash used was 6 mJ, and the low-intensity level was 0.03 mJ. These levels correspond to 80 and 4 units each using the photoconversion parameter (PCP) of Eisfeld et al. (*30*). Twenty-five spectra were averaged for each experiment using the high-intensity flash, and 400 were averaged for the low-intensity flash.

RESULTS

The restoration of native photocycle behavior to Triton-treated PM preparations by incubation with unfractionated PM lipids in the presence of high salt has been shown previously (*19*, *20*). In Figure 1 (left side), we show a similar recovery of normal photocycle characteristics by a mixture of lipids isolated from the PM lipid extract. The 0 for the time-course is taken at the peak of M formation following a

Table 1: Reconstitutions of Triton-Treated PM with PM Lipids (Omissions of Single Fractions)

	lipid fraction(s) added or omitted							
				omitted				
	none	total extract	all fractions	SQ	GLS	PG	PGS	PGP
ΔmOD[a] BR	68	96	100	98	98	95	92	93
ΔmOd[a] M	40	52	56	54	55	53	52	55
average τ (ms)	61	4.1	7.3	4.7	5.2	6.3	14	41
fraction M_f								
H[2] [b]	*c*	0.64	0.54	0.58	0.57	0.55	0.45	0.21
L[2] [b]		0.88	0.79	0.85	0.73	0.78	0.66	0.46
ratio L/H[b]		1.38	1.46	1.46	1.28	1.42	1.46	2.19

[a] ΔmOD is the maximum change in absorbance resulting from the laser flash expressed in milliunits of OD. [b] H and L refer to high- and low-intensity actinic laser flashes as described in the Experimental Procedures. [c] There is no true M_f as previously defined in Mukhopadhyay et al. (1996). The principal characteristics of true M_f are τ near 2 ms, a decay compensated by formation of O, and a mole fraction well above 0.1 which consistently increases with decreasing laser intensity.

Table 2: Reconstitutions of Triton-Treated PM with PM Lipids (Omissions of GLS and One Other Lipid Fraction)[a]

	lipid fraction(s) added or omitted							
					omitted GLS and			
	none	total extract	total (−) GLS	PGP	PG	PGS	SQ	PGP
ΔmOD BR	56	96	98	92	94	96	96	80
ΔmOD M	34	52	54	51	53	53	55	47
average τ (ms)	65	4.1	4.7	19	5.1	5.5	6.4	46
fraction M_f								
H		0.64	0.58	0.35	0.56	0.58	0.65	0.12
L		0.88	0.85	0.52	0.83	0.78	0.86	0.25
ratio L/H		1.38	1.46	1.48	1.48	1.34	1.32	2.08

[a] Refer to Table 1 for additional information.

laser flash. The changes in absorbance at the single wavelengths at 412, 641, and 569 nm reflect the time courses for decay of the M intermediates (top panels), the formation and decay of the O intermediate (middle panels), and the regeneration of the ground state, BR (bottom panels). In all cases where recovery of normal behavior is achieved, the final plateau level attained is the same as the dark level prior to laser light activation of the cycle.

The traces showing M decay and BR recovery are made up of a fast component (M_f) which accounts for the initial rapid phase and a slow component (M_s) accounting for the slower phase. Because the decay of M_f is always accompanied by a rise in the O intermediate, whereas the decay of M_s is not, the solid line in the middle panel shows an initial peak at ∼5 ms. Although all O decays back to BR, the curves shown in the middle panel do not decrease to 0. This is because the spectrum for the increasing BR contributes to the 641 nm absorbance. The Triton-treated samples (dotted lines) in these three panels show that the M_f component is completely absent. The addition of a combination of the fractionated PM lipids essentially restored all lost functions (short dashed lines). The lipids of *Halobacteria* differ significantly from those of other more common species in at least two major respects. The long-chain aliphatic moiety is derived from the highly branched phytanol rather than fatty acid, and the linkage to glycerol is by ether rather than ester bonds. Lipids make up 25% of the weight of PM, and BR accounts for the remaining 75%. There are five major lipid classes present, listed in order of decreasing mole percents (*19*, *24*): PGP (49%), GLS (22%), SQ (10%), PG (5%), and PGS (4%). In addition, there is retinal (12%) and traces of vitamin MK-8. The PGP is present as methyl phosphate ester. For general structural formulas see Kates et al. (*24*).

