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Regeneration of bacteriorhodopsin in mixed micelles

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Regeneration of bacteriorhodopsin from bacterioopsin and all-*trans*-retinal was studied in a mixed micelle system consisting of dodecyl sulfate, CHAPS and a water-soluble phospholipid dihexanoylphosphatidylcholine (hex_2-PhosChol). Regeneration to approximately 40000 $M^{-1} \cdot cm^{-1}$ extinction at 550 nm (ε_{550}) was obtained with either 2.3 mM or 6.5 mM CHAPS along with 6.9 mM dodecyl sulfate and 4.5 mM hex_2-PhosChol in 0.16 M NaCl and 40 mM phosphate (pH 6.0). Without CHAPS, the regeneration in 4.5 mM Hex_2-PhosChol gave $\varepsilon_{555} = 27800$; without PhosChol, the 1:3 CHAPS/dodecyl sulfate mixture gave $\varepsilon_{550} \approx 20000$; and without PhosChol the nearly equimolar CHAPS/dodecyl sulfate mixture gave $\varepsilon_{550} \approx 10000$. The composition of the mixed micelles was estimated from fluorescence spectroscopy using pyrene butyryl hydrazine. The molecular weight was estimated by molecular seive chromatography to be 87100 for 2.3 mM CHAPS, 6.9 mM dodecyl sulfate and 0.67 mM hex_2-PhosChol; and 83200 for 7.0 mM CHAPS, 6.9 mM dodecyl sulfate, and 1.1 mM hex_2-PhosChol. These results are consistent with the idea that at low concentrations of CHAPS and dodecyl sulfate, CHAPS organizes the dodecyl sulfate into disk shaped bilayer micelles that are favorable for bacterioopsin refolding. However, a high concentration of either detergent inhibits regeneration. Added hex_2-PhosChol can overcome the inhibitory effects of high concentrations of either CHAPS or dodecyl sulfate.

Regeneration of bacteriorhodopsin from bacterioopsin and all-*trans*-retinal occurs in solution with bile salt-based detergents combined with phospholipids [1] or with Triton X-100 [2]. These micelles have some limitations for physical studies, such as gel permeation chromatography or NMR spectroscopy, due to their large size. For example, the CHAPS/myr_2-PhosChol/bacteriorhodopsin micelle has a molecular weight of 210000 [3]; and the Triton X-100/bacteriorhodopsin micelle has a molecular weight of about 130000 [4]. Furthermore, the amphiphiles are difficult to exchange with other detergents: the bile salt/lipid system because of the insolubility of PhosChol; and Triton X-100 because of its low critical micelle concentration (cmc). These limitations prompted us to examine the possibility of regenerating bacteriorhodopsin in small micelles that also would be easily dialyzable.

Short-chain PhosChols are water-soluble and have been extensively characterized. Hex_2-PhosChol has a cmc in the 10 mM range and a micelle molecular weight of around 15000 [5,6], making it a promising candidate for regeneration. In this paper, we report our studies of regeneration of bacteriorhodopsin in mixed CHAPS/hex_2-PhosChol/dodecyl sulfate micelles.

Experimental methods

Materials

Purple membrane was isolated from *Halobacterium halobium* S9 by the method of Oesterhelt and Stoeckenius [7]. Lipid-free bacterioopsin was obtained from purple membrane by precipitation from acetone/NH_3 as described previously [8]. The dried precipitate (50 nmol) was resolubilized in 88% formic acid (0.1 ml), diluted with water (1.0 ml), and reprecipitated with concentrated ammonium hydroxide (0.3 ml). The precipitate was collected by centrifugation and then washed with 2 M Tris (pH 8), followed by deionized water. The final pellet was solubilized in 34.7 mM (1%) dodecyl sulfate (0.5 ml).

Abbreviations: CHAPS, 3-(cholamidopropyl)dimethyl[aminonio]-1-propane sulfonate; myr_2-PhosChol, dimyristylphosphatidylcholine; cmc, critical micelle concentration; hex_2-PhosChol, dihexanoylphosphatidylcholine.

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Detergents and lipids were obtained from the following sources: CHAPS: Calbiochem (La Jolla, CA) or Sigma (St. Louis, MO); myr_2-PhosChol: Calbiochem; hex_2PhosChol: Avanti (Birmingham, AL) or Sigma; and sodium dodecyl sulfate: Bio-Rad (Richmond, CA). All-*trans*-retinal was obtained from Sigma. 1-Pyrene butyryl hydrazine and other pyrene derivatives were obtained from Molecular Probes (Eugene, OR).

