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Direct reconstitution of bacteriorhodopsin into planar phospholipid bilayers — detergent effect

Mineo Ikematsu [a,*], Yukihiro Sugiyama [a], Masahiro Iseki [a], Eiro Muneyuki [b], Atsuo Mizukami [a]

[a] *Tsukuba Research Center, Sanyo Electric Co. Ltd., Tsukuba, Ibaraki 305, Japan*

[b] *Research Laboratory of Resources Utilization, Tokyo Institute of Technology, 4259 Nagatsuta, Midori-ku, Yokohama, Kanagawa 227, Japan*

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Abstract

This paper describes how the structure and concentration level of a detergent used for substitution after bacteriorhodopsin (bR) solubilization affect the reconstitution of the bR into phospholipid planar bilayers. A direct insertion method was used for the bR reconstitution into the bilayers. Two detergents representing the two major types were used: sodium deoxycholate with a cholane-ring structure, and octylglucoside with a linear (or chain) structure. We then characterized the reconstitution for the two detergents by considering the detergent separation profiles and the photocurrent variations upon addition of lanthanum chloride and the protonophore FCCP (carbonylcyanide-*p*-trifluoromethoxyphenylhydrazone). We found that for successful transmembrane reconstitution of bR the detergent with the cholane-ring structure was preferable to that with the linear structure when the detergent concentration was above its critical micellar concentration. This preference was explained by the ease with which the detergent with the cholane-ring structure was removed from protein compared to that with the linear structure. Finally, we proposed a scheme for the reconstitution of the protein.

Keywords: Bacteriorhodopsin; Phospholipid bilayer; Reconstitution; Detergent; Solubilization; Substitution

1. Introduction

To characterize membrane proteins, it is necessary to purify the proteins and reconstitute them into lipid bilayers. Reconstitution in a transmembraneous manner is particularly interesting because it is found in intrinsic biological membranes. Two approaches are being used in studies now in progress: one approach looks at proteoliposomes and the other at planar lipid bilayers. In proteoliposome studies [1–3], there are several advantages: high reproducibility, relatively simple experimental procedures, and high sensitivity of measurements when combined with the use of radio isotopes. However, it is rather difficult to establish a constant membrane potential or an ionic concentration gradient across the membrane, and to obtain satisfactory time resolution. In contrast, in planar lipid bilayer studies, it is quite easy to

* Corresponding author.

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control the membrane potential and the solution composition on both sides of the membrane [4,5]. However, the disadvantage in this system is that transmembraneous reconstitution of proteins is difficult.

Extensive work has been carried out to establish the transmembraneous reconstitution of membrane proteins into lipid bilayers, specifically reconstitution involving ion pumps [6–9]. In previous research we also have succeeded in the transmembraneous reconstitution of light-activated proton-pump bacteriorhodopsin (bR) extracted from the halophilic bacteria *Halobacterium halobium* [10–12]. In that work we found that to achieve the transmembraneous reconstitution of bR, it was important to maintain temperature control followed by perfusion of the bR solution. However, because it is still unclear how detergents used for substitution after the bR solubilization affect the bR reconstitution, no precise criteria have been established to determine which detergent should be used for the substitution. Concerning bR solubilization, it was recently inferred that a relationship exists between a detergent's structure and its function in the solubilization process [13].

In this paper, we describe how detergents used for substitution after solubilization affect bR reconstitution. Specifically, we discuss the structure–function and the concentration–function relationships for a detergent in reconstitution by looking at the two major types of detergent: sodium deoxycholate (Sodium DOC or SDOC) as a detergent with a cholane-ring structure, and octylglucoside (OG) as that with a linear- or chain-structure. After reconstituting bR, we measured the photoresponse (photoelectric current) of the resulting membrane under different treatment conditions to characterize the reconstitution. We then put forth a possible scheme for the reconstitution mechanism.

2. Materials and methods

We first solubilized bR with Triton X-100 using gel filtration. We then reconstituted the bR into planar bilayers using a Teflon chamber system. After the reconstitution, we measured the photoresponse of the resulting membrane under different treatment conditions to characterize the reconstitution.

2.1. Solubilization and gel filtration

Soybean lecithin was purchased from Sigma (type IV-S), and used without further purification.