The panels on the right side of Figure 1 illustrate the effects of leaving out one of each of the five native lipid classes that make up the mixture. Whereas the omission of SQ, GLS, or PG had minimal effect, the omission of PGP (dotted lines) produced a profound diminution in restoration of lost photocycle behavior. A small decrease in reconstitution resulted when PGS was omitted. Additional quantitative measures of the effects of the treatments illustrated in Figure 1 are shown in Table 1. The amount of turnover of BR and M (rows 1 and 2) was decreased by exposure to Triton (col 1) and restored by the total unfractionated lipids (col 2) and a mixture of the isolated fractions (col 3). A useful parameter of native photocycle kinetics is the average τ, which is the sum of products of mole fraction and τ for each form of M intermediate that is present. The average τ for M turnover was lengthened by exposure to Triton and restored to normal levels by both the unfractionated and reconstituted total lipids. The mole fraction of M_f was regulated by laser light intensity in both of the lipid reconstitution cases (col 2 and 3), but not in the Triton-treated case. Consistent with the results shown in Figure 1, either SQ or GLS could be eliminated from the total lipid mixture with little overall effect. It is worth noting, however, that there is evidence for a close structural association of GLS with tryptophan and of tyrosine of BR (*31*). The only omission which markedly decreased the degree of restoration of normal behavior (especially for the loss of the fast M component, M_f) was PGP.

Next, we tested the effects of leaving out GLS plus each of one of the remaining four lipid classes (i.e. PG, PGS, SQ, PGP). The results are illustrated in Figure 2 and Table 2.

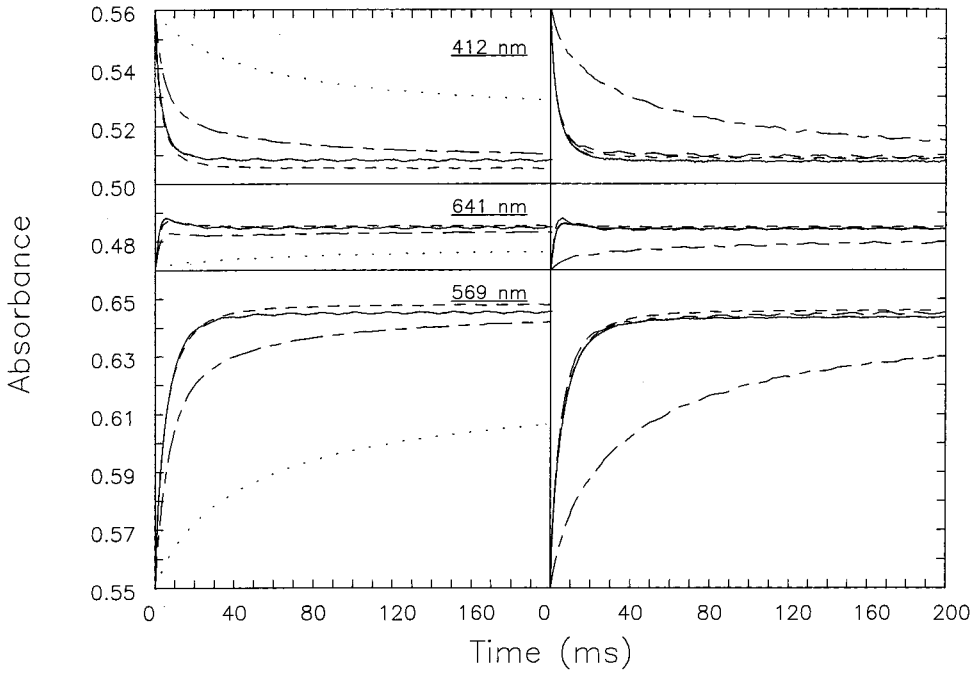

FIGURE 2: Reconstitutions using different lipid combinations. (Left-side panels) total lipids (solid lines), total lipids minus GLS (short dashed lines), PGP alone (alternating long and short dashed lines), no additions (dotted lines). (Right-side panels) Reconstitutions with total lipids minus GLS and one additional lipid as follows: PG (solid lines), PGS (short dashed lines), SQ (long dashed lines), and PGP (alternating long and short dashed lines). Additional information is in the legend to Figure 1 and in the text.