Methods

Bacteriorhodopsin was regenerated after a 5-fold dilution of bacterioopsin (solubilized in 34.7 mM dodecyl sulfate) with buffer containing 50 mM sodium phosphate (pH 6.0) and the appropriate detergent and lipid. All-*trans*-retinal (7 mM in ethanol) was added in slight molar excess over bacterioopsin and the solutions were stored in the dark for 18–24 h. Absorbance spectra were measured on an Aviv/Cary 14 spectrophotometer. Critical micelle concentrations were measured by a method similar to that of Chattopadhyay and London [9]. Fluorescence spectra of pyrene butyryl hydrazine in detergent solutions were measured on a Farrand MKI spectrofluorimeter. Gel permeation chromatography was performed on a Pharmacia FPLC, using two 1 × 30 cm superose 12 columns in tandem.

Results

Regeneration of bacteriorhodopsin in hex_2-PhosChol

High concentrations of hex_2-PhosChol regenerated bacteriorhodopsin from all-*trans*-retinal and dodecylsulfate solubilized bacterioopsin in 40 mM phosphate (pH 6.0), either in 2.4 M NaCl (Fig. 1, inverted triangles) or in water (not shown). Regeneration was observed in hex_2-PhosChol mixed with 6.9 mM (0.2%) dodecyl sulfate only at concentrations of the PhosChol above its cmc, despite the fact that the cmc for dodecyl sulfate in 2.4 M NaCl is less than 1 mM. The cmc of hex_2-PhosChol is 15.5 mM (6.9 mg/ml) in water and 2.5 mM (1.1 mg/ml) in 3 M NaCl [5]. We found that in 2.4 M NaCl, 4.5 mM hex_2-PhosChol, the absorbance maximum of the regenerated bacteriorhodopsin was at 555 nm with the molar extinction coefficient, ε_{555}, equal to 27800. In 16.8 mM hex_2-PhosChol without added NaCl, the absorbance maximum of regenerated bacteriorhodopsin was at 550 nm, with $\varepsilon_{550} = 31400$.

Mixed micelles containing hex_2-PhosChol, dodecyl sulfate, and CHAPS gave regeneration similar to that previously reported for similar mixed micelles containing myr_2-PhosChol [2,10], with values of ε_{550} close to 40000. Our result was obtained in a completely soluble detergent/PhosChol system with 6.5 times less PhosChol and 3.5 times less CHAPS than used by Liao et al. [10]. All of the pigments regenerated in micelles containing CHAPS had absorbance maxima at 550 nm. The regenerated pigment was stable for weeks in the mixed micelles containing short chain PhosChol. The variation of the amount of regeneration with hex_2-PhosChol concentration is shown in Fig. 1. In 6.5 mM (0.4%) CHAPS, 6.9 mM dodecyl sulfate (Fig. 1, triangles), not much regeneration was observed in the absence of lipids. However, addition of increasing amounts of hex_2-PhosChol gave increasing regeneration, up to 4.5 mM. Similar results were obtained with the same detergent and lipid concentrations with added 0.16 M NaCl, although higher regeneration was obtained at lower PhosChol concentrations (Fig. 1, diamonds). By contrast, in 2.3 mM (0.14% CHAPS, 6.9 mM dodecyl sulfate (Fig. 1, circles and squares), substantially more regeneration was obtained with no added lipids. The regeneration was approximately doubled by addition of 4.5 mM hex_2-PhosChol. Little difference in the effect of hex_2-PhosChol was observed between regeneration in 2.4 M (Fig. 1, squares) and 0.16 M NaCl (Fig. 1, circles) at the lower concentration of CHAPS. The regeneration at low concentrations of CHAPS depended on the amount of dodecyl sulfate. Not much regeneration was observed with 2.8 mM CHAPS, 0.19 M NaCl at a dodecyl sulfate concentration of 1.7 mM ($\varepsilon_{550} = 6300$).