Purple membrane sheets were isolated from *Halobacterium halobium* strain ET-1001 according to the method described by Oesterhelt and Stoeckenius [14]. Purple membranes were solubilized with Triton X-100 and applied to gel filtration [15]. A purple membrane pellet containing 25 μg of the bR was homogenized with 1.3 ml of 5% Triton X-100 in a 0.1 M sodium acetate buffer (pH 5.0) and gently stirred at room temperature in the dark for three days. After that, the solution was centrifuged at $100\,000 \times g$ for 30 min at 4°C. The supernatant (i.e., the solubilized bR) was collected and then applied on a column of Bio-Gel A-05m (2.5 cm × 100 cm) pre-equilibrated with a buffer containing 0.025% NaN_3, 10 mM Tris–HCl (pH 8.0), and a detergent. The concentration of detergent was varied: for SDOC, the levels used were 0.08, 0.15, and 0.25% (wt/v); and for OG, 0.25, 0.5, and 1.0%. The column was eluted with the same buffer solution at a flow-rate of 5 ml/h at 4°C in the dark. Separation of Triton X-100 from bR was confirmed by measuring the absorbance of fractions both at 280 nm and 570 nm. Peak fractions of solubilized bR were collected and stored at −150°C until use.

2.2. Planar bilayer formation and photoresponse measurements

Planar bilayers were formed according to the method described by Montal and Mueller [16]. A Teflon film (25 μm thick) with a hole of 200 μm in diameter was placed between two Teflon chambers (each chamber had an internal volume of 1.5 ml). Photoresponse or photoelectric current (I_p) measurements were taken with a patch/whole-cell clamp amplifier (CEZ-2300, Nihon Koden) under different treatment conditions. The signals were filtered with a multifunction decade filter (E-3201A, NF Electric Instruments) and sent to a strip-chart recorder. The chamber being at electrical ground was defined as the *trans*-side and the other as the *cis*-side. The membrane system was illuminated by a xenon lamp (300 W) along with a heat absorption filter.

The steps in the bilayer formation and subsequent I_p measurements (taken at room temperature) were as follows.

(1) *Membrane formation.* Only membranes having an electrical resistance above 1000 GΩ just after the formation were used.

(2) *Membrane stabilization.* A 1-h incubation period allowed the membrane to stabilize.

(3) *Bacteriorhodopsin reconstitution.* Thirty microliters of bR suspension (corresponding to approximately 8 μg of bR) was added to the *cis*-chamber, and left untouched for one hour during which solutions in both chambers were stirred.

(4) *Photocurrent measurement No. 1.* The membrane was illuminated with the xenon lamp, and the I_p was measured and recorded.

(5) *Perfusion of the cis-chamber.* The bR that was not reconstituted into the lipid bilayer was removed by perfusion with a buffer solution using a peristaltic pump.

(6) *Photocurrent measurement No. 2.* The I_p was measured again to assure successful perfusion.

(7) *Addition of lanthanum ions.* Ten microliters of a 1 M $LaCl_3$ solution was added to the *cis*-chamber to assure that the bR oriented properly in the membrane, because the La^{3+} site directly binds to bR (to the cytoplasmic side) and inhibits the proton pump activity of bR.

(8) *Photocurrent measurement No. 3.* The I_p was measured again to assure successful inhibition.

(9) *Perfusion of the cis-chamber.* The La^{3+} ions were removed using the method mentioned in Step 5.