Table 3: Reconstitutions of Triton-Treated PM with PM Lipids (Comparisons of Lecithin and PGP)

	lipid fraction(s) added						
	PGP		all (−)		lecithin^M		lecithin^P
	+GLS	+SQ	(PG + PGS)	lecithin^M	+SQ	lecithin^P	+SQ
ΔmOD BR	95	98	93	70	76	84	62
ΔmOD M	53	55	52	41	43	49	37
average τ (ms)	28	5.3	6.2	62	15	32	21
fraction M_f							
H	0.27	0.57	0.54	0.09	0.45	0.30	0.44
L	0.24	0.77	0.72	0.12	0.62	0.48	0.65
ratio L/H	0.89	1.35	1.33	1.33	1.38	1.60	1.48

[a] Lecithin^M is equimolar to PGP; lecithin^P is equal in PO_4 to PGP.

The left-hand panels confirm that GLS omission does not decrease the reconstitution of photocycle behavior in Triton-treated PM and that PGP alone affords appreciable reconstitution. The right-hand panels show that, in addition to GLS, either PG, PGS, or SQ could also be eliminated without serious deleterious effects. Once again, it was seen that eliminating PGP caused a marked decrease in the recovery of lost photocycle behavior. Table 2 provides additional support for the picture seen in Figure 2 and confirms the loss of the fast M component by omitting PGP.

The above experiments show that the presence of three lipid classes alone can re-establish most of the photocycle characteristics lost by brief exposure to Triton, *provided that one of the three is PGP*. PGS and PG are minor lipid components, present at less than 1 molecule/BR monomer and less than 10% of the total phospholipid. When both PG and PGS were eliminated, good reconstitution was still obtained (Figure 3, left panels, long dashed line). Attention was then focused on trying to reduce the number of lipids to just PGP and either squalene or GLS. Figure 3 (left panels) shows partial reconstitution with the PGP + GLS mixture that is comparable to the results obtained with PGP alone (compare dotted lines in left and right-hand panels and data column 1, Table 3 with data column 4, Table 2). Complete restoration was obtained with the PGP + squalene mixture. Further documentation for these experiments is provided in Table 3.

The question of PL specificity was addressed by the substitution of egg lecithin for native PGP. The tests were conducted in two different contexts. In one case, an equimolar amount of lecithin to PGP was used. Because PGP has two phosphates per molecule, this condition provided only $^1/_2$ the amount of phosphate. The other condition provided an equimolar concentration of phosphate which required a double molar amount of phospholipid. Figure 3 (right-hand side, short dashed line) shows that although equimolar lecithin provided for some reconstitution, it was far less effective than PGP (dotted line). At the double molar concentration, lecithin was still less effective than native PGP. It is also shown that lecithin potentiated the ability of SQ to support reconstitution and that SQ did alter the effects of lecithin by enhancing the relative amount of M_f characteristics. These conclusions are supported further by the quantitative parameters shown in Table 3.