The difference in regeneration between low and high CHAPS concentrations suggests that CHAPS inhibits

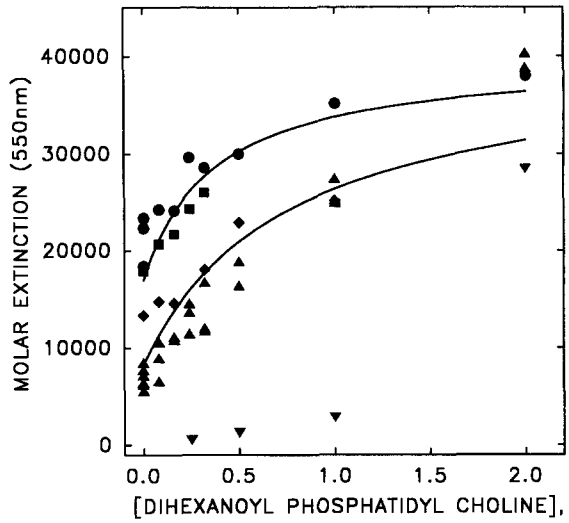

Fig. 1. Variation of bacteriorhodopsin regeneration with hex_2-PhosChol concentration. Absorbance was measured at 550 nm (except inverted triangles, which were measured at 555 nm) 18 to 24 h after regeneration (see Methods). Protein concentration was calculated from the absorbance of bacterioopsin at 280 nm in dodecyl sulfate [10]. Molar extinction of the regenerated pigment was calculated by dividing the 550 nm (or 555 nm) absorbance by the protein concentration. All samples were in 40 mM phosphate buffer (pH 6.0), 22–24 °C. Circles: 2.3 mM CHAPS, 6.9 mM dodecyl sulfate, 0.16 M NaCl. Squares: 2.3 mM CHAPS, 6.9 mM dodecyl sulfate, 2.4 M NaCl. Triangles, 6.5 mM CHAPS, 6.9 mM dodecy sulfate. Diamonds, 6.5 mM CHAPS, 6.9 mM dodecyl sulfate, 0.16 M NaCl. Inverted triangles: 6.9 mM dodecyl sulfate, 2.4 mM NaCl. Lines were calculated from Eqn. 12, assuming that maximum regeneration gives $\varepsilon_{559} =$ 40000, $K_h = 0.35$ mM, and $K_c' = 1.7$ mM.

the regeneration of bacteriorhodopsin. However, this inhibition appears to be overcome by high concentrations of hex_2-PhosChol. This is most likely due to an inhibition of folding or a destabilization of bacteriorhodopsin by CHAPS, and a parallel promotion of folding or a stabilization of bacteriorhodopsin by hex_2-PhosChol. The effect is complex, because hex_2-PhosChol alone in dodecyl sulfate does not show a simple promotion of regeneration.

Characterization of mixed micelles containing dodecyl sulfate and CHAPS

The regeneration results in Fig. 1 suggested a significant difference between mixed micelles formed with 2.3 mM CHAPS and 6.5 mM CHAPS, and also indicated an important role for dodecyl sulfate. We used a fluorescence assay to probe the properties of these mixed micelles. We found that the fluorescence of pyrene butyryl hydrazine appears to be a suitable probe of micelle formation in dodecyl sulfate and CHAPS (Fig. 2), similar to 1,6-diphenyl-1,3,5-hexatriene [9] and phenyl-1-naphthylamine [11]. Other pyrene derivatives (hydrazine, sulfonic, acid, carboxylic acid, butyric acid) were less sensitive. Both detergents enhanced the pyrene butyryl hydrazine fluorescence sharply above the cmc. The observed break in fluorescence occurred near the literature values for the cmc of dodecyl sulfate and CHAPS [9,11]. The fluorescence signal appeared to become saturated as the concentration of micelles increased. We interpret this saturation effect as evidence of the equilibrium between pyrene butyryl hydrazine in the micelle phase and in the aqueous phase. The quantum yield of the probe in dodecyl sulfate is lower than in CHAPS micelles by a factor of 2.1. The graphs of fluorescence against total detergent concentration (Fig. 2) indicate that the cmc of dodecyl sulfate in 0.16 M NaCl is 0.8 mM, and the cmc of CHAPS in 0.16 M NaCl is 5.8 mM. The apparent dissociation constants of pyrene butyryl hydrazine from micelles (in concentration units of detergent monomer in micelles) is 0.3 mM for dodecyl sulfate and 1 mM for CHAPS.