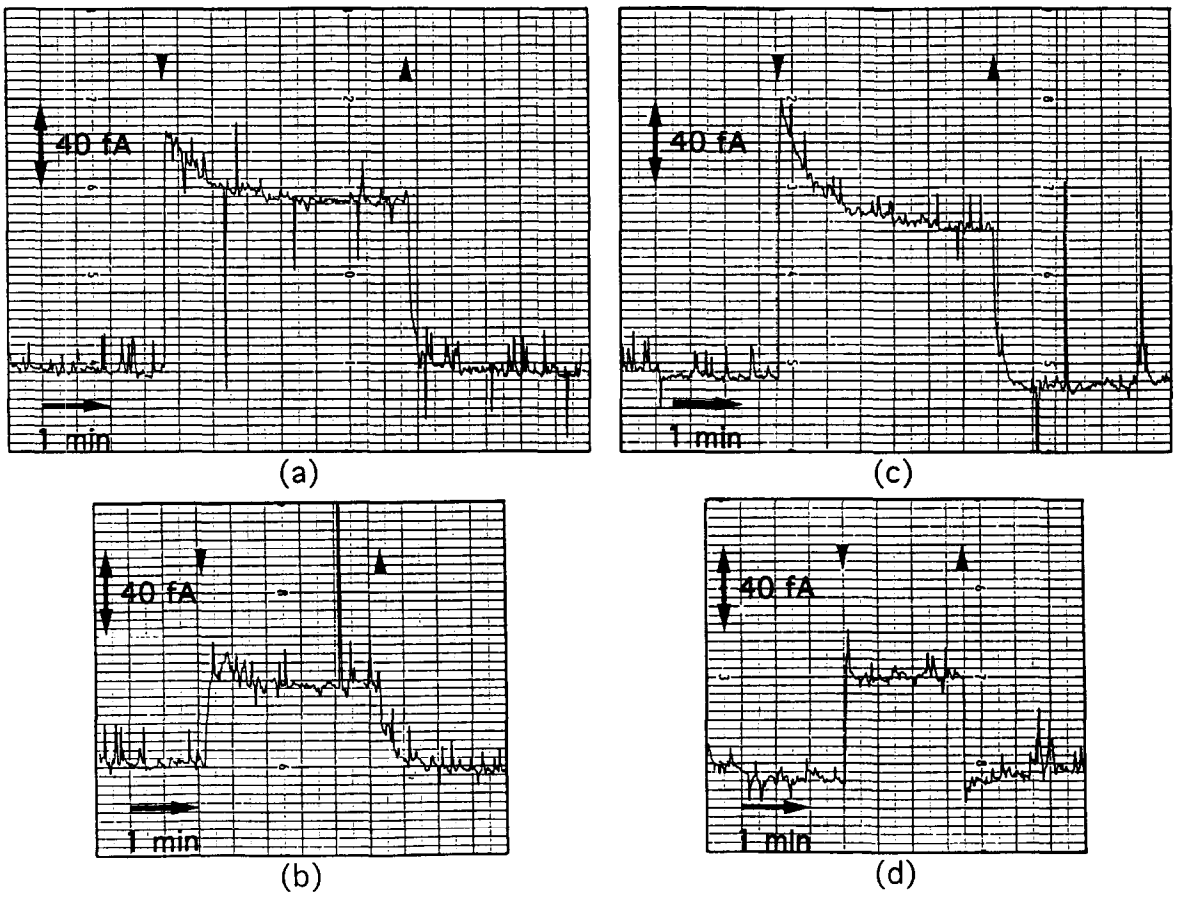


Fig. 1. Photocurrent I_p measured under different treatment conditions after the bR reconstitution process. (a) After perfusion of the non-reconstituted bR (Step 6). (b) After addition of La^{3+} (Step 8). (c) After removal of La^{3+} (Step 10). (d) After final addition of FCCP, totaling 4–6 ng. Up and down arrows represent turning the light source on and off, respectively.

(10) *Photocurrent measurement No. 4.* The I_p was measured to assure successful removal of La^{3+}.

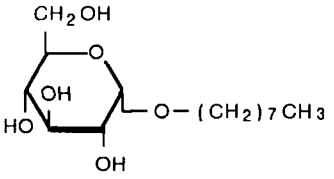
(11) *Addition of FCCP (carbonylcyanide-p-trifluoromethoxyphenylhydrazone).* Two microliters of an ethanolic solution of FCCP (1 μg/ml) was successively added to the *trans*-chamber a total of 2 to 4 times.

(12) *Photocurrent measurement No. 5.* The I_p was measured at each FCCP addition to determine the completion of the reconstitution. No increase in I_p indicated successful transmembraneous reconstitution, because FCCP is a protonophore that makes the underlying lipid bilayer permeable to proton.