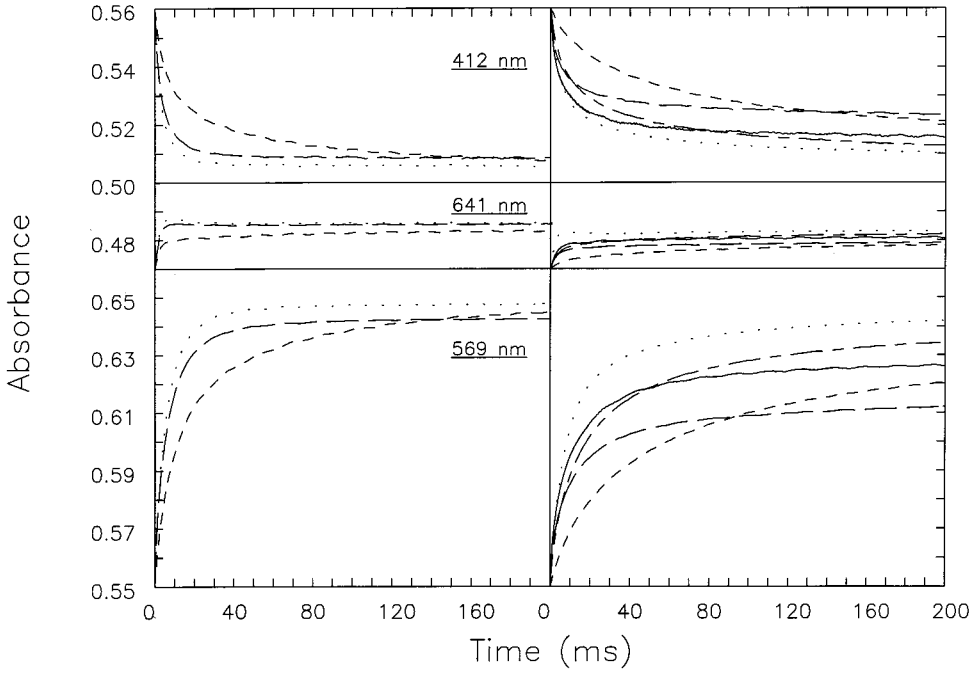

FIGURE 3: Additional reconstitutions using different lipid combinations. (Left-side panels) PGP and SQ (dotted lines), total lipids minus PG and PGS (long dashed lines), and PGP plus GLS (short dashed lines). (Right-side panels) PGP alone (dotted lines), Lecithin, equimolar to PGP (short dashed lines), Lecithin, equimolar to PGP, plus SQ (solid lines), Lecithin, equimolar to PO_4 in PGP (alternating long and short dashed lines), and Lecithin, equimolar to PO_4 in PGP, plus SQ (long dashed lines).

