Non-Proton Ion Release in Purple Membrane

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ABSTRACT Large conductivity changes have been measured during the photocycle of bacteriorhodopsin in purple membrane. These phenomena were explained as being due to the occurrence of large-scale non-proton ion release. Here we show that these conductivity changes do not appear if the purple membrane is immobilized. We propose an alternative hypothesis that explains the presence of conductivity change in suspensions and their absence in gels, as well as several related effects suggesting that the observed conductivity changes are due to alteration of the polarizability of purple membrane during the photocycle.

INTRODUCTION

In a paper, Marinetti (1987a) pointed out that evidence exists that shows large-scale conductivity changes independent of proton release from purple membrane (PM) after light excitation of bacteriorhodopsin (bR) molecules. The onset of the large-scale change occurs if pH is increased through pH 7 in a solution containing 20 mM NaCl, or at pH 7 if the NaCl concentration is raised from 20 to 150 mM.

Marinetti (1987a) interpreted these results to mean that non-proton ions are released from PM into the suspension. The time-resolved conductivity change at the proper pH or NaCl concentration range corresponds to ≈10 non-proton ions released per photon absorbed. Since the quantum efficiency of the photocycle is ≈0.6 (Govindjee et al., 1990), nearly 20 ions/photocycle would be involved in the conductivity change. The onset of the large change in released charges was attributed to the phenomenon of the counterion condensation at the surface of the purple membrane, which would change dramatically due to the activity of the bR proton pump. The critical charge density required for condensation may depend on pH or NaCl concentration as predicted by the theory of Manning (1978). This would explain, according to Marinetti (1987a), the abrupt pH and salt concentration dependence of the conductivity changes.

Arguments based on simple principles of energetics may be raised against this interpretation. The binding energy of an ion should be larger than the thermal energy ≈0.025 eV at room temperature. This requires 0.5 eV for 20 ions. About 30% of the photon energy (0.7 eV) is used to drive the photocycle (Birge et al., 1989). It is rather improbable that >0.5 eV energy from 0.7 eV is used for ion release.

We show in this paper that conductivity changes do not occur if the PM is immobilized in gel. An alternate explanation, which takes into account the rotational motion of PM, is presented.

MATERIALS AND METHODS

PM was prepared from *Halobacterium salinarium* (formerly *halobium*) strain ET 1001 using standard procedures (Oesterhelt and Stoeckenius, 1974). In setting the bathing solution, size of cuvette, and electrodes, the protocol of Marinetti (1987a) and Marinetti and Mauzerall (1983) was followed as closely as possible. An important difference was the preparation of gel samples of 1 × 1 × 1-cm size in which PM was immobilized in a random distribution (Dér et al., 1985). A suspension of PM and the gel sample (bR content ≈ 8 μM in both cases) were put into an 1 × 1 × 4-cm cuvet. (The gel sample was stored in the given bathing solution of ≈20 ml overnight.) Platinized Pt electrodes set at the diagonal corners of the cuvette were connected through a measuring resistance (see Fig. 1) to the frequency output of a lock-in amplifier (KFKI NV-255B, Budapest, Hungary), and from this output 1 V at 100 KHz was switched to the circuit.

The photocycle was initiated by a dye laser (rhodamine 6G) excited by an excimer laser (Lambda Physik EMG101 MSC, Göttingen, Germany). Laser flashes of ≈2 mJ energy were focused on the sample in a spot of 3-mm diameter. The voltage at the resistance was registered by the lock in amplifier with a time constant of 100 μs, and digitized by a transient recorder (KFKI, NEO 200-B, Budapest, Hungary). In general 100–120 repetitions were averaged.

RESULTS AND DISCUSSION

It was thought that data at the limiting pH and NaCl concentration values for suspension and gel samples provide enough information to decide whether immobilization influences the "non-proton ion" release or not. In Fig. 2, traces measured for suspension at pH 6.43 and 8 are shown. The data reproduce the main features of the measurement of Marinetti (1987a). Both figures show an increase in the conductivity constant in time due to an increase in the temperature of the solution. At pH 8, the additional large conductivity change that is missing at pH 6.43 is remarkable.

On the other hand, similar traces in the case of gel samples at pH 6.38 and 8.9 do not show such conductivity change due to a rise in the pH (Fig. 3), although the temperature-dependent conductivity change is readily apparent.

Similar data were obtained when the NaCl concentration was changed from 20 mM to 150 mM at pH 7 in solution and gel, respectively (not shown).

We have to conclude that the observed large conductivity change (Marinetti, 1987a; Marinetti and Mauzerall, 1983) does not appear if the PM is immobilized. If the conductivity changes were the consequence of free charges released from

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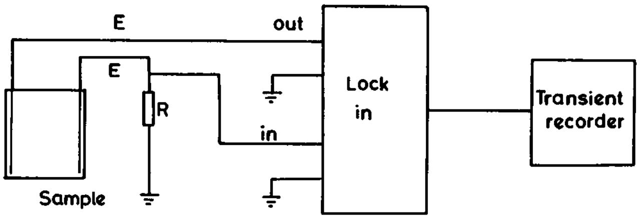

FIGURE 1 Scheme of the measuring system.

PM, they should also be manifested in gels (the polyacrylamide content of the gels is ≈5% (w/w) related to water). Therefore a different explanation, based on the free mobility of PM in suspension, has to be found.