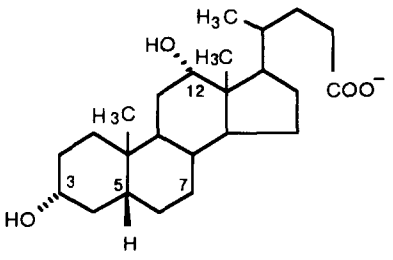
3. Results

3.1. Photoresponse of the bR-reconstituted planar phospholipid bilayers

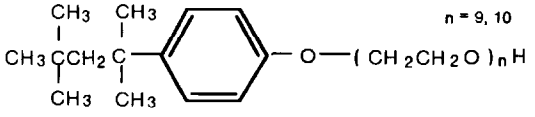
Fig. 1 shows a typical photoresponse of bR reconstituted into lipid bilayers. In this case, the SDOC concentration was 0.25%. Fig. 1a–d correspond to Steps 6, 8, 10, and 12 above, respectively. When La^{3+} was added to the *cis*-chamber in which bR had been added, a steady-state photocurrent I_s decreased (Fig. 1b), clearly indicating that the proton pumping mechanism of bR was inhibited by La^{3+}. However, this inhibition, represented by the decrease in I_s, was limited, that is, I_s was still about half of that for the initial steady-state photocurrent (Fig. 1a). We assumed that this 'partial' inhibition was due to the stacking of bR in which the underlying layers of bR were not easily inhibited by La^{3+}. This assumption is also supported by the fact that I_s totally disappeared when the system was left untouched for 10 to 30 min after the La^{3+} addition. After removal of La^{3+}, I_s was usually restored, however, in some cases, not entirely restored to its initial value (Fig. 1c). Because perfusion seems capable of removing the bR stacked on the lipid bilayers [11,12], incomplete restoration of I_s after the perfusion may occur. However, when La^{3+} removal proceeded without removing the bR on the membrane system, I_s was restored. Upon addition of FCCP, I_s decreased (Fig. 1d), and in some cases, remained unchanged. As



(a) Octylglucoside (OG)



(b) Deoxycholate (DOC)



(c) Triton X - 100

Fig. 2. Structure of the detergents. (a) Octylglucoside, OG (linear structure). (b) Deoxycholate, DOC (cholane-ring structure). Sodium DOC or SDOC was used in this study. (c) Triton X-100 (linear structure) used for solubilization of bacteriorhodopsin, bR.

mentioned earlier in Step 12, no increase in I_s after FCCP addition indicates that bR had been reconstituted in a transmembraneous manner. The decrease in I_s seen after the FCCP addition was possibly due to a significant decrease in the membrane resistance (less than one-tenth that without FCCP addition), meaning a shunting of the electrical circuit involving the membrane system.

3.2. Effects of detergent on the photoresponse of the bR-reconstituted phospholipid bilayers

Fig. 2a and b shows the structures of the two detergents, OG and DOC, respectively, used for the substitution after solubilizing bR with Triton X-100 (whose linear structure is shown in Fig. 2c as a comparison). Linear-structured detergents have often been used for bR-solubilization from purple membranes [17–19]. In contrast, cholane-ring structured detergents have not been used, because they show poor ability in solubilization of integral membrane proteins, especially bR [20,21]. Del Rio et al. [13] showed that the difference in the solubilizing ability between the two detergent types results from the difference in their structures. Their study clearly indicated that the linear-structured detergent tightly bound with the bR, making it difficult to be removed from bR. In contrast, their study found that the cholane-ring structured detergent could be easily removed from bR.

The critical micellar concentration (CMC) of OG is 0.73% (wt/v), and that of SDOC is 0.2%. In this study, we used OG concentrations of 0.25, 0.5, and 1.0% (wt/v), and sodium DOC concentrations of 0.08, 0.15, and 0.25% (wt/v). No photoresponse was observed when OG concentration was 1.0%, in many cases because the lipid bilayer significantly destabilized, resulting in membrane rupture. However, even when no membrane rupture occurred, no photoresponse was observed. In addition, we often observed denaturation of bR molecules. The observed membrane rupture and denaturation were probably due to the strong interaction of OG with bR and with lipid bilayers. Such interaction results from the linear structure of OG [13].

Figs. 3 and 4 show the variation in I_s of the bR-reconstituted lipid bilayer in which OG and SDOC, respectively, were used for the detergent