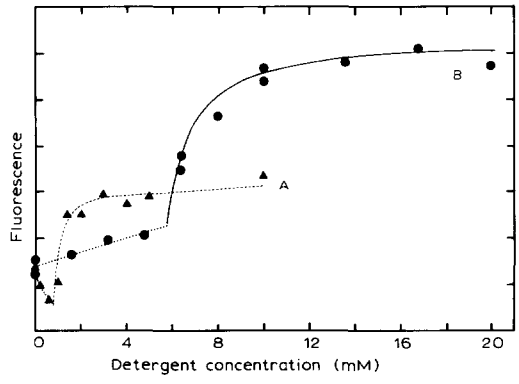

Fig. 2. Measurement of critical micelle concentration of detergents. Concentrations of dodecyl sulfate (A) or CHAPS (B) in 0.16 M NaCl were varied. The fluorescence of pyrene butyryl hydrazine (1 μM, added from a 0.1 mM stock solution in ethanol) was measured at 377 nm (excitation at 340 nm). Both A and B are scaled to the same arbitrary fluorescence units. Dotted lines are used to estimate CMC [9,11]. Solid line is calculated for a dissociation constant of pyrene butyryl hydrazine from CHAPS micelles of 1 mM. Dashed line is calculated for a dissociation constant of pyrene butyryl hydrazine from dodecyl sulfate micelles of 0.3 mM. Temperature = 22° C.

The composition of mixed CHAPS/dodecyl sulfate micelles may be estimated from the probe fluorescence as follows. The fluorescence quantum yield of probe in mixed micelles, Q, consists of separate aqueous and micelle components. We assume that the fluorescence of the micelle component is proportional to the mole fraction of CHAPS in micelles, X_{cm}, times the probe quantum yield in pure CHAPS micelles, Q_c, plus the mole fraction of dodecyl sulfate in micelles, X_{sm}, times the probe quantum yield in pure dodecyl sulfate micelles, Q_s. Thus, the fluorescence signal is:

$$Q = \{[P]Q_w + [PM](X_{cm}Q_c + X_{sm}Q_s)\}/([P] + [PM]) \quad (1)$$

where [P] is the concentration probe in the aqueous phase, Q_w is the corresponding quantum yield, and [PM] is the concentration of probe in micelles. We assume (considering Fig. 2) that the dissociation of probe from mixed micelles is a simple equilibrium, described by a single constant K′:

$$nK' = [M][P]/[PM] \quad (2)$$

where n is the micelle aggregation number and [M] is the concentration of monomer in micelles lacking the probe. Eqn. 2 is valid at low probe concentrations. The total probe concentration is 1 μM, whereas the detergent concentrations are in the mM range. Therefore, [M] is approximately equal to $S_m + C_m$, respectively, the concentrations of CHAPS and dodecyl sulfate monomers in micelles. Then Eqn. 1 may be written:

$$Q = (KQ_w + c_mQ_c + s_mQ_s)/(K + C_m + s_m) \quad (3)$$

where $K = nK'$. Assuming ideality of mixing in micelles [12],

$$X_{sw} = X_{sm}cmc_s' \quad (4)$$

$$X_{cw} = X_{cm}cmc_c' \quad (5)$$

where X_{sw} and X_{cw} are, respectively, the mole fractions of dodecyl sulfate monomer and CHAPS monomer in the aqueous phase, and cmc_s' and cmc_c' are, respectively, the cmc of dodecyl sulfate of CHAPS alone (in mole fraction units). Since $X_{sm} + X_{cm} = 1$,

$$X_{sw}/cmc_s' + X_{cw}/cmc_c' = 1$$

Thus, in molar concentration units,

$$S_w/cmc_s + C_w/cmc_c = 1 \quad (6)$$

where S_w and C_w are, respectively, the concentrations of dodecyl sulfate or CHAPS in the aqueous phase. Combining Eqns. 3 and 6, the micelle concentrations are:

$$C_m = \frac{S_T - K(F_w - F)/(F - F_s) - cmc_s + C_T cmc_s/cmc_c}{(F_c - F)/(F - F_s) + cmc_s/cmc_c} \quad (7)$$

$$S_m = [K(F_w - F) + C_m(F_c - F)]/(F - F_s) \quad (8)$$

where $S_T = S_w + S_m$ and $C_T = C_w + C_m$, and F, F_w, F_s, and F_c are fluorescence intensities, in arbitrary units, proportional to Q, Q_w, Q_s, and Q_c, respectively.