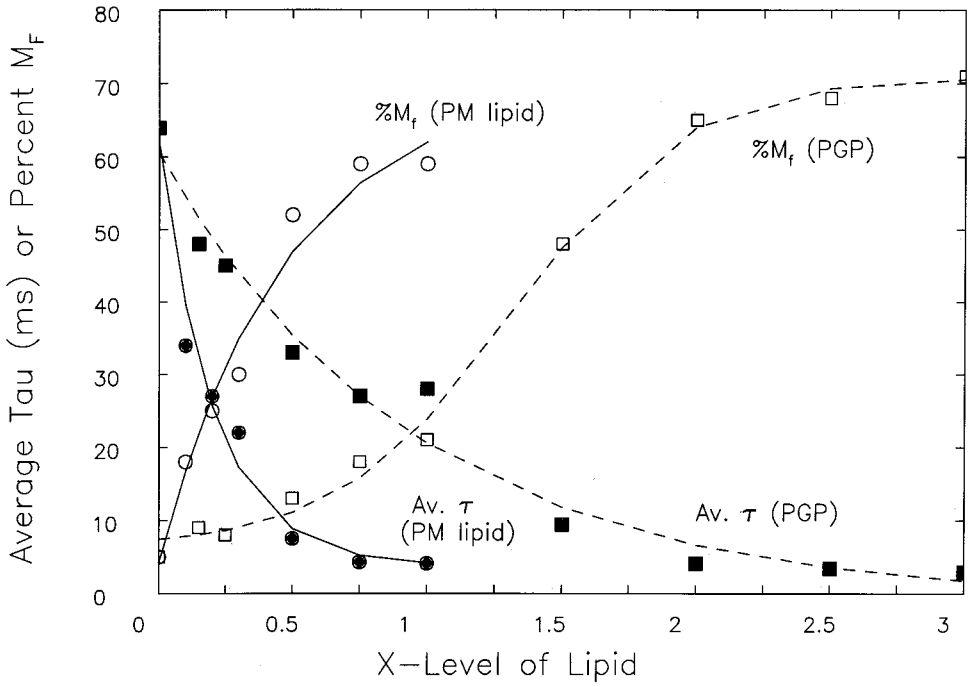

FIGURE 4: Comparison of reconstitutions using either total PM lipid extract (solid lines and circle points) or PGP (dashed lines and square points). Two quantitative parameters of normal photocycle behavior are used: average tau (solid points) and M_f as a percent of total M (open points). See text for further details.

Although PGP alone can re-establish a significant amount of lost photocycle behavior, it was not as effective as the combination of PGP and SQ (compare dotted lines in left and right panels in Figure 3). In fact, it was seen that the addition of SQ to PGP mostly affected the reappearance of M_f characteristics as evidenced by the rapid initial phases for M decay and BR recovery and for the $M_f \rightarrow O$ pathway indicated by the early rise in the trace at 641 nm for the O intermediate. Further support for the improvement in reconstitution by the addition of SQ to PGP is seen by comparing data column 2 in Table 3 with data column 4 in Table 2. In similar experiments where GLS, instead of SQ, was added to a 1× level of PGP, no enhancement in reconstitution was obtained (data not shown).

The results described above demonstrated that the single most important added lipid for reconstitution is PGP, but for complete restoration, SQ is required. All of the above-described experiments were conducted at the 1× level of lipids, defined as the amount of lipid normally present in PM relative to BR. If an excess of PGP is added, additional

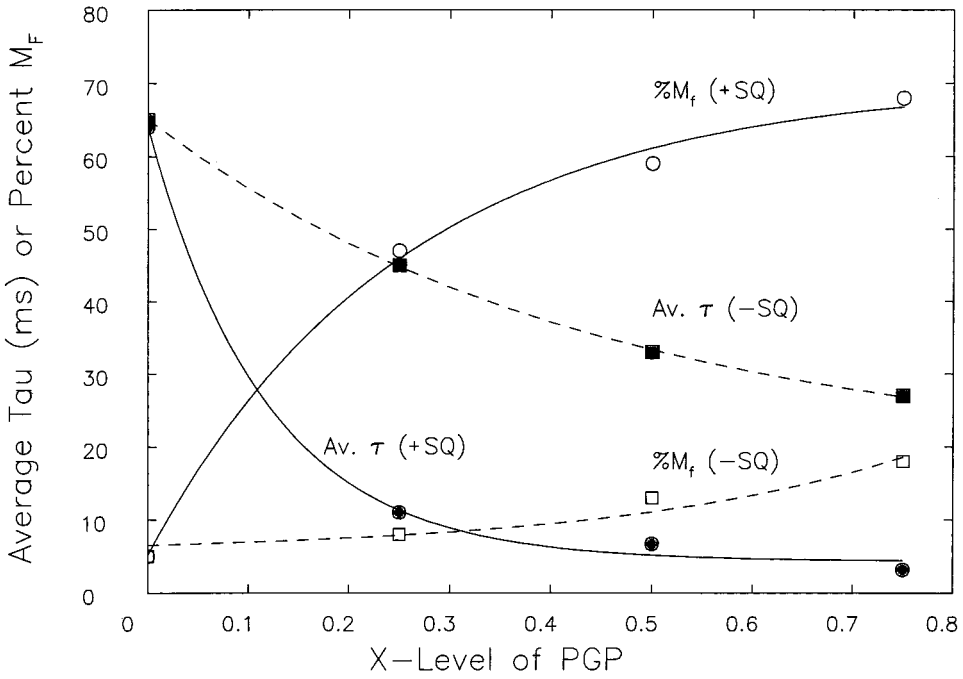

FIGURE 5: Influence of SQ on reconstitutions with PGP. Reconstitutions in absence of SQ (dashed lines and square points), in the presence of 1.5× SQ (solid lines and circle points), average tau (solid points), percent M_F (open points).