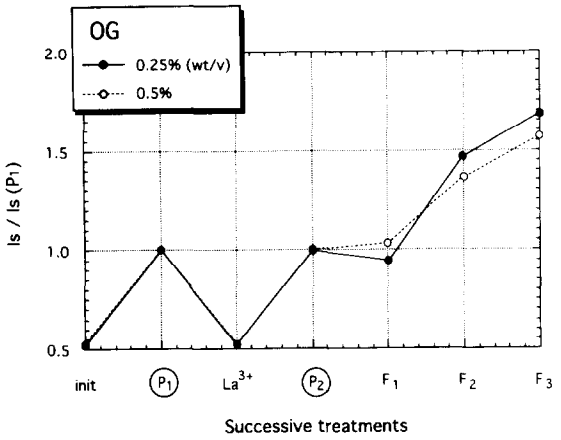


Fig. 3. Treatment-dependent variation in I_s of the bR-reconstituted system in which OG was used as a substitute for Triton X-100. 'init' represents after 1 h incubation that followed the addition of bR. P represents after perfusion (P_1 and P_2 represent after the perfusion of bR and La^{3+}, respectively). La^{3+} represents after the addition of La^{3+}. F_{1-3} represent after successive addition of FCCP. Each I_s was normalized by the value at P_1 (considered the steady-state photocurrent I_s).

substitution. In both figures, I_s was obtained after averaging four measurement data and normalized by the I_s obtained at Step 6, which was after the first perfusion and prior to the La^{3+} addition. When the OG concentration was below its CMC (Fig. 3), the variation in I_s was similar for both concentration levels (0.25% and 0.5%): (1) I_s decreased upon

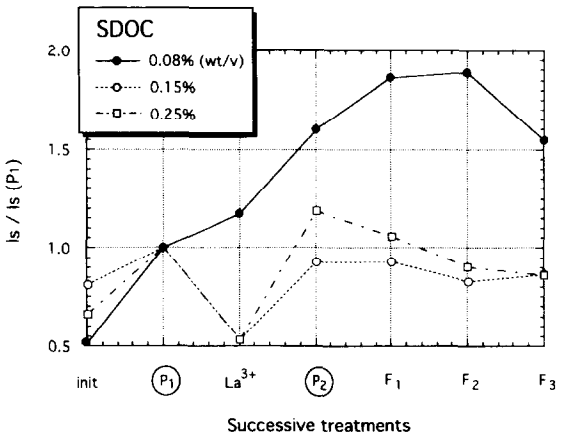


Fig. 4. Treatment-dependent variation in I_s of the bR-reconstituted system in which SDOC was used as a substitute for Triton X-100. For abbreviations of the treatments, refer to the legend for Fig. 3.

addition of La^{3+}; (2) the decreased I_s was restored after removal of La^{3+} by perfusion; and (3) I_s increased upon addition of FCCP. In contrast, for DOC (Fig. 4), the variation in I_s differed depending on the concentration. For a 0.25% SDOC concentration (above its CMC), the variation in I_s was similar to that shown in Fig. 1. As mentioned earlier, such variation in I_s indicated the establishment of the transmembraneous reconstitution of bR being oriented so that its C-terminus side faced the chamber to which bR was added, because FCCP did not cause I_s to increase and because La^{3+} caused it to either decrease or totally disappear depending on how long the membrane was left untouched. On the other hand, when the SDOC concentrations were 0.08% and 0.15%, their photoresponses were quite different, even though the concentration levels were both below the CMC of SDOC. The 0.15% level exhibited the following variation in I_s : (1) a decrease in I_s upon addition of La^{3+}; (2) restoration in I_s after removal of La^{3+} by perfusion; and (3) no change upon addition of FCCP. This variation was similar to that of 0.25% SDOC level, however I_s was always relatively small compared with the I_s for the other two concentration levels, namely, about 20–25% of the I_s seen for the 0.08% or 0.25% levels. This small I_s probably resulted from a photocurrent flowing in the reverse direction caused by a comparatively large amount of reverse-oriented bR, (even though this amount was smaller than that of normal-oriented bR). For 0.08% level, I_s exhibited the following variation: (1) an increase upon addition of La^{3+}; (2) another increase after removal of La^{3+} by perfusion; (3) a further increase upon addition of FCCP.

4. Discussion

4.1. A proposed bR-reconstitution scheme

Considering the results shown in Figs. 3 and 4, we propose a scheme for the bR reconstitution into lipid bilayers schematically illustrated in Fig. 5. We need to remember that a bR molecule has a C-terminus and an N-terminus side, and when reconstituted, the C-terminus tends to face the side to which bR is added; we call this condition *unidirectional* or *normal-oriented* when every bR molecule has the same condition (i.e., surrounding detergent and its quantity) [22]. The mechanism of this unidirectional reconstitution is still unclear. In addition, we assume that the detergent surrounding the bR must be removed upon reconstitution into the lipid bilayer because the hydrophobic region of the lipid bilayer requires bR to expose its hydrophobic portion so that bR can be in contact with the inner (hydrophobic) region of the lipid bilayer.