The fluorescence of pyrene butyryl hydrazine in mixed micelles is shown in Fig. 3. We have used these results and Eqns. 7 and 8 to estimate the concentrations of dodecyl sulfate and CHAPS in the micelles. K was assumed to be 0.3 mM, the measured apparent dissociation constant for the probe from dodecyl sulfate micelles (Fig. 2). This value is a reasonable approximation since the mixed micelles being used appear to have a considerable excess of dodecyl sulfate over CHAPS (see below). The solutions to Eqns. 7 and 8 were constrained by the requirement that concentrations could not be negative, nor could micellar detergent concentrations exceed total detergent concentrations. These boundaries were exceeded for three points out of eight. A negative concentration was taken to be 0 (one point), and excessively high micellar concentrations of dodecyl sulfate were set equal to the total concentration of dodecyl sulfate (2 points). The modified values of S_m or C_m satisfied Eqn. 3 to within 1%, except for the zero point, which was approx. 11% high.

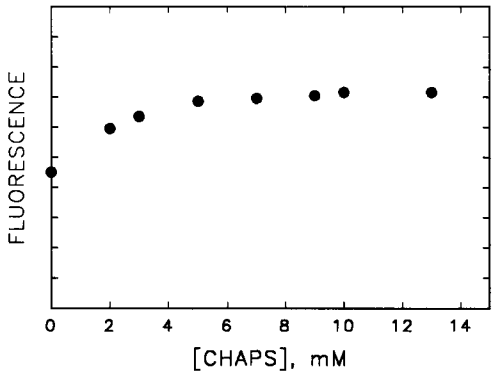

Fig. 3. Fluorescence of pyrene butyryl hydrazine in mixed micelles. Similar to Fig. 2 except samples were mixed micelles: 6.9 mM dodecyl sulfate, varying CHAPS. Fluorescence was used to calculate the micellar concentrations of dodecyl sulfate and CHAPs in mixed micelles from Eqns. 7 and 8.

TABLE I

Calculated compositions of mixed dodecyl sulfate/CHAPS micelles

Calculated from Eqns. 7 and 8 and Figs. 2 and 3 (see text). $S_T = 6.9$ mM, $K = 0.3$ mM, $cmc_s = 0.8$ mM, $cmc_c = 5.8$ mM. On the fluorescence scale of Fig. 3 (F varied from 90 to 143), $F_w = 48$, $F_s = 103$ and $F_c = 220$.

C_T (mM)	S_m (mM)	C_m (mM)
0	6.10	0
2	6.21	1.19
3	6.26	1.87
5	6.38	2.94
7	6.61	3.28
9	6.84	3.64
10	6.90	3.97
13	6.90	4.17

The calculated micelle compositions are shown in Table I. Interpolation for 2.3 mM total CHAPS, 6.9 mM total dodecyl sulfate, gives a micelle ratio S_m/C_m of 4.6. The 6.9 mM dodecyl sulfate and 7 mM CHAPS results give a micelle ratio S_m/C_m of 2.0. Thus, doubling the micellar dodecyl sulfate to CHAPS ratio appears to enhance regeneration of the bacteriorhodopsin chromophore in the absence of lipids (Fig. 1) by a factor of two.

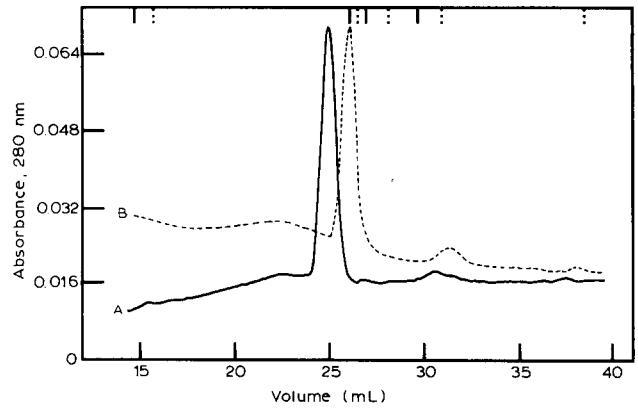

Fig. 4. Gel permeation chromatography of regenerated bacteriorhodopsin. Elution profiles of bacteriorhodopsin regenerated in: 2.3 mM CHAPS, 6.9 mM dodecyl sulfate, 0.16 M NaCl, 0.72 mM hex_2-PhosChol, 40 mM phosphate (pH 6.0) (A) or 7 mM CHAPS, 6.9 mM dodecyl sulfate, 1.1 mM hex_2-PhosChol, 0.16 M NaCl, 40 mM phosphate (pH 6.0) (B). Samples were chromatographed in elution buffer that was the same as regeneration buffer except it contained 0.02% NaN_3. Column: two tandem superose 12 columns, 1×30 cm each. Flow rate, 0.5 ml/min. Vertical marks at top of figure are peak elution positions of calibration molecules. Solid lines refer to A and dashed lines refer to B. From left for A: blue dextran, catalase monomer (60000), ovalbumin (43000), and horse myoglobin (18800); for B: blue dextran, bovine serum albumin (68000), ovalbumin, myoglobin, and $K_3Fe(CN)_6$. The major 280 nm peaks for both A and B were the only pink-colored fractions eluting from the column. For B, A_{280}/A_{550} of the major peak was 3.1.