If PM is in suspension and an alternating electric field is applied, the membranes will be aligned through their inducible dipole moment, which is parallel to the membrane surface (Barabás et al., 1983). In such cases ions move on the surface of the PM inducing a current in the external circuit. Papp and Fricsovszky (1990) have shown that with the switching on of a given alternating electric field, 1) the current and consequently the conductivity increase in time from 0 to a saturation value, and 2) the saturation value increases with the field strength. These conductivity changes are parallel to the optically observed alignment of PM, meaning thereby that the ordering of the membranes may be observed by measuring either the conductivity or the absorption of polarized light. The current is

$$I_a \sim \alpha E \phi(\gamma) \cos \omega t \qquad (1)$$

where α is the polarizability of membranes, E is the amplitude of the electric field, $\phi(\gamma)$ is the orientation function ($\gamma = \alpha E^2$; Shah, 1963), and ω is the frequency of the alternating field. Similarly, the conductivity

$$G_a \sim \alpha \phi(\gamma) \qquad (2)$$

Papp and Fricsovszky (1990) also observed that at a given external electric field and ω the conductivity changes during the photocycle nearly proportionally to the alignment, i.e.,

$$\Delta G_a \sim C(t) \phi(\gamma) \qquad (3)$$

The function $C(t)$ depends on time: it rises with the time constant of the decay of the M intermediate and decays with the lifetime of the O intermediate of the bR photocycle. According to the measurements of Papp and Fricsovszky (1990) the value of $\Delta G_a/G_a$ is ~3–4%. We think that $C(t) \approx \Delta\alpha(t)$, i.e., the polarizability of PM changes during the photocycle. Such a small change could occur due to protons appearing on the surface or due to changes in the shape of PM. $\Delta\alpha(t)$ has been previously measured and found to be small (Kimura et al., 1984). The absence of any conductivity signal in gels where the PM is immobilized requires that the essential part of $\Delta\alpha(t)$ should originate from changes in the shape of PM, because otherwise the component of $\Delta\alpha$ of the randomly oriented PM parallel to the direction of the electric field should produce a current. It is known that such shape and size alterations due to the photocycle exist (Czégé and Reinisch, 1990, 1991) and also that they do not appear in gels (Czégé et al., 1982).

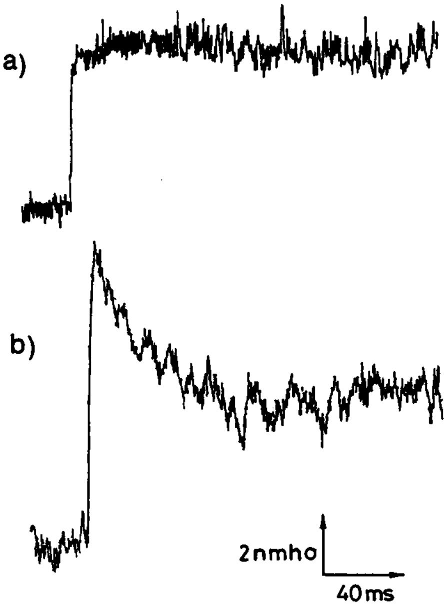

FIGURE 2 Time dependence of the conductivity change in bR suspension after flash excitation. 8 μM bR in a solution of 2 mM TEMED, 20 mM NaCl at (*a*) pH 6.43 and (*b*) pH 8. Temperature 21°C.

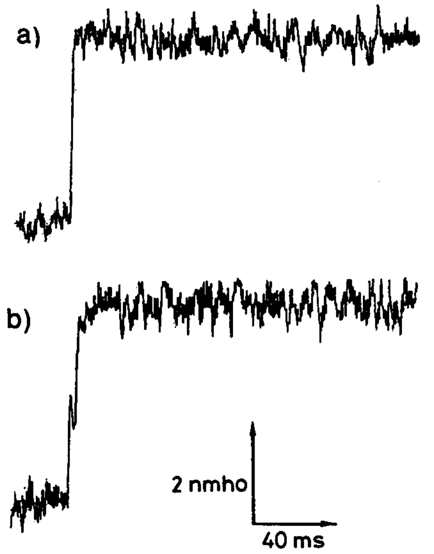

FIGURE 3 Time dependence of the conductivity change in gel containing immobilized, randomly ordered bR of ≈8 μM. Solution contained 2 mM TEMED, 20 mM NaCl at (*a*)pH 6.38 and (*b*) pH 8.9. Temperature 22°C.

This model explains the other related results of Marinetti (1987a, 1988) as well. $\Delta\alpha$, because of shape and size alterations, may depend on the condensation phenomenon. Since alignment is needed for the observation of $\Delta\alpha$ according to this model and it depends on the size of the membrane (Kimura et al., 1984), it is not surprising that solubilization abolishes the effect (Marinetti and Mauzerall, 1986). Bacteriorhodopsin in vesicles does not exhibit the large-scale non-proton ion release (Marinetti, 1987b). In contrast to the explanation of the author that the disruption of the native PM structure in vesicles causes the absence of the effect, our model simply states that the closed vesicles do not align in the electric field. Consequently, the alteration of α may not induce current. The change of shape or size is a cooperative effect (Czégé and Reinisch, 1990, 1991); therefore the cooperativity found by cross-section measurement (Marinetti, 1988) is not surprising.

In conclusion, we think that the explanation assigned to large-scale non-proton ion release during the photocycle of bR, which contradicts energy conservation, may be understood simply by assuming a change in the polarizability of the PM due to shape and size alterations.

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