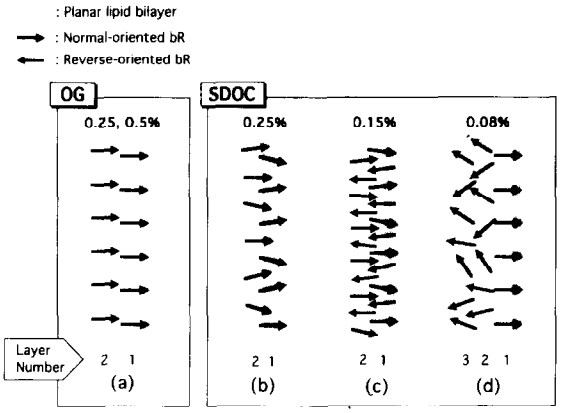


Fig. 5. Proposed scheme for the reconstitution of bacteriorhodopsin (bR) into phospholipid bilayers for different types and concentrations of substitution detergent. (a) OG concentrations of 0.25 and 0.5%. (b) SDOC concentration of 0.25%. (c) SDOC concentration of 0.15%. (d) SDOC concentration of 0.08%.

4.2. OG case and the proposed bR-reconstitution scheme

The similarity in photoresponse for the OG concentrations of 0.25 and 0.5% (i.e., independent of concentration) was possibly due to the similarity in structure between OG and the Triton X-100 used for solubilization. Because both OG and Triton X-100 have linear structures (see Fig. 2), the strength of their interaction with bR is the same. Hence, the detergent-substituted bR may be similar in terms of difficulty in removing the surrounding detergent, independent of how much Triton X-100 was substituted with OG. Using Triton X-100 separation measured both at 280 and 568 nm (see Materials and Methods section), we were not able to identify the detergent substitution because the two detergents show a large absorbance at 280 nm resulting from their similarity in structure (not shown).

Considering this similarity, we see in Fig. 5a for the OG case that the first layer was assumed to be composed of bR that had not been sufficiently reconstituted (i.e., not in a transmembraneous manner) into the lipid bilayer because neither OG nor Triton X-100 could be easily removed from bR. In this case, the hydrophilic portion of the detergent surrounding bR was considered to interact electrostatically with the polar (i.e., hydrophilic) region of the lipid bilayer. The bR was fully surrounded by the detergent, and hence we can anticipate a relatively stable reconstitution state. In this OG case, because bR was not considered reconstituted into the lipid bilayer, we may call this state adhesive reconstitution. We need to consider the second layer to clearly explain the photoresponse results seen in Fig. 3. We assumed that this layer was reconstituted in the same manner as the first layer (i.e., hydrophilic interaction between the surrounding detergents). The addition of La^{3+} inhibited the second layer and resulted in the decrease in I_s. The successive removal of La^{3+} restored I_s, indicating no bR was affected or removed by the perfusion, possibly due to the stable reconstitution. When FCCP was added to the *trans*-chamber, the pathway for protons to flow was opened because FCCP is a protonophore. Therefore, I_s increased because the flow of protons translocated by the stacked bR (in the first and second layers) was facilitated across the lipid bilayer through the FCCP-induced pathways.

Concerning the FCCP-induced increase in I_s, we found that it is more than ten times larger than that found when purple membranes other than solubilized bR were used for the reconstitution. Because purple membranes are large enough not to be reconstituted into the lipid bilayer, they possibly only adhere to the bilayer, thus leading to the large increase in I_s upon addition of FCCP.