Molecular sieve chromatography of regenerated bacteriorhodopsin in CHAPS and dodecyl sulfate

Regenerated bacteriorhodopsin was studied by molecular sieve chromatography on crosslinked dextran (Superose 12). In 2.3 mM CHAPS, 6.9 mM dodecyl sulfate, 0.67 mM hex_2-PhosChol, and 0.16 M NaCl, bacteriorhodopsin migrated as a homogeneous band with a relative molecular weight of 87100 (Fig. 4A). In 7 mM CHAPS, 6.9 mM dodecyl sulfate, 1 mM hex_2-PhosChol, 0.16 M NaCl, bacteriorhodopsin migrated as a homogeneous band with a relative molecular weight of 83200 (Fig. 4B).

Discussion

Previous studies of bacteriorhodopsin regeneration in mixed micelles showed that no regeneration was obtained in dodecyl sulfate/myr_2-PhosChol in the absence of bile salt-based detergents; and inhibition of regeneration was observed at high concentrations of cholate [1]. We have extended these results by investigating regeneration of bacteriorhodopsin in the water-soluble phospholipid, hex_2-PhosChol. We find that with dodecyl sulfate/hex_2PhosChol mixed micelles, regeneration is observed above the cmc of the PhosChol. Regeneration comparable to the dodecyl sulfate/CHAPS/myr_2-PhosChol system of Liao et al. [10] was obtained in 4.5 mM hex_2-PhosChol along with either 6.9 mM dodecyl sulfate and 2.3 mM CHAPS, or 6.9 mM dodecyl sulfate and 6.5 mM CHAPS (Fig. 1). We also observe regeneration in mixed micelles of CHAPS (2.3 mM) and dodecyl sulfate (6.9 mM) in the absence of PhosChol. However, higher CHAPS concentrations (6.5 mM) with dodecyl sulfate (6.9 mM) do not support much regeneration in the absence of PhosChol.

We studied the dodecyl sulfate/CHAPS micelle system by fluorescence spectroscopy, using pyrene butyryl hydrazine as a probe. Both detergents show sharp breaks in the probe fluorescence quantum yields at the cmc, with a 2-fold quantum yield difference above the cmc (Fig. 2). We have used these properties to estimate the composition of the dodecyl sulfate/CHAPS mixed micelle, based on some simplifying assumptions. Probe fluorescence in mixed micelles (Fig. 3) indicates that the micellar ratio of dodecyl sulfate to CHAPS is 4.6 (Fig. 4) when the total detergent concentrations are 6.9 mM and 2.3 mM, respectively; and the micellar ratio of dodecyl sulfate to CHAPS is 2.0 when the detergent concentrations are 6.9 mM and 7.0 mM, respectively (Table I). Micelle size was measured by gel permeation chromatography of regenerated bacteriorhodopsin. In 6.9 mM dodecyl sulfate, 2.3 mM CHAPS and 0.67 mM hex_2-PhosChol bacteriorhodopsin elutes at a relative molecular weight of 87100 (Fig. 4A). Thus, the detergent component of the micelle has a molecular weight of 87100–27100 = 60000, if there is one bacteriorhodopsin per micelle, or 32900 if there are two bacteriorhodopsins. Because of the small mole fraction of total PhosChol under these conditions, it is unlikely to significantly influence the micelle composition. We assume the PhosChol is represented in the micelle to the same extent as its total mole fraction, and that the detergent components have the same composition estimated from Eqns. 7 and 8. Thus, the micelle appears to contain an average of 128 dodecyl sulfate molecules, 27 CHAPS, 14 hex_2-PhosChol, and one bacteriorhodopsin, or 70 dodecyl sulfate molecules, 15 CHAPS, 6 hex_2-PhosChol, and two bacteriorhodopsins.