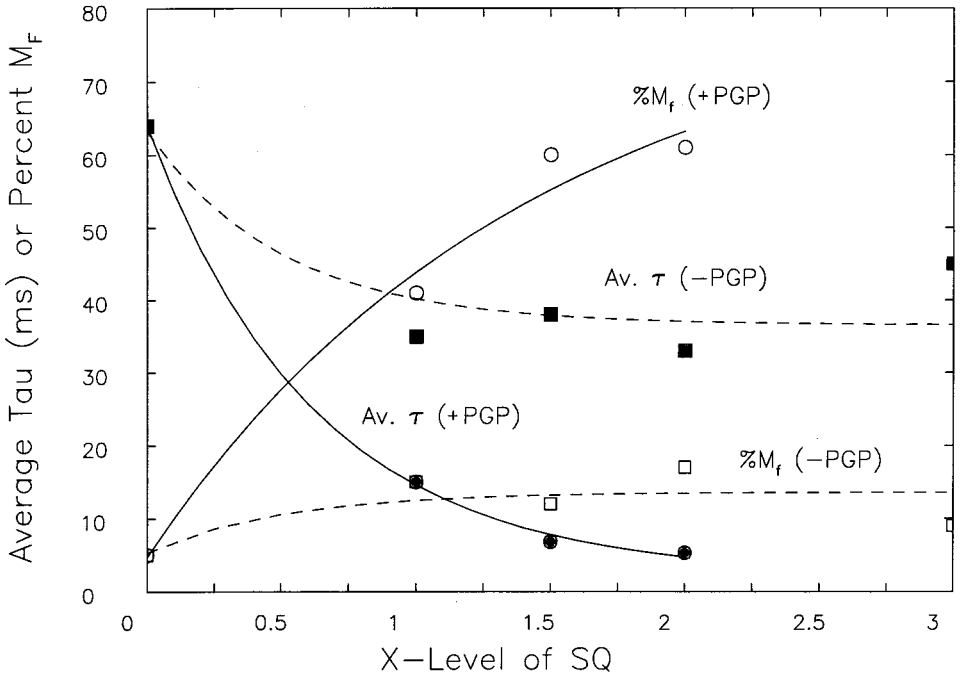

FIGURE 6: Influence of PGP on reconstitutions with SQ. Reconstitutions in absence of PGP (dashed lines and square points), in the presence of 0.15× PGP (solid lines and circle points), average tau (solid points), percent M_F (open points).

SQ is no longer required. Figure 4 shows the recovery of two important parameters of normal photocycle behavior, average τ and fraction of M_f. Complete recovery to normal values for these parameters is obtained with a 1× amount of total PM lipid extract (solid lines) or with a 2× level of PGP alone (dashed lines). The reconstitution with PGP shows a sigmoidal shape which suggests the possibility that more than a single molecule of PGP is involved in the process. The effectiveness of SQ at $<$1× levels of PGP is shown in Figure 5. A 1.5× level of SQ markedly improves reconstitution with suboptimal levels of PGP (solid lines compared to dashed lines). It is also interesting that complete reconstitution can be achieved with SQ alone provided that a 0.15× level of PGP is present (Figure 6, solid lines compared to dashed lines).

DISCUSSION

In contrast to earlier studies (see Introduction), the disruptions of the photocycle that we have probed occur with minimum delipidation of the membrane and while the trimer structure remains intact. They involve the ability of laser light to regulate the levels of M_f and M_s in the photocycle, the alteration of photocycle pathways (loss of $M_f \rightarrow O$), the marked slowdown in overall kinetics, and a decrease in the amount of BR turnover.

There are five distinct lipids present in PM. Per molecule of BR, there are approximately 5 PGP, 2 GLS, 1 SQ, 0.5 PG, and 0.5 PGS molecules (*19*, *25*). High-resolution electron microscopy of normal and deoxycholate-treated PM with a resolution of 2.6 Å shows an intimate juxtaposition of membrane lipids with the BR trimer (*32*). The image was further refined using electron diffraction data (*33*) to reveal opportunities for specific lipid−protein interactions.