4.3. SDOC case and the proposed bR-reconstitution scheme

The photoresponses for SDOC (Fig. 4) can be explained by taking the Triton X-100 separation (Fig. 6) into account. When the SDOC concentration was 0.25%, most of the Triton X-100 was substituted with SDOC as indicated by Fig. 6a. Therefore, the bR reconstitution proceeded, with the normal orientation for bR being preferred. Hence, normal-oriented bR was most likely to be present. The existence of at least two stacked layers of reconstituted bR (Fig. 5b) explains the photoresponse results. Upon addition of La^{3+}, the second layer was inhibited, resulting in the decrease in I_s. The successive removal of La^{3+} restored I_s, however, FCCP did not cause the response to increase again. From this, we conclude that the reconstituted bR bound the inner portion of the lipid bilayer with hydrophobic interaction unlike the hydrophilic one seen in the OG case. In addition, we conclude that the bR-reconstituted lipid bilayer was

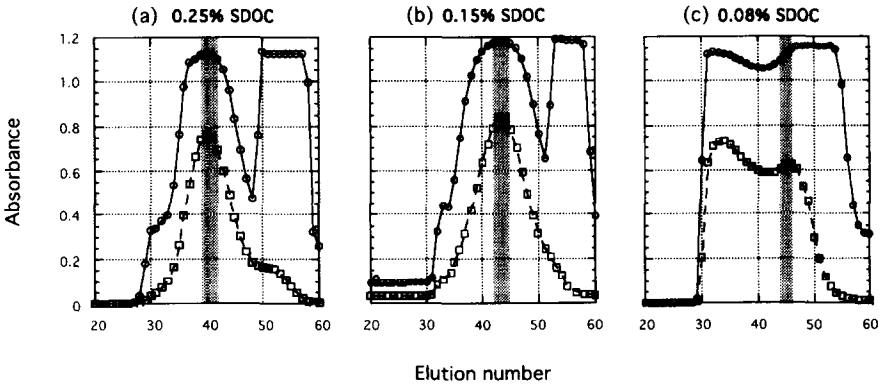


Fig. 6. Absorbance of the gel-filtrated fractions, showing removal of Triton X-100 from the solubilized bR. The detergent substitute was SDOC varied at the following concentration levels: (a) 0.25% (w/v); (b) 0.15%; and (c) 0.08%. Absorbance at 280 nm (○) indicates the existence of both Triton X-100 and bacteriorhodopsin. Absorbance at 568 nm (□) indicates the existence of bacteriorhodopsin. The fractions indicated by the shaded area were collected as peak fractions to be used in the bR reconstitution.

in a rather disturbed condition because the transmembraneous insertion of bR had been established, and hence the proton translocation by bR in the second layer was not entirely inhibited by the lipid bilayer.

When the SDOC concentration was 0.15%, the degree of the separation of the Triton X-100 was less than that observed for 0.25%, as seen in the elution profiles in Fig. 6a and b. This was judged from the fact that the ratio of the 280 nm absorbance at separation point to that at peak for 0.15% level (i.e., fraction numbers 43 and 51 in Fig. 6b; ratio: 0.56) is larger than the ratio for 0.25% level (i.e., fraction numbers 40 and 48 in Fig. 6a; ratio: 0.42). This indicates the existence of larger amount of residual Triton X-100 in 0.15% level compared with 0.25% level.

In the proposed reconstitution scheme for 0.15% (Fig. 5c), the first and second layers were formed by both bR on which DOC is bound around the N- and C-terminus (N- and C-bR). This is because most of the surrounding detergent was DOC (however, less than the 100% (by surface area) speculated for the 0.25% SDOC level). This resulted in the simultaneous reconstitution of C-bR with N-bR in a reverse-orientation manner, because, as we mentioned earlier, DOC is easily removed from bR, and we assumed that detergent removal is necessary for the reconstitution into the lipid bilayer. Again, the small I_s must result from the reversed current contribution caused by C-bR. It is quite natural that N-bR (which is normal-oriented bR) was larger in number than C-bR, due to the preference for the normal orientation. Consequently, La^{3+} could inhibit N-bR, leading to the decrease in I_s. Because both layers consisted of bR on which most of its surface area was reconstituted (after removal of DOC), the first layer was fit rather snugly into the lipid bilayer by the hydrophobic interaction as stated for the 0.25% DOC case, and the second layer into the first. Therefore, the perfusion contributed only to the removal of La^{3+}, and not to a simultaneous removal of the second layer, resulting in the restoration of I_s. Because the first layer had distorted the resulting reconstituted lipid bilayer, the addition of FCCP did not increase I_s (similar to the 0.25% concentration case).