Gel permeation chromatography of regenerated bacteriorhodopsin in 6.9 mM dodecyl sulfate, 7 mM CHAPS, and 1.1 mM hex_2-PhosChol, gives a relative molecular weight for the bacteriorhodopsin micelle of 83200 (Fig. 4B). Thus, the detergent component of the micelle has a molecular weight of 56100 if there is one bacteriorhodopsin per micelle, or 29100 if there are two bacteriorhodopsins per micelle. Using the same assumptions as above, the micelle composition is 84 molecules of dodecyl sulfate, 42 CHAPS, 14 hex_2-PhosChol, and one bacteriorhodopsin, or 43 dodecyl sulfate molecules, 22 CHAPS, 7 hex_2-PhosChol, and two bacteriorhodopsins.

The 3.5 × 2.5 nm ellipsoidal bacteriorhodopsin molecule has a circumference of about 9.5 nm in the plane of the purple membrane, whereas two bacteriorhodopsin molecules in a closely packed dimer would have a circumference of approximately 17 nm. In both types of mixed micelles (i.e., either low (2.3 mM) or high (7 mM) CHAPS) there appears to be only one bacteriorhodopsin per micelle, since the number of CHAPS molecules is insufficient to surround two bacteriorhodopsin molecules in a bilayer (assuming a molecular width of 0.80 nm for CHAPS and a disk-shaped micelle edged by CHAPS [13]).

The difference in extent of regeneration in low and high CHAPS is striking. Micelle size cannot explain the difference, since the estimated molecular weights (Fig. 4) are similar. The micelle compositions for both sets of conditions (Figs. 3 and 4, and Table I) indicate aggregation numbers in the expected range for dodecyl sulfate micelles. Since bacteriorhodopsin cannot be regenerated in dodecyl sulfate alone, it seems likely that CHAPS alters the organization of dodecyl sulfate around bacteriorhodopsin. For example, CHAPS may convert a globular dodecyl sulfate micelle into a disk-shaped bilayer micelle (of the type originally proposed by Small [13] for mixtures of bile salts and PhosChol). There are enough hydrocarbon chains from the dodecyl sulfate (and hex_2-PhosChol, if present) to form two or three double layer rings around the bacteriorhodopsin, with the CHAPS coating the edges.

However, this does not explain why higher amounts of CHAPS inhibit bacteriorhodopsin regeneration. The

results in Fig. 1 show that hex_2-PhosChol has a stabilizing effect on bacteriorhodopsin, suggesting a very simple (but speculative) competitive inhibition model. The model postulates that there are, in addition to the non-specific protein-micelle interactions, two specific lipid/detergent binding sites, one on the bacterioopsin (BO) surface, and the other on the bacteriorhodopsin (BR) surface. The first may be occupied by either CHAPS (C) or retinal (R) and the second by hex_2-PhosChol (H). Binding of CHAPS to the first site blocks retinal binding, whereas hex_2-PhosChol binding to the second site stabilizes regenerated bacteriorhodopsin (BR):

$$BR \rightleftharpoons BO + R \quad (9)$$

$$BOC \rightleftharpoons BO + C \quad (10)$$

$$BRP \rightleftharpoons BR + H \quad (11)$$

All of the protein concentration terms refer to the protein-micelle complex, assuming one bacteriorhodopsin per micelle. The free detergent or lipid terms in Eqns. 9–11 refer to monomers. The respective dissociation constants for Eqns. 9–11 are K_r, K_c, and K_h. Thus, the fraction of regenerated bacteriorhodopsin, X_r, is given by:

$$X_r = (1 + [H]/K_h)/(1 + [H]/K_h + [C]/K_c') \quad (12)$$

where $K_c' = K_c[R]/K_r$. The solid lines in Fig. 1 are obtained from Eqn. 12, with $K_h = 0.35$ mM and $K_c' = 1.7$ mM.

The model does not explain the results obtained in the absence of CHAPS, suggesting that the stabilizing effect of hex_2-PhosChol may require a particular micelle structure. The present data cannot distinguish between whole-micelle effects and specific binding sites in the stabilization (Eqn. 11) and destabilization (Eqn. 9) postulated in the above model. If the competition is at a specific site, further studies might provide information about the mechanism of retinal binding to bacteriorhodopsin. If the effects are exerted through the whole micelle, the mixed micelles described in this paper may be useful in studies of the folding and stability of membrane proteins.

Acknowledgments

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