The extraction of PM with 0.1% Triton for 2 min removes ∼25% of the SQ, ∼20% of GLS, and ∼6% of the phospholipids (*19*). Restoration of lost BR photocycle behavior can be obtained with an amount of extracted lipid equal to that originally present in the membrane. It is interesting that although most of the original lipid is still present after brief treatment of PM with dilute Triton, the full reconstitution of photocycle behavior requires the addition of an amount of lipid equal to the full normal amount.

By a series of gradual lipid omissions and the testing of different combinations, it was found that full reconstitution can be obtained with 1× levels of just two lipids, PGP and SQ. The most effective single lipid is PGP, but reconstitution obtained with 1× PGP is markedly lacking in the fast kinetic form of M. Addition of 1× SQ preferentially restores the M_f component. Higher levels of PGP alone or SQ in the presence of a small amount of PGP can restore full activity. Suboptimal levels of either PGP or SQ greatly stimulate the ability of the other in achieving full reconstitution. The requirement for lipids shows specificity both with respect to the individual native lipid components of PM and with respect to Archeobacterial lipids (*20*). We do not believe that the ability to restore to full activity with a high level of PGP alone is indicative of a lack of specificity. It is more likely that the additional PGP plays a part in the mobilization of the nonextracted lipids (principally SQ) so that they can reform important structural associations with BR. Our finding that SQ appears to be crucial for the appearance of the M_f species is consistent with the conclusion that a structural basis for BR cycle heterogeneity exists and depends on specific lipid−protein associations (*34*). The potential importance of SQ in PM integrity and BR function was indicated in very early studies where it was concluded that SQ might be involved in Mg^{2+}- or Ca^{2+}-mediated PL−protein binding (*35*).

A further indication of discrete lipid−BR interaction necessary for normal photocycle activity and control is the observation that, in order to obtain reconstitution, charge−charge repulsion must be overcome by either high salt or titration of a group with an apparent p*K* near 5 (*36*). This p*K* is too high for a lipid phosphate, and we suspect that it could be in an acidic amino acid residue of BR. In future studies, we hope to test this possibility and to identify a particular acidic amino acid residue as the partner for interaction with the negatively charged PGP by use of site-directed mutants.

ACKNOWLEDGMENT

The authors appreciate the contribution of Latisse Mays in the growth of *H. salinarium*, isolation of BR and PM lipids, and the performance of certain chemical assays.