When the SDOC concentration was 0.08%, the addition of La^{3+} increased I_s, while the addition of FCCP increased it even further. According to the elution profile (Fig. 6c), Triton X-100 was not clearly separated and most of the detergent remained bound to bR even after detergent separation. From this, we inferred the co-existence of bR with a small amount of DOC and bR with no DOC (Tri-bR). Concerning bR with the small amount of DOC, we assumed that the substituted portion on bR was rather randomly distributed. Therefore, the amount of C-bR and N-bR was assumed to be equal. We should note again that the quantity of DOC bound to bR was smaller compared to those of 0.15% and 0.25% level, even though the terms C- and N-bR are referred in three concentration levels of SDOC.

Fig. 5d shows the proposed reconstitution for an SDOC concentration of 0.08%. This reconstitution scheme consists of three layers to explain the results. In the first layer of the reconstitution, normal-oriented bR was considered predominant (as in the case of OG seen in Fig. 6a), because the bR molecules that were fully surrounded by Triton X-100 were assumed major in number. Tri-bR contributed to the formation of the first layer driven by the preference for normal orientation in the bR reconstitution onto lipid bilayers. Because Tri-bR is basically the same as the bR for the OG case (i.e., the linear structured detergent surrounds bR), the first layer was formed by Tri-bR with the same mechanism for the OG case (i.e., with hydrophilic interaction). N-bR and C-bR were considered to participate in the formation of the first layer, however N-bR must have been predominantly reconstituted due to the preference of the normal orientation. Though N-bR was considered to be involved in the first layer, it was much smaller in number compared with Tri-bR. Upon reconstitution, N-bR was considered to interact with the bilayer in a different manner from the other cases mentioned earlier. That is, N-bR was considered to be reconstituted by the hydrophobic interaction between the inner portion of the bilayer and the DOC-removed portion of bR, because the DOC-removed portion must be kept away from an aqueous solution. For this scheme, it was necessary for the bilayer to expose its inner portion to N-bR in order to interact with N-bR, and hence those two hydrophobic regions could not interact closely. Therefore, we considered that N-bR was not reconstituted stably. In addition, as mentioned earlier, because the DOC-substituted

portion was considered randomly distributed, the orientation of the reconstituted N-bR in the first layer was considered to deviate from the normal to the bilayer plane. When DOC is removed from the C-bR after the formation of the first layer, the C-bR must either aggregate or be reconstituted so that its C-terminus side faces the first layer (i.e., reverse orientation) in the same manner as N-bR, overcoming the preference for the normal orientation. The second layer was formed first, and then the third layer. The aggregated C-bR might participate in the formation of these layers. Again, because the DOC-substituted portion was considered randomly distributed, the reverse-oriented bR in the second and third layers must have a rather disturbed orientation, as shown in Fig. 5d. Hence, we assume that the C-terminus of bR, to which La^{3+} was to bind, was considerably exposed to the *cis*-chamber so that La^{3+} could inhibit the proton pump mechanism of bR. This assumption clearly explains the results for the SDOC concentration of 0.08%. That is, La^{3+} inhibited the bR in the third layer, even though bR was reverse-oriented, resulting in the increase in I_s, because the protons pumped in the reverse direction had contributed to the decrease in I_s. The perfusion that followed this inhibition then removed the third layer and then the second layer as well as La^{3+}, because, as mentioned for the N-bR contribution to the first layer, the detergent-free portion of bR was considered to distantly or weakly interact with the inner portion of the bilayer. The first layer was formed in the same manner as those for the OG case by Tri-bR which was considered predominant in number, therefore, the increase in I_s upon the addition of FCCP does not contradict our assumption of the three-layered structure.

5. Conclusions

In this paper, we described how detergents used for the substitution after solubilization of bacteriorhodopsin affect the reconstitution of the protein into planar phospholipid bilayers. A detergent with a linear structure showed a strong interaction with the protein and caused the protein to denature when the concentration of the detergent was above its critical micellar concentration. However, when used at below this critical concentration, the detergent showed characteristics in the reconstitution of the protein (determined by measuring the photoresponse under different treatment conditions) that were independent of its concentration. On the other hand, a detergent with a cholane-ring structure showed various characteristics in the reconstitution depending on its concentration. The major cause for this variation was clearly explained by considering the ease at which the detergent can be removed from the protein.

Our results suggest that, for successful transmembraneous reconstitution of bacteriorhodopsin, detergents with cholane-ring structure are preferable to those with linear structure when used at above its critical micellar concentration. This study should be expanded to include other detergents so that successful reconstitution of various membrane intrinsic proteins other than bacteriorhodopsin can be attained and characterized.

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