REFERENCES

1. Michel, H., Oesterhelt, D., and Henderson, R. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 338−342.
2. Popot, J.-L., Gerchman, S.-E., and Engelman, D. (1987) *J. Mol. Biol. 198*, 655−676.
3. Sternberg, B., L′Hostis, C., Whiteway, C. A., and Watts, A. (1992) *Biochim. Biophys. Acta 1108*, 21−30.
4. Sternberg, B., Watts, A., and Cejka, Z. (1993) *J. Struct. Biol. 110*, 196−204.
5. Mitra, A. K., Miercke, L. J. W., Turner, G. J., Shand, R. F., Betlach, M. C., and Stroud, R. M. (1993) *Biophys. J. 65*, 1295−1306.
6. Swords, N. A., and Wallace, B. A. (1993) *Biochem. J. 289*, 215−219.
7. Polovnikova, O. Yu., Simonova, T. N., Tarakhovsky, Yu. S., Nikonova, T. N., Sukhanov, S. V., Dubachev, G. E., Borovyagin, V. L., Vasilenko, I. A., and Barsukov, L. I. (1995) *Membrane and Cell Biol. 9*, 259−278.
8. Szundi, I., and Stoeckenius, W. (1987) *Proc. Natl. Acad. Sci. U.S.A. 84*, 3681−3684.
9. Hartsel, S. C., and Cassim, J. Y. (1988) *Biochemistry 27*, 3720−3724.
10. Jang, D.-J., and El-Sayed, M. A. (1988) *Proc. Natl. Acad. Sci. U.S.A. 85*, 5918−5922.
11. Fukada, K., Ikegami, A., Nasuda-Kouyama, A., and Kouyama, T. (1990) *Biochemistry 29*, 1997−2002.
12. Uraga, T., Hamanaka, T., Wakabayashi, K., and Ameniya, Y. (1990) *J. Biochem. 108*, 938−946.
13. Milder, S. J., Thorgeirsson, T. E., Miercke, L. J. W., Stroud, R. M., and Kliger, D. S. (1991) *Biochemistry 30*, 1751−1761.
14. Huang, K.-S., Bayley, H., and Khorana, H. G. (1980) *Proc. Natl. Acad. Sci. U.S.A. 77*, 323−327.
15. Lind, C., Hojeberg, B., and Khorana, H. G. (1981) *J. Biol. Chem. 256*, 8298−8305.
16. Rigaud, J.-L., Paternostre, M.-T., and Bluzat, A. (1988) *Biochemistry 27*, 2677−2688.
17. Szundi, I., and Stoeckenius, W. (1988) *Biophys. J. 54*, 227−232.
18. Mukhopadhyay, A. K., Bose, S., and Hendler, R. W. (1994) *Biochemistry 33*, 10889−10895.
19. Dracheva, S., Bose, S., and Hendler, R. W. (1996) *FEBS Lett. 382*, 209−212.
20. Mukhopadhyay, A. K., Dracheva, S., Bose, S., and Hendler, R. W. (1996) *Biochemistry 35*, 9245−9252.
21. Barnett, S. M., Dracheva, S., Hendler, R. W., and Levin, I. W. (1996) *Biochemistry 35*, 4558−4567.
22. Oesterhelt, D., and Stoeckenius, W. (1974) *Methods Enzymol. 31*, 667−679.
23. Bligh, E. G., and Dyer, W. J. (1959) *Can. J. Biochem. Physiol. 37*, 911−917.
24. Kates, M., Kushwaha, S. C., and Sprott, G. D. (1982) in *Methods in Enzymology* (Packer, L., Ed.) Vol. 88, pp 98−193, Academic Press, New York.
25. Kates, M. (1986) *Techniques of lipidology: isolation, analysis, and identification of lipids*, Elsevier Press, Amsterdam, New York.
26. Rouser, G., Slakotos, A. N., and Fleisher, S. (1966) *Lipids 1*, 85−86.
27. Cole, J. W., Hendler, R. W., Smith, P. D., Fredrickson, H. A., Pohida, T. J., and Friauf, W. S. (1997) *J. Biochem. Biophys. Methods 35*, 161−174.
28. Hendler, R. W., Dancshazy, Zs., Bose, S., Shrager, R. I., and Tokaji, Zs. (1994) *Biochemistry 33*, 4604−4610.
29. Hendler, R. W., and Shrager, R. I. (1994) *J. Biochem. Biophys. Methods 28*, 1−33.
30. Eisfeld, W., Pusch, C., Diller, R., Lohrmann, R., and Stockburger, M. (1993) *Biochemistry 32*, 7196−7215.
31. Weik, M., Patzelt, H., Zaccai, G., and Oesterhelt, D. (1998) *Mol. Cell 1*, 411−419.
32. Grigorieff, N., Beckmann, E., and Zemlin, F. (1995) *J. Mol. Biol. 254*, 404−415.
33. Grigorieff, N., Ceska, T. A., Downing, K. H., Baldwin, J. M., and Henderson, R. (1996) *J. Mol. Biol. 259*, 393−421.

34. Shrager, R. I., Hendler, R. W., and Bose, S. (1995) *Eur. J. Biochem. 229*, 589−595.
35. Lanyi, J. K., Plachy, W. Z., and Kates, M. (1974) *Biochemistry 13*, 4914−4920.
36. Bose, S., Mukhopadhyay, A. K., Dracheva, S., and Hendler, R. W. (1997) *J. Phys. Chem. B 101*, 10584−10587.

BI980965J